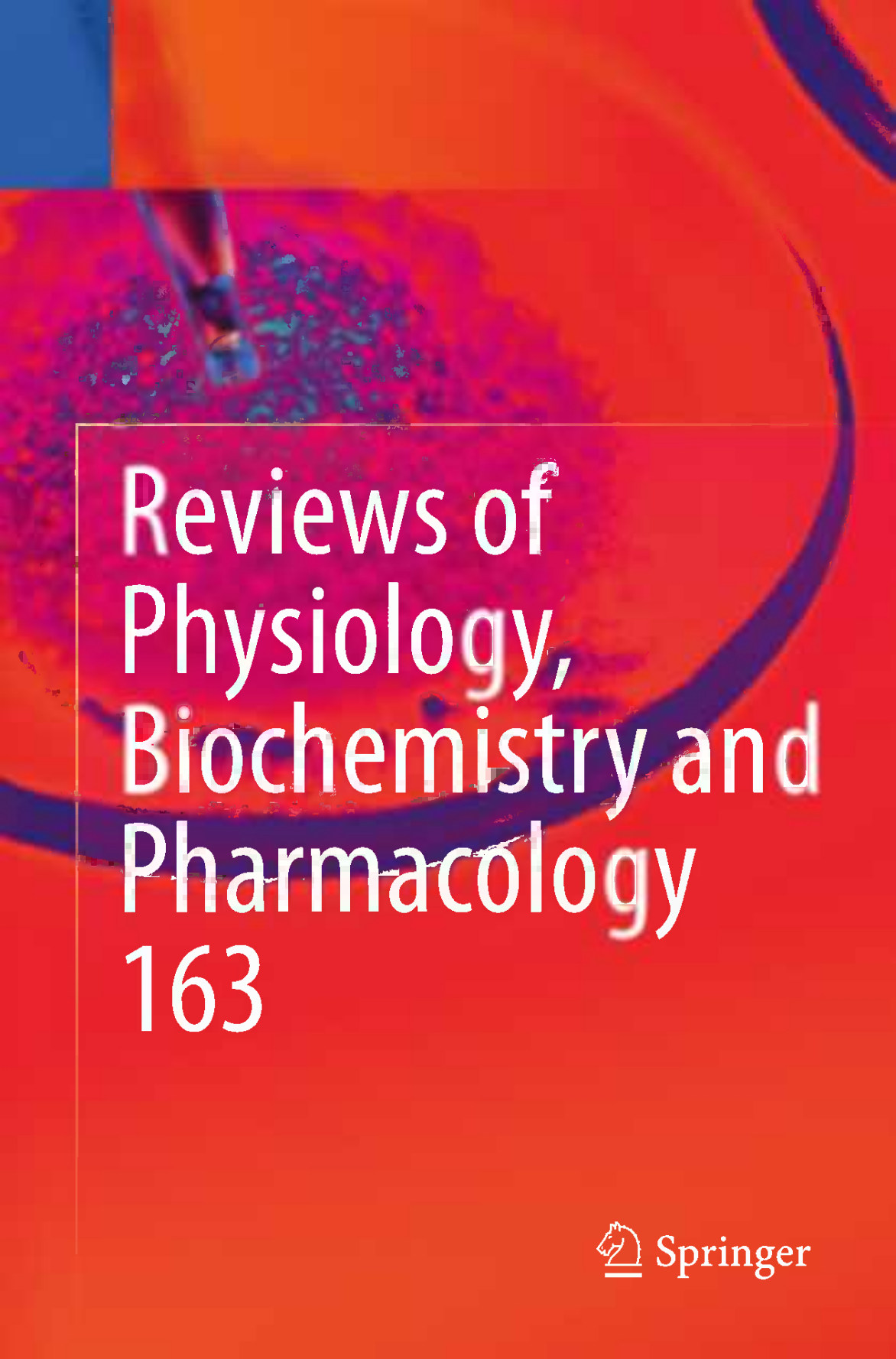
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Induced Pluripotent Stem Cells in Cardiovascular Research

Daniel Sinnecker, Ralf J. Dirschinger, Alexander Goedel, Alessandra Moretti, Peter Lipp, and Karl-Ludwig Laugwitz

Abstract The discovery that somatic cells can be reprogrammed to induced pluripotent stem cells (iPSC) by overexpression of a combination of transcription factors bears the potential to spawn a wealth of new applications in both preclinical and clinical cardiovascular research. Disease modeling, which is accomplished by deriving iPSC lines from patients affected by heritable diseases and then studying the pathophysiology of the diseases in somatic cells differentiated from these patient-specific iPSC lines, is the so far most advanced of these applications. Long-QT syndrome and catecholaminergic polymorphic ventricular tachycardia are two heart rhythm disorders that have been already successfully modeled by several groups using this approach, which will likely serve to model other mono- or polygenetic cardiovascular disorders in the future. Test systems based on cells derived from iPSC might prove beneficial to screen for novel cardiovascular drugs or unwanted drug side effects and to individualize medical therapy. The application of iPSC for cell therapy of cardiovascular disorders, albeit promising, will only become feasible if the problem of biological safety of these cells will be mastered.

1 Introduction

Among the organs that constitute the human body, the heart has always been regarded as extraordinary. William Harvey, the seventeenth century anatomist known for the discovery of the systemic blood circulation, poetically addressed

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the heart as "the sun in the animal's microcosm", "from which all power and vitality emanates" (Harvey 1628). While cardiac catheterization, open-heart surgery and even heart transplantation have become commonly-performed procedures nowadays, cardiomyocytes from human patients are still not easily obtained in order to use them for physiological experiments designed to illuminate the pathophysiology of the patient's diseases. While cardiomyocytes isolated from animals might be used instead, interspecies differences in cardiac physiology often hamper the extrapolation of the results of such studies to human physiology. Thus, a method to safely generate patient-specific human cardiomyocytes would be extremely valuable.

In contrast to the hearts of some lower animals, which have a great potential for regeneration after damage, mammalian hearts almost completely lack this ability. Accordingly, heart failure represents a major cause of mortality in western societies. The evolving field of regenerative medicine might provide a cure for these patients, for example by transplanting in vitro-generated cardiomyocytes into the failing myocardium. However, methods to effectively generate such cells still have to be developed. The induced pluripotent stem cells described in the next section might provide new approaches to the above-mentioned problems.

2 Induced Pluripotent Stem Cells

The derivation of embryonic stem cells (ESC) from the inner cell mass of early embryos has spawned a technological revolution in biology and medicine. The two major characteristics of ESC are pluripotency and self-renewal. This means that they can differentiate into all kinds of somatic cells that constitute an adult organism, and, on the other hand, proliferate without losing this potential. ESC culture techniques are the basis of state-of-the-art genetic methods such as the generation of genetically-modified mice.

2.1 A New Type of Pluripotent Stem Cells

In 2006, Takahashi and Yamanaka published a seminal paper showing that mouse fibroblasts could be reprogrammed to ESC-like pluripotent cells by expressing a specific cocktail of transcription factors in these cells (Takahashi and Yamanaka 2006). They termed this new cell type "induced pluripotent stem cells" (iPSC). It was demonstrated that these iPSC share key features with the embryo-derived ESC, including the potential to contribute to an embryo by chimera formation and to transmission through the germ line to the next generation (Okita et al. 2007). This methodology was soon also applied to human cells (Takahashi et al. 2007; Yu et al. 2007). These human iPSC bear the potential to fulfill the needs of scientists interested in the development of new cardiovascular disease models as well as physicians searching for a source of cells for regenerative medicine.

2.2 Generation of Human Induced Pluripotent Stem Cells

In the original report of the generation of murine iPSC, fibroblasts have been reprogrammed to a pluripotent state (Takahashi and Yamanaka 2006). When the same group published the first report of the generation of human iPSC, they again used fibroblasts as starting material (Takahashi et al. 2007). Accordingly, when this technology was adopted by other groups and exploited to generate patient-specific iPSC lines, most investigators decided to also use skin fibroblasts that can be easily isolated from skin biopsies. A skin biopsy is a small, not very invasive procedure that can be performed in local anesthesia. However, newer developments like the reprogramming of keratinocytes from plucked hair follicles (Novak et al. 2010) or the reprogramming of T-lymphocytes isolated from blood (Brown et al. 2010) might render iPSC generation even more convenient in the future.

The classical "cocktail" used for reprogramming consists of the transcription factors Oct3/4, Sox2, c-Myc and Klf-4. The classical method to deliver these factors into the cells is the use of retroviral gene transfer. Several attempts have been made to modify these protocols (reviewed recently in Sidhu 2011), mainly to generate iPSC that neither overexpress the known oncogene c-Myc nor have retroviruses randomly integrated into their genome, potentially leading to the activation of endogenous oncogenes. These approaches are especially important for future applications in human cell therapy, where the safety of the cells and the integrity of their genomic DNA are important issues.

2.3 Differentiation Protocols

Several protocols have been developed to direct the differentiation of iPSC towards cardiomyocytes. Most of these protocols are based on the differentiation of the cells in "embryoid bodies" (EB), which form spontaneously when undifferentiated ESC or iPSC aggregate under appropriate culture conditions (Keller 1995). In these three-dimensional structures, spontaneous differentiation into lineages representing all three germ layers starts, eventually leading to the differentiation of some cells to cardiomyocytes. Cardiac differentiation can be promoted by adding supplements such as ascorbic acid (Takahashi et al. 2003), Wnt3a (Tran et al. 2009) or several others to the cell culture medium. The areas of the EB in which the cells differentiate into cardiomyocytes can be easily identified by spontaneous contractions that appear usually after 10–20 days of differentiation. These spontaneously contracting areas can be dissected manually from the rest of the EB and cultured further to allow additional maturation of the cells. To perform experiments that require single cells, these cells can be dissociated by collagenase digestion.

Immunofluorescence stainings of human iPSC-derived cardiomyocytes are shown in Fig. 1. Cardiomyocytes can be identified by the expression of the cardiac isoform of troponin T (cTnT) in ordered sarcomeric structures. When looking more closely at those cells, different subpopulations can be identified. One subpopulation

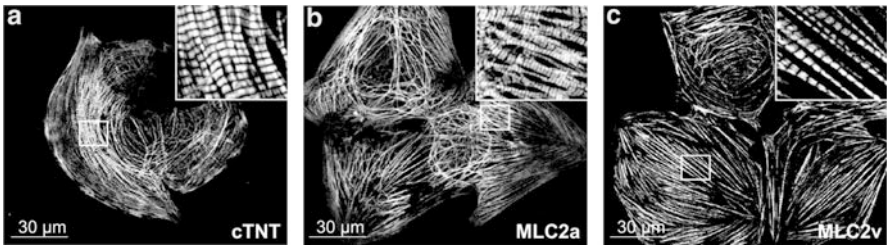

Fig. 1 Cardiomyocytes generated from human induced pluripotent stem cells. Immunofluorescence images of human iPSC-derived cardiomyocytes stained for cardiac troponin T (A) as well as the atrial (B) and the ventricular isoform (C) of myosin light chain are shown. The insets show magnifications of the areas indicated by the boxes

of these cells stains positive for the atrial isoform of myosin light chain (MLC2a), while another subpopulation expresses the ventricular isoform MLC2v (see Fig. 1). Co-expression of both isoforms was observed in a fraction of cells. It should be noted that these cells are morphologically more similar to embryonic than to adult cardiomyocytes. This also holds true in respect to RNA expression profiles and should be considered in the interpretation of experiments relying on these cells. Accordingly, protocols that lead to the generation of more mature cardiomyocytes from iPSC would be extremely valuable.

2.4 The Concept of Patient-Specific Stem Cells

The reprogramming of somatic cells to iPSC offers the opportunity to generate patient-specific iPSC lines from somatic cells of a patient affected by a genetically-caused disease. The resulting iPSC lines are genetically identical to the patient, which makes them an ideal source of cells for further experiments in the fields of disease modeling, drug development or cell therapy (Fig. 2). If the cells are differentiated to the somatic cell that is typically affected by the patient's disease, these patient-specific iPSC-derived differentiated cells can be used to study pathophysiological aspects of the disease in vitro. Furthermore, these cells might be used as a platform to develop new drugs, to screen for unwanted drug side effects, or to select a drug that fits best to the genetic background of a specific patient. This concept appears especially beneficial for disciplines like cardiovascular biology, where samples of human tissue for research purposes are not easily obtained, often requiring invasive procedures like myocardial biopsy. Finally, the use of such patient-specific cells in cell therapy might represent a means to circumvent the problem of immunological rejection, based on the principle that the cells are genetically identical to the patient (see Fig. 2).

3 A New Type of Disease Models

The availability of patient-specific stem cells offers the possibility to differentiate these cells to the type of cells or tissues that are normally affected by the patient's disease to study the pathophysiology of the disease in vitro (see Fig. 2). While the first human diseases that were successfully modeled with iPSC technology were neurodegenerative (Ebert et al. 2009; Lee et al. 2009) and hematological (Ye et al. 2009) disorders, the potential of this new type of disease models has been soon recognized by scientists working in the area of cardiology. The first cardiac disease that was studied by several research groups using this new methodology was the long-QT syndrome, an inherited arrhythmogenic disease.

3.1 General Considerations

Animal models have been used widely to gain insight into the pathophysiology of cardiovascular disorders. Especially genetically-modified mouse models have proven to be extremely valuable in this context. However, differences between human and rodent physiology preclude the generalization of these results to human disease. This becomes particularly evident when looking at arrhythmogenic disorders. The heart rate of a mouse is about ten times faster than that of a human, requiring the cardiac action potential to be much shorter. Accordingly, major differences exist in the shape of the cardiac action potential and in the underlying ionic currents between murine and human cardiomyocytes (London 2001; Nerbonne et al. 2001).

When iPSC-derived patient-specific cardiomyocytes are used as a disease model, the observations in the patient-specific cells must be compared with observations in control cardiomyocytes. These cells should be iPSC-derived cells generated by the same differentiation protocol. The ideal source for these control iPSC is still a matter of debate. Up to date, the usual practice is to use control iPSC lines generated from healthy probands unrelated to the patient who are unaffected by the disease under study (Moretti et al. 2010b; Itzhaki et al. 2011; Yazawa et al. 2011). However, this approach bears the risk that differences between patient and control cardiomyocytes arise from confounding genetic factors unrelated to the disease. One way to circumvent this problem would be to rely on not just one control iPSC line but on a panel of control lines derived from genetically diverse subjects. The major drawback of this approach is the increased cost and labor associated with the generation and maintenance of multiple control cell lines and with the performance of multiple control experiments with cells derived from the different lines. Another way to limit the genetic variance between patient and control cells would be the derivation of control iPSC lines from a close relative of the patient who is unaffected by the disease. When a monogenetic disease is investigated, the ultimate control iPSC line could be constructed by correcting

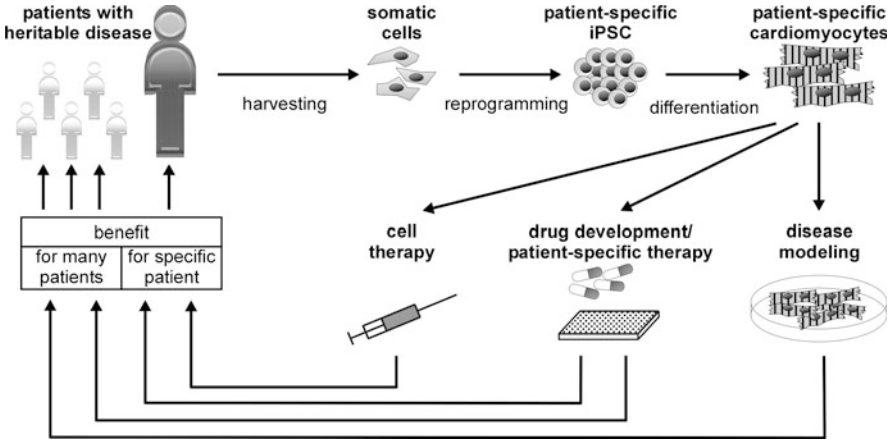

Fig. 2 The concept of patient-specific stem cells. Patient-specific iPSC are generated by reprogramming of somatic cells harvested from a patient affected by a heritable disease. These iPSC are differentiated e.g. to patient-specific cardiomyocytes that can be used in a wealth of applications ranging from disease modeling to drug development, the development of patient-specific drug therapies or cell therapy. These applications may be beneficial either to the specific patient who has donated the cells for reprogramming or to a greater number of patients affected by the same disease

the disease-causing mutation in the patient iPSC by a gene-targeting approach. Furthermore, such an experiment could unequivocally prove that the mutation under consideration is the sole cause of the phenotypic differences between patient and control cells. Advances in gene targeting of human iPSC may make this approach more feasible in the future by reducing the amount of time, labor and money necessary for the generation of a genetically-corrected control cell line.

3.2 Long-QT Syndrome as a Paradigm for iPSC-Based Disease Modeling

The first cardiovascular diseases that were successfully modeled using iPSC technology were different forms of the long-QT syndrome (Moretti et al. 2010b; Itzhaki et al. 2011; Yazawa et al. 2011; Matsa et al. 2011). Patients suffering from long-QT syndrome present clinically with a prolonged QT interval in the ECG and an increased susceptibility to ventricular arrhythmias. The underlying causes are channelopathies which lead to a dysregulation of the electrophysiological properties of the cardiomyocytes. According to the affected gene, the long-QT syndrome is classified into different subgroups (LQT1–LQT13).

The decision of several groups to model the long-QT syndrome with the new methodology of patient-specific stem cells was likely based on the following aspects of the disease that make it a promising candidate for iPSC-based disease

modeling: First, the long-QT syndrome is a quite common disorder. The incidence of the genetically caused congenital forms was classically estimated to range from 1:20,000 to 1:5,000, while a recent report based on ECG screening of neonates suggests an even higher prevalence of 1:2,000 (Schwartz et al. 2009). The acquired forms, typically unwanted side effects of pharmacotherapy, are even more frequent and represent a common problem in daily clinical practice. Second, based on what is known to date, the disease phenotype of congenital long-QT syndromes develops in a paradigmatically cell-autonomous manner caused by aberrations in the action potentials of single cardiomyocytes. Third, the molecules affected by the disease-causing mutations are plasma membrane ion channels that are easily accessible to electrophysiological investigations, even (by means of patch clamp recordings) at the single-molecule level. Finally, despite invaluable insights gained by animal models, electrophysiological differences between human and non-human cardiomyocytes call for disease models that better represent the electrophysiology of a human heart. Thus, a new class of disease models based on human iPSC was expected to add new insights into the pathophysiology of these disorders.

The common feature of the different types of the long-QT syndrome is that, at least under specific conditions, the plateau phase of the cardiac action potential is prolonged, leading to a prolonged QT interval in the surface ECG. This clinically apparent feature is linked to an increased susceptibility to life-threatening ventricular arrhythmias like ventricular tachycardia or ventricular fibrillation. The typical arrhythmia observed in patients with long-QT syndrome was first described by François Dessertenne in 1966 and termed "torsade de pointes" (Dessertenne 1966), meaning "twisting spikes". This terminus was chosen to describe the typical electrocardiographic pattern of this polymorphic ventricular tachycardia, in which a progressively changing amplitude and shape of the QRS complex gives the impression of the electrical axis rotating around the isoelectric line.

Two basic mechanisms seem to contribute to arrhythmogenesis in long-QT syndrome patients (Eckardt et al. 1998; Antzelevitch 2005): early afterdepolarizations (EAD) and the so-called dispersion of repolarization. EADs, which frequently develop under conditions of a prolonged action potential duration, can give rise to premature action potentials or even series of action potentials, which are then called triggered activity. The physiological dispersion of repolarization – meaning that the action potential is longer in the midmyocardial cells than in the subepicardial or subendocardial cells – is exagerated in long-QT syndrome patients because the midmyocardial cells are particularly sensitive to a prolongation of the action potential duration in response to physiological stimuli or drugs. This provides the substrate on which EAD can trigger reentrant tachycardias (like torsades de pointes).

At the time of the preparation of this manuscript, four iPSC-based model systems for the long-QT syndrome have been published. Our group has focused on LQT1 (Moretti et al. 2010b), which is caused by mutations in the potassium channel subunit *KCNQ1*. Other groups were successful in modeling LQT2, caused by mutations in the potassium channel subunit *KCNH1* (Itzhaki et al. 2011; Matsa et al. 2011), as well as the Timothy syndrome, a disease caused by mutation of the

calcium channel *CACNA1C*, which leads to QT prolongation, syndactyly, immune deficiency and autism (Yazawa et al. 2011). Since the approaches used by the different groups were similar in many aspects, we will focus on our LQT1 model (Moretti et al. 2010b) in the following section.

To model LQT1 with patient-specific iPSC (Moretti et al. 2010b), dermal fibroblasts from two patients (father and son) who were clinically apparent with a prolonged QT interval and proven by genomic sequencing to carry a LQT1-associated mutation of the KCNQ1 locus (R190Q) were reprogrammed to generate patient-specific R190Q-iPSC lines. Control iPSC lines were derived from fibroblasts of an unrelated healthy proband without history of cardiac disease. The patient-specific iPSC lines as well as the control lines were differentiated to cardiomyocytes using an EB-based differentiation protocol. When action potentials of ventricular-like myocytes were elicited by electrical pacing at 1 Hz in R190Q and control cells, it was obvious that the R190Q myocytes displayed prolonged action potentials as compared to control cells (Fig. 3A). The same observation was made for spontaneously occurring action potentials in unstimulated ventricular cells. When the control cells were paced at different rates, it was observed in control cells that increasing the pacing rate led to a decrease of the action potential duration (APD, see Fig. 3A). This is consistent with normal cardiac physiology, where the QT interval (which reflects the action potential duration of ventricular myocytes) decreases with increasing heart rates. In the R190Q myocytes, the action potentials were already prolonged at a slow pacing rate (1 Hz) and the decrease of the APD at increased pacing rates was blunted (see Fig. 3A). Similarly, catecholamine stimulation led to a much lesser reduction of the APD in R190Q cells than in control myocytes. This points out that in the R190Q myocytes, the APD is not only prolonged under basal conditions, but the normal regulation mechanisms which lead to a shortening of the action potential at situations of increased catecholamine stimulation or heart rate (which are the situations in which LQT1 patients typically develop torsades de pointes) are malfunctional. This was corroborated by an experiment in which spontaneously beating myocytes were subjected to stimulation with the catecholminergic agonist isoproterenol (Fig. 3B). In control cells, this led to an increased beating rate, but concomitantly to a decreased APD, resulting in a reduction of the ratio between the APD90 and the beat-to-beat interval. In the R190Q cells, however, the increased beating rate could not be compensated by a shortening of the action potentials, indicated by a increase in the APD90/beat-to-beat interval ratio. Moreover, under conditions of catecholamine stimulation, the cells frequently displayed EAD. The β receptor antagonist propranolol (reflecting a class of medications that is typically beneficial for patients affected by LQT1) reversed both effects in the R190Q cells (see Fig. 3B).

The *KCQ1* gene mutated in the LQT1 patients encodes the α-subunit of the ion channel responsible for the repolarizing I_{Ks} current. When measuring I_{Ks} in the R190Q myocytes, we found it to be reduced to about 25% as compared to control myocytes. This reduction by more than 50% indicated that the mutation might exhibit a dominant-negative effect in the cells heterozygous for the R190Q mutation. Indeed, we could demonstrate that the R190Q mutation exerts a

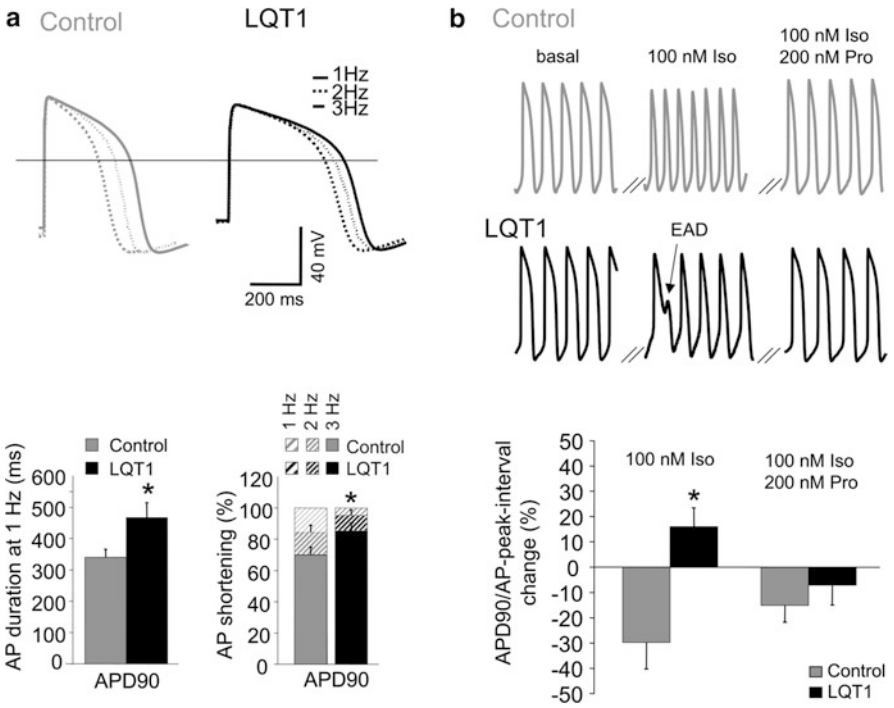

Fig. 3 Modeling the long-QT syndrome type 1 with patient-specific iPSC. *Panel A* shows representative tracings of action potentials (AP) recorded from control and KCNQ1-R190Q (LQT1) myocytes at three different pacing rates (1, 2, and 3 Hz) as indicated. The *left bar graph* shows statistics for the absolute value of APD90 (the duration from the beginning of the action potential until repolarization is accomplished by 90%) at 1 Hz pacing in control and LQT1 myocytes. *The right bar graph* shows the relative shortening of the APD90 upon increasing the pacing rate from 1 Hz to 2 or 3 Hz (as indicated) in control and LQT1 myocytes. *Panel B* shows representative membrane potential recordings of spontaneously beating control and LQT1 myocytes before and after incubation with 100 nM isoproterenol (Iso), in the presence or in the absence of 200 nM propranolol (Pro). In the tracing from the LQT1 cell, an early afterdepolarization (EAD) is indicated. The *bar graph* shows statistics for the ratio of the APD90 divided by the interval between two action potentials under isoproterenol stimulation in the presence and in the absence of propranolol (Adapted from data published in Moretti et al. 2010b)

dominant-negative effect by forming multimers with wild type subunits and interfering with their trafficking to the plasma membrane (Moretti et al. 2010b).

3.3 Other Promising Target Diseases

Disease modeling with patient-specific iPSC can be principally applied to all types of chromosomally-inherited diseases, ranging from monogenetic to complex polygenetic disorders. The decision of several groups to first apply this new technology to

monogenetic diseases was presumably based on the simplicity of this approach. Inherited long-QT syndromes, besides being monogenetic disorders, are paradigmatic cell-autonomous diseases, in which key features of the disease phenotype can be recapitulated in single cells affected by the disease-causing mutation. Modeling diseases with such a cell-autonomous pathophysiology can be expected to give the clearest results. Thus, other monogenetic diseases with a cell-autonomous pathophysiology, like short-QT syndrome, Brugada syndrome or catecholaminergic polymorphic ventricular tachycardia (reviewed below), appear to be promising future targets for iPSC-based disease modeling.

In other monogenetic heart diseases, the phenotype does not arise in a cell-autonomous manner. This is presumably the case in dilated and hypertrophic cardiomyopathy as well as in arrhythmogenic right ventricular cardiomyopathy (reviewed below), where alterations in the myocardial tissue are key features of the pathophysiology. To model such diseases with iPSC technology, it might become necessary to resort to modern tissue engineering techniques (Tiburcy et al. 2011; Tulloch et al. 2011) to construct artificial human myocardium from patient-specific iPSC. Such engineered heart tissue might be also useful to model more complex aspects of arrhythmogenic disorders like the generation of reentrant arrhythmias, which are not accessible to single-cell experiments.

Finally, possible applications of this technology to the more common multifactorial diseases will be reviewed.

3.3.1 Catecholaminergic Polymorphic Ventricular Tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT; reviewed recently by Priori and Chen 2011) is another interesting target for iPSC-based disease modeling. The typical clinical presentation of this inheritable heart rhythm disorder is the development of ventricular tachycardias in situations of increased catecholamine secretion, e.g. physical exercise or emotional stress. The tachycardias observed in CPVT patients are often so-called bidirectional ventricular tachycardias, in which two different morphologies of the QRS complex alternate from beat to beat, suggesting two alternating arrhythmogenic foci. In contrast to the disorders mentioned above, in which the primary pathomechanism is the disruption of the normal function of plasma membrane ion channels, CPVT is a disorder of intracellular calcium cycling. One of the key functions of the cardiac action potential is to regulate the entry of calcium ions into the cytoplasm, which then act as a trigger to activate calcium release from the intracellular calcium stores, constituted mainly by the sarcoplasmic reticulum (SR). The resulting increase in the cytoplasmic calcium concentration is a major regulator of cardiac contraction. This system of calcium storage and release, which results in coordinated oscillations of the cytoplasmic calcium concentration from beat to beat, is tightly regulated. The CPVT-causing mutations known to date are either recessive mutations in the gene encoding the cardiac ryanodine receptor (RyR2), which is the calcium release channel in the

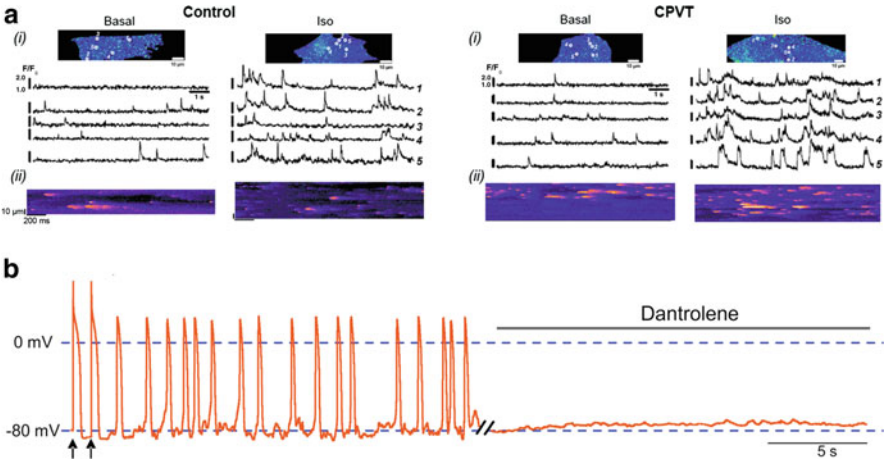

Fig. 4 Modeling catecholaminergic polymorphic ventricular tachycardia with patient-specific iPSC. *Panel A* shows typical results of calcium spark imaging in fluo-4-AM-loaded control and CPVT myocytes in the absence (Basal) or in the presence (Iso) of 1 μM isoproterenol. *Part (i)* shows pseudo-colored images (*upper row*) together with typical Ca^{2+} traces corresponding to each of the five individual regions of interest marked in the top images, imaged at 105 images/s (*lower row*). *Part (ii)* shows line-scan images of Ca^{2+} sparks at a higher temporal resolution (1,000 lines/s). Statistical analysis revealed that the spark frequency was similar in CPVT and control myocytes under basal conditions, but increased to a much larger extent in CPVT cells after isoproterenol application. *Panel B* shows a typical membrane potential recording from a CPVT cardiomyocyte during and after electrical pacing (indicated by the *arrows*) and after application of dantrolene. Note that after termination of pacing, spontaneous action potentials arise, which disappear after application of dantrolene (Adapted from data published in Jung et al. 2011)

membrane of the SR, or dominant mutations in calsequestrin, which is a calcium-buffering protein located in the SR lumen. A unifying concept of the pathophysiology of CPVT is that in patients, overactive mutated ryanodine receptors or a lowered SR calcium buffering capacity due to mutated calsequestrin lead to a lowered threshold for calcium release from the SR under conditions of increased SR calcium loading which arise during catecholaminergic stress. This in turn leads to increased calcium release from the SR resulting in arrhythmogenesis (see Priori and Chen 2011).

We generated iPSC lines from a CPVT patient affected by a mutation in the RyR2 locus (S406L) and analyzed the dynamics of intracellular calcium handling in cardiomyocytes generated from these patient-specific iPSC lines (Jung et al. 2011). We found that, compared to control cells, patient-specific cardiomyocytes displayed elevated diastolic calcium concentrations, a reduced SR calcium content and an increased susceptibility to arrhythmias under conditions of catecholaminergic stress. On the molecular level, this was caused by an increased frequency of elementary calcium release events from small groups of clustered ryanodine receptors, so-called calcium-"sparks" (Berridge et al. 2000), as investigated by high-speed confocal calcium imaging (Fig. 4A). Moreover, we could demonstrate

that the drug dantrolene restored normal calcium spark properties and suppressed arrhythmogenic triggered activity in patient-specific cardiomyocytes (Fig. 4B).

Fatima and colleagues have generated iPSC lines from a CPVT patient affected by another mutation (F2438I) in the RyR2 gene (Fatima et al. 2011). Similar abnormalities in intracellular calcium cycling were found in cardiomyocytes generated from these cells, which were abolished by forskolin, implicating a role of cAMP-mediated regulation in the pathogenesis of this mutation.

CPVT caused by a mutation in the cardiac calsequestrin gene (CASQ2 D307H) was also studied using a patient-specific iPSC approach (Novak et al. 2011). The patient-specific iPSC-generated cardiomyocytes displayed an increased susceptibility to catecholamine-mediated arrhythmia and calcium overload as well as an altered ultrastructure of the sarcoplasmic reticulum.

3.3.2 Short-QT Syndrome

Intriguingly, also a shortening of the QT interval has been found to be associated with syncope, susceptibility to ventricular fibrillation and to sudden cardiac arrest in a rare familial syndrome with autosomal dominant inheritance, termed short-QT syndrome (SQTS; Gaita et al. 2003; Giustetto et al. 2006). Gain-of-function mutations in KCNH2 (encoding HERG; SQTS1), KCNQ1 (SQTS2), and KCNJ2 (SQTS3) as well as loss-of-function mutations in genes encoding the l-type calcium channels CACNA1C and CACNB2 (Giustetto et al. 2006; Antzelevitch et al. 2007) have been found in patients suffering from this disease. It is not well understood why patients affected by these mutations are susceptible to life-threatening arrhythmias, particularly since healthy individuals with relatively short QT intervals do not have a comparable risk for sudden cardiac arrest (Gallagher et al. 2006). Induced pluripotent stem cell-based disease modeling might help to shed some light on the so far poorly understood pathophysiology of this disease by analyzing the electrophysiological properties of affected human cardiomyocytes in vitro.

3.3.3 Brugada Syndrome

Brugada syndrome is another cause of sudden cardiac death in persons with structurally normal hearts, defined by specific ECG patterns and typical clinical features (Wilde et al. 2002; Antzelevitch et al. 2005). Sudden unexpected nocturnal death syndrome (SUNDS), described in males in southeast Asia, is considered to be the same disease (Vatta et al. 2002). Mutations in the gene SCN5A have been found in patients with Brugada syndrome, a gene that is also affected in LQT3 (Antzelevitch et al. 2005) which encodes subunits of a cardiac sodium channel. Also mutations in other cardiac ion transport genes have been described in patients with Brugada syndrome (Weiss et al. 2002; Watanabe et al. 2008). Penetrance of this autosomal dominant disorder is highly variable and the typical ECG pattern is

present more often in men than in women for reasons not completely understood (Benito et al. 2008). The known mutations are only present in a fraction of patients with Brugada syndrome and the mutation status is not sufficient to predict the risk of sudden cardiac arrest in affected individuals (Antzelevitch et al. 2005). Thus, patients with Brugada syndrome appear to be a genetically heterogeneous population, and risk stratification of individuals has been proven difficult (Priori et al. 2002; Probst et al. 2010). Studying SCN5A mutation-positive Brugada syndrome with patient-specific iPSC models might help to understand the pathogenesis of the disease as well as the mechanisms of arrhythmogenesis and to develop specific therapies. Analysis of the electrophysiological features of iPSC-derived human cardiomyocytes, such as ion channel function and action potential properties, from patients with Brugada syndrome not carrying a known mutation might help to define a functional cellular phenotype leading to Brugada syndrome, regardless of the genotype. Knowledge of this cellular phenotype might help to improve risk stratification, identify exogenic factors that promote arrhythmia, and identify or develop drugs to prevent sudden cardiac arrest in affected patients.

3.3.4 Dilated Cardiomyopathy

Dilated cardiomyopathy is a clinical disease entity defined by dilation of the cardiac chambers and ventricular dysfunction, accompanied by myocyte loss and fibrosis. Pathophysiologically, dilated cardiomyopathies are a heterogeneous group of diseases with different etiologies, with familial disease being responsible for one third to half of the cases (reviewed by Watkins et al. 2011).

Many disease genes and patterns of inheritance have been identified. Interestingly, although the phenotypes of affected individuals are usually very similar, mutations causing the disease have been identified in genes affecting many different cellular functions, pathways, and compartments, e.g. calcium handling proteins, sarcomeric proteins, force transduction apparatus, nuclear envelope, gene transcription, splicing, and energy metabolism. Examples of genes that have been found mutated in cases of dilated cardiomyopathy include phospholamban, β-myosin heavy chain, cypher, δ-sarcoglycan, and lamin A and C (see Watkins et al. 2011). Several of the known mutations are considered to cause decreased myocyte contraction or impaired structural integrity of the cells, thereby causing the disease. However, the mechanism of disease is not clear in many of the mutations. Human iPSC-based disease models might be a useful tool to improve our understanding of the molecular mechanisms underlying dilated cardiomyopathy caused by different mutations. As stated earlier, while some mutations may affect cell function at a cell-autonomous level, this might not be the case in other mutations, where cell–cell interactions are required for a phenotype to develop. Therefore, tissue-engineering approaches modeling heart tissue could play an important role in human iPSC based disease models of dilated cardiomyopathies. These models might also help to understand the conditions that lead to myocyte death and fibrosis in all mutations.

3.3.5 Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is characterized by hypertrophy of the left ventricular myocardium that is not explained by an exogenous factor like arterial hypertension. The disease can be complicated by a dynamic obstruction of the left-ventricular outflow tract caused by the thickened intraventricular septum, a condition termed hypertrophic obstructive cardiomyopathy (HOCM). By predisposing affected patients to lethal arrhythmias, HCM is the most common cause of sudden cardiac death in young athletes (Maron et al. 1996). Other typical symptoms are shortness of breath, angina pectoris and syncope. Mutations in genes encoding for sarcomeric proteins are the most common cause of HCM, with mutations in *MYH7* (encoding the β-myosin heavy chain) and *MYBPC3* (encoding the cardiac myosin-binding protein C) together accounting for about half of all cases (Richard et al. 2003). A common feature of HCM-causing mutations is that they increase the calcium affinity and sensitivity of the contractile apparatus, leading to subsequent alterations in intracellular calcium homeostasis. Moreover, the increased sarcomeric calcium sensitivity leads to an increased myocytic energy consumption due to inefficient ATP utilization that may ultimately trigger left ventricular hypertrophy (Ashrafian et al. 2003). This hypertrophic response is the common point of convergence of several intracellular signal transduction pathways (Heineke and Molkentin 2006).

An intriguing feature of HCM is the high degree of, sometimes age-related, penetrance among different patients affected by the same mutation, indicating that other genetic or environmental factors are necessary to trigger the development of the disease phenotype (Ashrafian et al. 2011). It would be, thus, a promising approach to modify these precipitating factors to prevent the disease or to slow its progression in affected patients. Modeling hypertrophic cardiomyopathies with patient-specific iPSC might provide a means to identify these factors and to evaluate therapies directed against them.

3.3.6 Arrhythmogenic Right Ventricular Cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a mainly autosomal dominantly-inherited disease which leads to a fatty degeneration of the myocardium, especially in the right ventricle. Clinically, patients with ARVC suffer from episodes of ventricular tachycardia, syncopes and are at high risk of sudden cardiac death. Because of the progressive nature of the disease, the therapeutic options are limited and the overall prognosis remains poor. So far, mutations in 12 different genes which lead to the phenotype have been described. The most common mutations are found in plakophilin-2 (*PKP-2*), a gene associated with the cardiac desmosome that plays an important role in cell-cell adhesion (van Tintelen et al. 2006). Moreover, mutations in other desmosomal genes like plakoglobin (*JUP*),

desmoplakin (*DSP*) and desmoglein-2 (*DSG-2*) are also described to cause ARVC (Azaouagh et al. 2011). Also mutations in genes not directly involved in the cell-cell contact like transforming growth factor (*TGF)-β3*, the human ryanodine receptor 2 and transmembrane protein 43, a response element for PPAR gamma, have been described in patients suffering from ARVC (Beffagna et al. 2005; Tiso et al. 2001; Merner et al. 2008). The large number of genes involved and the highly variable penetrance within families affected by ARVC already suggest a complex nature of the disease. Since the available methods to directly study the disease in affected human individuals are limited, animal disease models are fundamental for such kind of research. Several mouse and zebrafish models have been established which successfully recapitulate parts of the phenotype and have brought many new insights into the pathophysiology of ARVC. However, large parts remain unknown. Especially considering non-desmosomal gene mutations, there have been difficulties in modeling the disease, suggesting that the affected intracellular signaling pathways differ a lot between human and non-human cells (McCauley and Wehrens 2009).

Using iPSC technology with cells from patients affected by ARVC could provide a new tool for studying the disease. This becomes especially interesting as recent research suggests that certain mutations in ARVC interfere with the differentiation of cardiac progenitors to cardiomyocytes and might promote adipocyte development (Lombardi et al. 2011).

3.3.7 Multifactorial Diseases

The initial attempts on iPSC-based disease modeling have focused on monogenetic disorders. However, this is not a necessity for iPSC-based disease models. Patient-specific stem cells, which are by definition genetically identical to the somatic patient cell that was used for reprogramming, might also provide a platform to model complex multifactorial diseases, in which genetic factors act together with lifestyle and environmental factors to precipitate the disease phenotype in a single patient. Diseases like atherosclerosis, diabetes mellitus, or arterial hypertension, which are responsible for most of the cardiovascular morbidity in industrial countries, belong to this group of disorders. By using iPSC technology, it would be possible to investigate the genetic factors in patient-specific cells while keeping the environmental factors constant in patient and control cells.

3.4 Outlook

Most of the reports of cardiovascular disease modeling with patient-specific iPSC published to date have been, at least to a large extent, proof-of-principal studies that

did not reveal fundamentally new aspects of the underlying pathophysiology. Given the novelty of the field, this is not surprising. It is wise to first apply a new technology to an area of research that has been already mapped well using conventional methodology. This facilitates the interpretation of results gained by the new method and allows the correlation of the new model system with the known aspects of the world it is supposed to represent. However, as the methodology becomes used by more and more scientists working in different fields all over the world, it will hopefully not only lead to more refined maps of well-known territories, but also to the discovery of unknown shores that were inaccessible before the advent of iPSC technology.

To date, the generation of cardiomyocytes from human iPSCs is a laborious task. The amount of qualified manual cell culture work and the cost of cell culture reagents make iPSC-derived cardiomyocytes a precious material. Accordingly, labor-intensive and time-consuming techniques such as whole-cell patch clamp recordings or single-cell-RT-PCR have been applied for the analysis of the physiology of these cells (Moretti et al. 2010b). However, one important goal of current research in the iPSC field is to increase the efficiency of the cell culture and differentiation protocols (Fujiwara et al. 2011; Shafa et al. 2011), which will eventually make patient-specific cardiomyocytes a more common good. Alongside, it will be helpful to also increase the throughput of the methodology used to study these cells. One step in this direction already taken is the use of multielectrode arrays (Itzhaki et al. 2011), which allow the recording of field action potentials from larger numbers of myocytes. Other medium- to high throughput analysis methods like automated patch clamping (Jones et al. 2009) will likely aid iPSC-based disease modeling in the future.

4 Applications in Drug Development

4.1 Drug Screening

Cardiomyocytes derived from patient-specific iPSC could be used in the development of new drugs by setting up a platform for drug screening in these cells. So far, such experiments are mainly performed in non-human cell systems or in vivo models. Recently, new small molecules which shorten the QT interval were identified using high-throughput screening in a zebrafish long-QT model (Peal et al. 2011). The results from these studies are partially limited by the electrophysiological differences between zebrafish and human cardiomyocytes. Newly-developed models of human long-QT syndromes (Moretti et al. 2010b; Itzhaki et al. 2011; Yazawa et al. 2011; Matsa et al. 2011) might offer a platform for similar screening experiments without this limitation.

4.2 Drug Safety

More common than the monogenetic congenital long-QT syndromes is the so-called "acquired" long-QT syndrome. Several conditions, such as heart failure, electrolyte disturbances, thyroid disorders or, most importantly, treatment with specific drugs, lead to a prolonged QT interval in the ECG, indicative of prolonged single-cardiomyocyte action potentials. Similar to congenital long-QT syndrome, this condition leads to an increased susceptibility to potentially fatal ventricular arrhythmias. Although the precise pathophysiology of acquired long-QT syndrome is far from being completely understood, it has been demonstrated that most drugs known to cause acquired long-QT syndrome are inhibitors of the HERG potassium channel, which is responsible for a repolarizing potassium current occurring during the repolarization phase of the cardiac action potential, called the rapid component of the delayed rectifier potassium current (I_{kr}; Sanguinetti et al. 1995).

Drug-induced QT interval prolongation is a major and increasingly recognized problem of drug safety and was the most important cause for restrictions of use or the withdrawal of drugs from the US market in the recent time (Lasser et al. 2002). A drug-induced QT interval prolongation – in some or all patients – might increase the mortality risk of these patients by increasing the likelihood of fatal ventricular arrhythmias. However small this risk may be, it can be only tolerated if the drug is needed to treat a serious condition and if no safer alternative exists. Intriguingly, the problem of drug-induced QT interval prolongation is not limited to drugs or drug candidates intended for cardiac use, but it is also a highly relevant problem of substances intended for non-cardiac use. For the companies involved in drug development, this poses the risk of tremendous financial losses, since the investments during the early phases of drug development are lost if during the clinical trials the drug candidate turns out to lead to acquired long-QT syndrome. Therefore, and also as a requirement imposed by the regulatory agencies of many countries, several preclinical test systems are regularly used before the drug candidates are used in clinical trials (Giorgi et al. 2010). Since most of these tests so far rely on surrogate parameters like the extent of I_{Kr} blockade induced by the drug candidate, their predictive value is limited.

Given the impact of QT interval prolongation on the development of new drugs, human cell-based systems to screen for QT interval prolongation would be a desirable goal. It is easy to envision a test system consisting of human iPSC-derived cardiomyocytes whose action potentials are monitored (e.g. by whole-cell patch clamp recordings) before and after exposure to a candidate drug. However, when it comes to the question which human iPSC lines should be used for such a test system, several different strategies are feasible. Since a modulation of HERG channel activity is a frequent mechanism of drug-induced QT interval prolongation, iPSCs derived from a LQT2 patient carrying a HERG mutation could be used to generate the test cardiomyocytes (Itzhaki et al. 2011). However, drug-induced QT interval prolongation frequently occurs in patients who do not carry mutations in their HERG (alias KCNH2) locus (Yang et al. 2002). Thus, another strategy would

be to use a panel of several iPSC lines derived from a random sample of (at best genetically diverse) subjects. Finally, since a genetic predisposition seems to play a role for the susceptibility to drug-induced QT interval prolongation (although the involved genes are not known; Kannankeril et al. 2005), a panel of iPSC lines derived from patients who have already reacted to different drugs with a prolonged QT interval might be used to generate the cardiomyocytes that constitute the test system.

5 Patient-Specific Therapy

It is a basic principle of pharmacotherapy that genetic variants may lead to a varying degree of efficacy of a specific drug on different patients. Accordingly, it often has to be empirically tested which of several drugs fits best with the specific needs of a single patient. By using patient-specific iPSC-derived cardiomyocytes as a test system for different drug candidates, one could predict, based on the individual genetic background of the patient, the potential hazardous and beneficial effects of several drugs for the individual patient and discriminate patients who will likely benefit from a certain therapy from those who are at risk for developing side effects.

6 A New Tool for Genetic Investigations

In the study of disorders with complex genetics, a common problem is to dissect genetically-defined aspects of the phenotype from aspects that are caused by environmental factors. Twin studies have proven useful in this context: since the environmental influences tend to be similar in twins that grow up together, the comparison of the phenotypic similarity in monozygotic and dizygotic twins can reveal the degree to which a certain phenotype is genetically determined. For example, a twin study could demonstrate that only about 25% of the variability of the QT interval in the ECG is explained by genetic factors (Carter et al. 2000). Patient-specific iPSC might be used to perform "in vitro twin studies" without the need to find twin pairs: by using identical reprogramming, cell culture and differentiation protocols for iPSC lines derived from different patients, the variance in the observed cellular phenotypes should be determined mainly by the genetic background of the patients.

Another problem that could be addressed by iPSC-based disease modeling is incomplete penetrance, meaning that not all individuals that carry a specific disease-causing mutation develop the disease phenotype to the same extent (Zlotogora 2003). The causes for incomplete penetrance can be environmental factors as well as additional genetic factors. Somatic cells from several members of a family affected by a disease with incomplete penetrance could be used to generate iPSC lines. These iPSC could then be differentiated to the cell type

affected by the disease and the in vitro phenotype of those cells could be correlated with the phenotype of the respective patient. By using this strategy, the genetic factors that contribute to incomplete penetrance could be investigated without confounding by environmental factors.

7 Cardiac Cell Therapy

The idea to replace the function of a failing organ by cells injected into the patient goes back to the work of Paul Niehans in the 1930s, who tried to replace the function of failing endocrine glands by preparations of animal cells (Niehans 1952). Today we know that these attempts were doomed to fail because an intact immune system successfully eliminates the immunologically incompatible graft cells. The great success of organ transplantation nowadays has been made possible by carefully matching donor and recipient and by the availability of several immunosuppressant drugs that interfere with the immunological rejection of the transplants. However, unwanted side-effects of these drugs are a common problem of patients living with transplanted organs and late rejection still remains an issue. Moreover, the demand for organs greatly surpasses the number of donors, limiting the availability of this treatment to patients with organ failure. The use of patient-specific autologous iPSC for cell therapy might become a means to address the problems of both immunological rejection and organ shortage.

A loss of viable cardiomyocytes is a major cause of heart failure. Accordingly, cell therapy directed at heart failure should be aimed at replacing these muscle cells. Moreover, in order to function properly and in order not to become a trigger for arrhythmias, the transplanted cells have to integrate physically into the heart muscle and need to couple electrically to the host myocardium. The best way to achieve this goal still has to be determined.

Hematopoietic stem cell transplantation in patients with hematological disorders has become a paradigm for successful human cell therapy. In the case of a patient in whom the endogenous bone marrow has been ablated e.g. by chemotherapy or irradiation, it is sufficient to inject bone marrow stem cells into the venous circulation, from where the cells home to the bone marrow, proliferate and reconstitute the hematopoietic organ. However, since this self navigation does not seem to work as straightforward with cardiomyocytes, it might be more complex to repair a damaged heart using autologous iPSC.

7.1 Transplantation of Undifferentiated iPSC

Following the example of hematopoietic cell transplantation, it seemed possible that the microenvironment of a heart might be sufficient to direct the differentiation of undifferentiated iPSC to cardiac cell types. However, when murine iPSC were

injected into the hearts of mice with an impaired immune system (to suppress transplant rejection), teratomas formed at the sites of injection (Moretti et al. 2010a). In a similar experiment, it was demonstrated that iPSC transplantation into murine hearts resulted in teratoma formation also in immunocompetent mice and, moreover, that washout of cells from the heart led to tumorigenesis also in non-cardiac tissues (Zhang et al. 2011). These results indicate that undifferentiated iPSC might not be a suitable cell type for human cell therapy.

7.2 Transplantation of iPSC-Derived Cardiomyocytes

In several animal models of heart failure, the transplantation of human ESC-derived cardiomyocytes restored the cardiac function (Laflamme et al. 2007; Caspi et al. 2007). Due to the similarity between ESC and iPSC, it appears feasible to use a similar approach to treat heart failure with autologous iPSC-derived cardiomyocytes. Since a large body of evidence indicates that iPSC-derived cardiomyocytes generated by state-of-the-art differentiation protocols are immature in respect to electrophysiology and calcium handling (recently reviewed by Poon et al. 2011), it would be a desirable goal do develop protocols that result in the generation of more mature cardiomyocytes for transplantation.

7.3 Transplantation of iPSC-Derived Cardiac Progenitors

The heart is composed not only of cardiomyocytes, but of several other cell types, most importantly vascular endothelial and smooth muscle cells that constitute the cardiac blood vessels. It is a relatively new concept that a common cardiovascular progenitor cell population, characterized by expression of the transcription factor Islet-1, can give rise to all those cell types and function as a multipotent cardiac progenitor cell (Moretti et al. 2006). These cells might represent an ideal source for cell therapy, since they are already determined to a cardiac fate. Indeed, when Islet-1-positive progenitors were generated and purified from a genetically-modified mouse iPSC line (in which these cells are marked by fluorescent protein expression) and transplanted into a mouse heart, in vivo differentiation into cardiomyocytes, vascular endothelial and smooth muscle cells was observed (Moretti et al. 2010a). Moreover, in contrast to transplantation of undifferentiated iPSC, no teratoma formation was observed.

The translation of these results to human iPSC is hampered by the problem that a method to isolate viable human Islet-1-positive cardiovascular progenitor cells from differentiating iPSC still has to be developed. Since Islet-1 is an intracellular protein, a simple approach like FACS sorting cannot be applied. One way to reach this goal would be to find a set of cell surface markers that can be used – by positive

or by negative selection – to define this cell population. Another way would be to genetically modify the iPSC to express a marker (e.g. a cell surface marker or a fluorescent protein) once they become cardiovascular progenitors.

7.4 Safety Issues

The safety of the applied cells is the major concern that has to be addressed before human cell therapy with iPSC or iPSC-derived cells is feasible. Some safety problems might be addressed by specific precautions. For example, as stated above, the administration of undifferentiated iPSC to a patient should be avoided because of the risk of tumor formation. Accordingly, progenitor populations considered to be used for cell therapy should be pure and contamination with undifferentiated cells should be excluded. Moreover, the classical reprogramming technique, which uses potentially tumorigenic retroviruses and the known oncogene c-myc, might be replaced by newer techniques that circumvent these problems. However, evidence accumulates that the reprogramming process compromises the genomic and epigenomic integrity of the cells and introduces mutations (Hussein et al. 2011; Gore et al. 2011; Lister et al. 2011), which might (e.g. via activation of endogenous oncogenes) lead to a tumorigenic potential of the iPSC and their progeny. It will be, thus, essential to develop tests that evaluate the safety of these cells prior to their use in cell therapy.

8 Concluding Remarks

The availability of human induced pluripotent stem cells has spawned a wealth of possible applications in cardiovascular biology, both in basic science and in applied medicine. Some of these applications, like cell therapy, are not more than a promise nowadays. While cell therapy with autologous iPSC-derived cells might become a treatment option for thousands of heart failure patients in the future, several obstacles have to be taken before such a treatment is feasible. Most importantly, it has to be determined whether it will be possible to control the tumorigenic potential of these cells.

However, already now, other applications like disease modeling are pursued to learn more about the pathophysiology of cardiovascular diseases. The knowledge gained from these experiments and the translation of this methodology to applications like drug development might provide another means by which iPSC technology will improve the treatment of patients with cardiovascular disorders.

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TRPs in the Brain

Rudi Vennekens, Aurelie Menigoz, and Bernd Nilius

Abstract The Transient receptor potential (TRP) family of cation channels is a large protein family, which is mainly structurally uniform. Proteins consist typically of six transmembrane domains and mostly four subunits are necessary to form a functional channel. Apart from this, TRP channels display a wide variety of activation mechanisms (ligand binding, G-protein coupled receptor dependent, physical stimuli such as temperature, pressure, etc.) and ion selectivity profiles (from highly Ca^{2+} selective to non-selective for cations). They have been described now in almost every tissue of the body, including peripheral and central neurons. Especially in the sensory nervous system the role of several TRP channels is already described on a detailed level. This review summarizes data that is currently available on their role in the central nervous system. TRP channels are involved in neurogenesis and brain development, synaptic transmission and they play a key role in the development of several neurological diseases.

1 The Transient Receptor Potential Family of Cation Channels

The transient receptor potential (TRP) multigene superfamily encodes integral membrane proteins that function as ion channels. Members of this family are conserved in yeast, invertebrates and vertebrates. All members TRP channels are subdivided into seven subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (poly-cystin), TRPML (mucolipin), TRPA (ankyrin) and TRPN (NOMPC-like), which is only found in invertebrates. Of the 6 mammalian subfamilies, 28 members are known, with only 27 in humans (TRPC2 is a pseudogene; see Fig. 1) (Nilius and Owsianik 2011).

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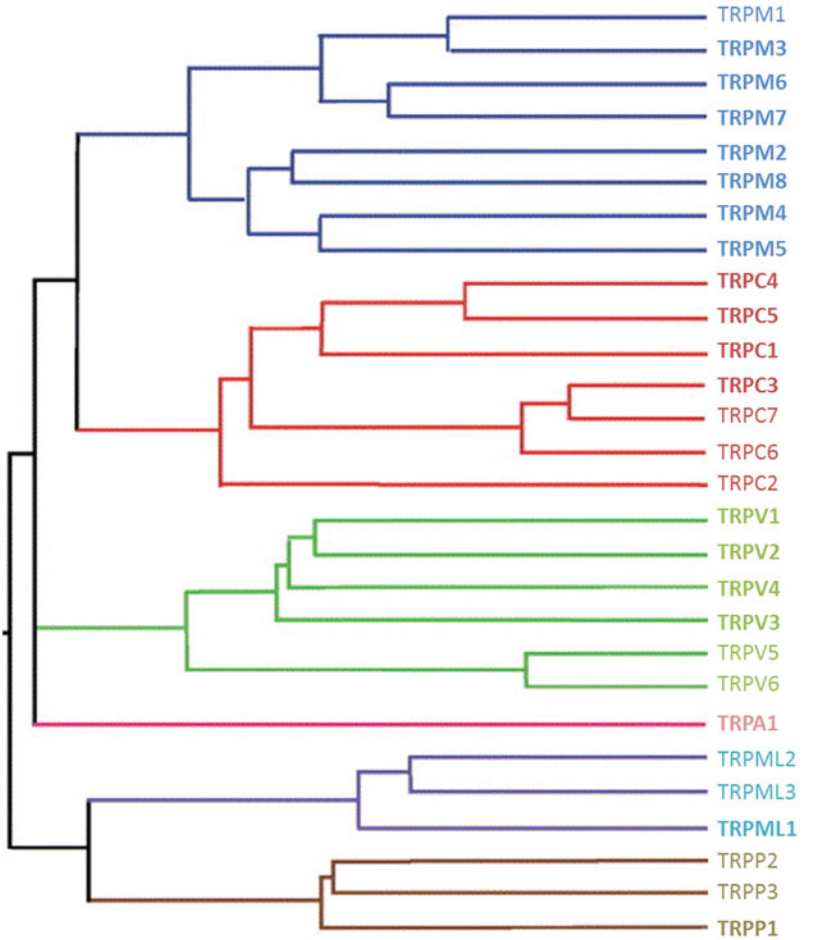

Fig. 1 Phylogenetic tree of the transient receptor potential family of ion channels. TRPC (canonical), TRPM (melastatin), TRPV (vanilloid), TRPA (ankyrin), TRPP (polycistin) and TRPML (mucolopid). *TRPC2* is a pseudogene in humans. The TRP channels reported to be expressed in brain are indicated in *bold*

It is clear from the current state of the literature that almost all 28 members of mammalian TRP channel play a key role in establishing the five classical senses described in De Anima (book II, 350 B.C.) by Aristotle, which allow humans to perceive the outside world: sight (visus), hearing (auditus), smell (olfactus), taste (gustus) and touch (contactus). For instance, recent studies have firmly established the role of temperature-sensitive TRPs (thermoTRPs) as the principal molecular thermometers in the peripheral sensory system, and provided the first molecular insight into the mechanisms underlying the exquisite thermo- and chemosensitivity of these channels. However, also for balance (or equilibrioreception), which is now

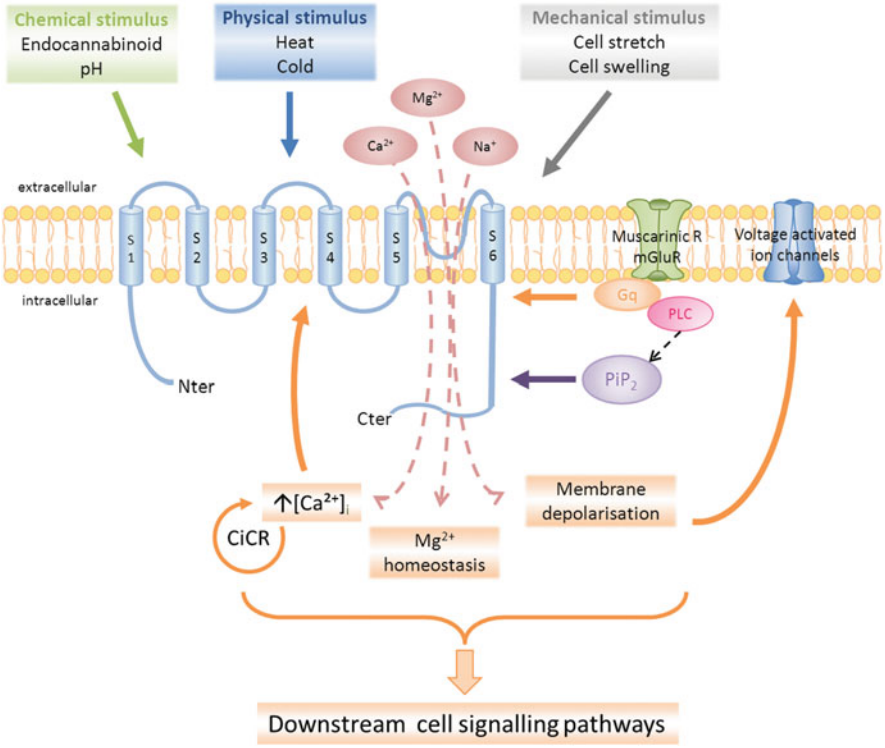

Fig. 2 Schematic representation of the proposed roles of TRP channels in neurons. TRP channels are cation channels that constitute an influx pathway for Ca^{2+}, Na^{+} and/or Mg^{2+}. Most TRP channels are Ca^{2+} permeable, except TRPM4 and TRPM5, which permeate exclusively monovalent cations. TRPM6 and TRPM7 are Mg^{2+} permeable. TRP channels are activated by endogenous ligands (e.g. Endocannabinois, pregnenolonsulphate), physical and mechanical stimuli (heat, cold, stretch) and/or through receptor-activated Gq coupled intracellular signalling pathways. Basically, TRP channels influence Ca^{2+} signalling by allowing Ca^{2+} to enter the cell directly, or through membrane depolarisation which provides the trigger for voltage-gated Ca^{2+} channels to activate, or which limits the driving force for Ca^{2+} entry. A depolarisation mediated by TRP channels as such will influence the firing of action potentials in neurons. All these principal effects will lead to downstream signalling events mediated by other proteins (including exocytosis, gene expression, growth cone migration, etc.). For more details, see the text

also considered as a sixth exteroceptive sense, and the interoceptive senses, that provide information from within the body (e.g. proprioception informs the brain about the relative position of muscles and joints), TRP channels play an essential role (for an extensive review see Damann et al. 2008).

Now, it is widely recognized that TRP channels play a much wider role in the nervous system. They are involved in many homeostatic functions and, importantly, play an essential role in our brain much beyond their function as cell sensors (see Fig. 2).

TRPCs are highly expressed in various parts of the brain (for a complete overview, see Table 1). They function generally as receptor-activated ion channels and have been implicated in the formation of synapses in the developing brain, amongst others. Among all 28 mammalian TRPs, TRPV1 is probably the best-studied TRP channel in neurons. In the peripheral nervous system it is critically involved in nociception via sensory C and A∂ fibres, and is activated by the 'hot' and pungent capsaicin and heat. This channel is also expressed in central neurons and plays a very important 'non-sensory role' in brain. The expression of other Vanilloid TRP channels has also been reported in different brain structures. TRPV2 expression has been shown in hippocampal neurons cultures and co-localized with TRPV1 in rat cortex (Liapi and Wood 2005). TRPV4 is detected in rat and mouse hippocampus (Gao et al. 2004; Shibasaki et al. 2007) and in substancia nigra (Guatteo et al. 2005).

The TRPM subfamily has eight members and has been named after the first identified member "Melastatin". Some of these channels are expressed in the central nervous system. TRPM2 is a Ca^{2+} permeable ion channel, expressed in hippocampal pyramidal neurons (Bai and Lipski 2010; Xie et al. 2012) and in dopaminergic neurons in substantia nigra (Freestone et al. 2009; Chung et al. 2011; Mrejeru et al. 2011). TRPM3 is highly expressed in the dentate gyrus, the hippocampus and likely plays a role during the development of the cerebellum (Lee et al. 2010) (Zamudio-Bulcock et al. 2011; Zamudio-Bulcock and Valenzuela 2011). TRPM4 and TRPM5 mRNA are also detected in the central nervous system. RT PCR experiments showed TRPM4 and TRPM5 expression in brain extracts from mouse and rat (Launay et al. 2002; Crowder et al. 2007; Yoo et al. 2010). TRPM5 is highly detectable by *ISH* and using reporter mice in the olfactory bulb and to a lesser extent in the thalamus (Lin et al. 2007). TRPM7 was detected on the mRNA and protein level in cell bodies from hippocampal neurons, cerebral neurons and cerebrospinal-fluid contacting neurons (Fonfria et al. 2006; Wei et al. 2007; Cook et al. 2009; Coombes et al. 2011; Zhang et al. 2011a). Finally, also TRPA1, TRPP1 and TRP-ML have been reported in the brain (see Table 1).

2 Clues for the Role of TRP Channels in the Development of the Brain and Neuronal Function

2.1 Axon Guidance, Growth Cone Tuning and TRPC's

Axon guidance and neurite outgrowth are essential processes in the developing brain. Establishment of functional and morphological polarity of the neuronal cell is an important step in the formation of synapses and neuronal networks. Several essential signalling pathways have been identified already in this process, including Gq coupled receptors and tyrosine kinase linked receptors, but a key feature is obviously the regulation of the intracellular Ca^{2+} signaling in the growth cone.

Table 1 Overview of TRP channels expressed in brain, their general features and assumed roles

TRP	Expression in brain	Detection methods	General features	Supposed role	References
TRPV1	Limbic system (M)	RT-PCR, ISH, western blot	Ca^{2+} permeable activation by noxious heat, capscaicin, endocannabinoid lipids	Control and modulation of synaptic plasticity	Mezey et al. (2000), Iida et al. (2005), Toth et al. (2005), Cristino et al. (2006), Marsch et al. (2007), Gibson et al. (2008), Cavanaugh et al. (2011)
	Hypothalamus (M, R)	Immunohistochemistry	Inhibition by PIP_2	Modulation of emotions and learning	
	Thalamus (M) Basal ganglia (M, R) Locus ceruleans (R) Cerebellum (H, M, R) Periductal grey matter (M)	Reporter mouse		Control of the locomotor pattern and body temperature	
TRPV2	Hypothalamus (R, M)	RT-PCR, immunohistochemistry	Weakly Ca^{2+} selective	Modulation of oxytocin release	Wainwright et al. (2004), Kunert-Keil et al. (2006)
	Hippocampus (M) Cortex (M) Cerebellum (M) Pons (M)		Activation by moderate temperature, cell swelling	Control of axon growth	
TRPV3	Whole brain (H)	RT-PCR	Weakly Ca^{2+} selective	Regulation of emotional response	Xu et al. (2002), Lipski et al. (2006), Moussaieff et al. (2008), Carreno et al. (2012), Hu et al. (2012)
	Brainstem (R)	Immunohistochemistry	Infrared heat activation Moderate temperature activation		
TRPV4	Hypothalamus (R, M) Hippocampus (M)	RT-PCR, immunohistochemistry, WB, ISH	Ca^{2+} permeable Activation by moderate heat, cell swelling and arachidonic acid derivative	Modulation of dopaminergic neurons excitability	Guler et al. (2002), Vriens et al. (2004), Guatteo et al. (2005), Kunert-Keil et al. (2006), Lipski et al. (2006), Shibasaki et al. (2007), Lowry et al. (2009)
	Basal ganglia (M)			Cellular stress and swelling after brain ischemia	
	Brainstem			Thermosensitivity and thermosensation	

(continued)

Table 1 (continued)

TRP	Expression in brain	Detection methods	General features	Supposed role	References
TRPC1	Hippocampus (M, R, H)	RT-PCR, ISH, immunohistochemistry, WB	Activation by mGluR1, Gq-PLC pathway, DAG	Modulation of glutamate release	Strubing et al. (2001), Riccio et al. (2002), Kunert-Keil et al. (2006), Martorana et al. (2006), Chung et al. (2007), Narayanan et al. (2008), Gasperini et al. (2009)
	Cortex (M, R, H) Cerebellum (M, R, H) Brain stem (M, R, H) Forebrain (M, R) Basal ganglia (M,R)		Heteromer with TRPC5	Growth cone tuning, and neurite outgrowth	
TRPC3	Substantia nigra (R, H)	RT-PCR, Immunohistochemistry	Non selective cation channel	Growth cone guidance through	Sylvester et al. (2001), Riccio et al. (2002), Li et al. (2005), Chung et al. (2007), Jia et al. (2007)
	Cerebellum (M)		Activation by Gq protein, BDNF pathway, diacylglycerol	BDNF pathway	
	Cortex (M) Hippocampus (M) Whole brain (R, H)		Inhibition by phosphorylation by cGK	Promotion of survival of cerebellar granule neurons	
TRPC4	Whole brain (H) Olfactory bulb (M) Hippocampus (M) Cerebellum (M)	RT-PCR, immunohistochemistry, WB	Activation by store depletion, Gq-PLC pathway and Trk receptors	Role in neuronal signalling and possible role in epileptiform seizure firing	Riccio et al. (2002), Chung et al. (2007), Fowler et al. (2007), Zechel et al. (2007), Zhang et al. (2011)
TRPC5	Whole brain (M, R)	RT-PCR, WB, Immunohistochemistry	Activation through Gq-PLC	Regulation of neurite length and growth cone morphology	Philipp et al. (1998), Strubing et al. (2001), Riccio et al. (2002), Greka et al. (2003), Chung et al. (2007), Riccio et al. (2009), Tai et al. (2011)
	Amygdala (M, R) Hippocampus (M, R)		pathway and Trk receptors	Fear response and synaptic plasticity	
TRPM2	Whole brain (M)	RT-PCR, WB, immunohistochemistry	Non selective cation channel	Functionally coupled to VDCC and NMDAR	Uemura et al. (2005), Fonfria et al. (2006), Lipski et al. (2006), Olah et al. (2009), Chung et al. (2011)
	Hippocampus (M)		Activation by reactive oxygen		

	Striatum (H) Substancia nigra (R)		Species and intracellular Ca^{2+} rise	Oxidative stress induced cell death and neurodegeneration	
TRPM3	Whole brain (M, H) Hippocampus (M) Cortex (M) Forebrain (M) Cerebellum (M) Brain stem (M)	RT-PCR, ISH	Ca^{2+}/Mn^{2}+ permeable Activation by steroid hormone	Regulation of Ca^{2+} homeostasis Modulation of glutamatergic communication	Lee et al. (2003), Kunert-Keil et al. (2006), Vriens et al. (2011), Zamudio-Bulcock et al. (2011)
TRPM4	Whole brain (M, R, H) Basal ganglia (M) PreBrötzinger complex (M)	RT-PCR, WB	Ca^{2+} impermeable Activation by high $[Ca^{2+}]_{cyt}$, PIP_2 Inhibition by ATP Voltage dependent	Activation of burst firing Generation of inspiratory burst	Launay et al. (2002), Mironov (2008), Mrejeru et al. (2011)
TRPM5	Whole brain (M, R, H) PreBrötzinger complex (M) Olfactory bulb (M)	RT-PCR, reporter mouse	Ca^{2+} impermeable Activation by intracellular calcium, PIP2 Voltage dependent	Processing of semiochemical signals	Fonfria et al. (2006), Kunert-Keil et al. (2006), Crowder et al. (2007), Lin et al. (2007)
TRPM7	Whole brain (M, R) Hippocampus (M, R) Cerebellum (M) Cortex (M)	RT-PCR	Mg^{2}+ permeable Modulation by NGF and ROS Inhibition by PIP_2	Microglial function Oxidative stress	Jiang et al. (2003), Lipski et al. (2006),Tian et al. (2007)
TRPM8	Mesencephalon (R)	immunohistochemistry	Non selective cation channel Activation by cold, menthol, PIP_2 Voltage dependent	Contribution to cold sensing	Fonfria et al. (2006), Du et al. (2009)

(continued)

Table 1 (continued)

TRP	Expression in brain	Detection methods	General features	Supposed role	References
TRPA1	Whole brain (M)	RT-PCR	Activation by noxious cold temperature, pungent compounds (mustard oil, cinnamaldehyde)	Modulation of glutamate release and modulation of the endocannabinoid effect	Kunert-Keil et al. (2006), Stokes et al. (2006), Sun et al. (2009), Koch et al. (2011), Shigetomi et al. (2011)
	Hippocampus (R) Brain stem (M)			Regulation of the resting calcium in astrocytes	
TRPP1	Hippocampus (M)	Reporter mouse	Non selective cation channel	Maintenance of dendritic arborisation and establishment of neuronal polarity	Yin et al. (2008), Czondor et al. (2009), Wodarczyk et al. (2009)
TRP ML1	Whole brain (M)	RT-PCR	Intracellular non selection cation channel Inhibition by lowering pH	Control of Zn^2+ homeostasis	Kunert-Keil et al. (2006)

Carreno O, Corominas R, Fernandez-Morales J, Camina M, Sobrido MJ, Fernandez-Fernandez JM, Pozo-Rosich P, Cormand B, Macaya A (2012) SNP variants within the vanilloid TRPV1 and TRPV3 receptor genes are associated with migraine in the Spanish population. Am J Med Genet B Neuropsychiatr Genet 159B:94–103.

In neuronal growth cones, spatiotemporally distinct Ca^{2+}waves can be detected upon receptor stimulation, and in their absence normal neuronal differentiation is prevented. Thus, these Ca^{2+} signals are in effect the link between external stimuli and processes such as growth-cone protrusion, axonal pathfinding and formation of synaptic contacts. These Ca^{2+} waves are largely dependent on the activity of Ca^{2+}permeable ion channels, and it's clear that TRPC channels are important candidates for a role in the developing brain (Tai et al. 2009). Indeed, Ca^{2+} influx via TRPC channels appears to be a critical component of the signalling cascade that mediates the guidance of growth cones and survival of neurons in response to chemical cues such as neurotrophins or Netrin-1 (Wang and Poo 2005) (Talavera et al. 2008). The role of TRPC in growth cone path finding has been reviewed already by several groups (Bezzerides et al. 2004; Moran et al. 2004; Wang and Poo 2005).

The first report on a TRPC channel as a regulator of neurite length and growth cone morphology (Greka et al. 2003) showed that TRPC5 expression is inversely related to hippocampal neurite length. Knockdown of channel activity by overexpressing a dominant-negative mutant channel allowed significantly longer neuritis and filopodia to form. TRPC5 knockout mice harbour long, highly branched granule neuron dendrites with impaired dendritic claw differentiation in the cerebellar cortex. Apparently, TRPC5 regulates dendrite morphogenesis in the cerebellar cortex in a cell-autonomous manner. Behavioral analyses reveal that TRPC5 knockout mice have deficits in gait and motor coordination and display diminished fear-levels in response to aversive stimuli. The protein kinase calcium/calmodulin-dependent kinase II beta (CaMKIIβ) is a critical effector of TRPC5 function in neurons. TRPC5 forms a complex specifically with CaMKIIβ, but not the closely related kinase CaMKIIα, and thereby induces the CaMKIIβ-dependent phosphorylation of the ubiquitin ligase Cdc20-APC at the centrosome. Accordingly, centrosomal CaMKIIβ signaling mediates the ability of TRPC5 to regulate dendrite morphogenesis in neurons (Puram et al. 2011). A role of TRPC5 in growth cone regulation also seems to involve Semaphorin 3A, a member of a class of growth-cone guidance – proteins. This protein mediates growth cone collapse, which is reduced in hippocampal neurons from *Trpc5*$^{-/-}$ mice. This effect is due to an inhibition of the calcium-sensitive protease calpain in wild-type neurons but not in *Trpc5*$^{-/-}$ neurons. Calpain-1 and calpain-2 cleave and functionally activate TRPC5. Semaphorin 3A initiates growth cone collapse via activation of calpain that in turn potentiates TRPC5 activity. Thus, TRPC5 acts downstream of semaphorin signaling and modulates neuronal growth cone morphology and neuron development (Kaczmarek et al. 2012).

Other TRPC channels implicated in modulating neurite outgrowth, include TRPC1 and TRPC6 (Li et al. 2005; Shim et al. 2009; Tai et al. 2009). Interestingly, though these ion channels, like TRPC5, each constitute Ca^{2+} permeable channels, their role in regulation of neurite outgrowth is often opposite; indicating that spatiotemporal regulation of these channels is critical for proper regulation of neuronal morphogenesis (Kumar et al. 2012).

TRPC1 seems to be specifically essential for early neurogenesis. In hippocampal development, proliferation of an adult neural progenitor cell (aNPC) is a critical first step. TRPC1 is the most significantly upregulated TRPC channel during neurogenesis and knockdown of TRPC1 markedly reduced the degree of aNPC proliferation. Specifically, suppression of aNPC proliferation was found to be associated with cell cycle arrest in G0/G1 phase (Li et al. 2012). Hence, TRPC1 plays probably an important role in hippocampal neurogenesis. Importantly, this mechanism is discussed as a tool for improving adult hippocampal neurogenesis and treating cognitive deficits (Li et al. 2012).

Furthermore, in a model system for neuritogenesis, i.e. nerve growth factor (NGF)-differentiated rat pheochromocytoma 12 (PC12) cells, it was shown that NGF markedly up-regulated TRPC1 and TRPC6 expression, but down-regulated TRPC5 expression, while promoting neurite outgrowth. Overexpression of TRPC1 augmented, whereas TRPC5 overexpression decelerated NGF-induced neurite outgrowth. Conversely, shRNA-mediated knockdown of TRPC1 decreased, whereas shRNA-mediated knockdown of TRPC5 increased NGF-induced neurite extension. TRPC6 overexpression slowed down neuritogenesis, whereas dominant negative TRPC6 (DN-TRPC6) facilitated neurite outgrowth in NGF-differentiated PC12 cells. Using pharmacological and molecular biological approaches, it was shown that NGF up-regulated TRPC1 and TRPC6 expression via a p75(NTR) -IKK(2) -dependent pathway that did not involve TrkA receptor signalling in PC12 cells. Similarly, NGF up-regulated TRPC1 and TRPC6 via an IKK(2) dependent pathway in primary cultured hippocampal neurons. Thus, it can be suggested that a balance of TRPC1, TRPC5, and TRPC6 expression determines neurite extension rate in neural cells, with TRPC6 emerging as an NGF-dependent "molecular damper" maintaining a submaximal velocity of neurite extension (Kumar et al. 2012).

In another study, the effects of TRPC channels and Stromal Interaction Molecule (STIM)1-induced store-operated Ca2+ entry on neurite outgrowth of PC12 cells were investigated. In general, it is now firmly established that upon depletion of intracellular Ca^{2+} stores, STIM1 activates store-operated channels in the plasma membrane (mainly members of the ORAI family). STIM1 and Orai assemble in puncta in the ER membrane upon Ca^{2+} store depletion and during growth cone turning. STIM1 knockdown perturbed growth cone turning responses to BDNF and semaphorin-3a (Sema-3a) (Mitchell et al. 2012). It was also shown that PC12 cell differentiation down-regulates TRPC5 expression, whereas TRPC1 expression is retained and transfection of TRPC1 and TRPC5 increased the receptor-activated Ca^{2+} influx that was in turn markedly augmented by the co-expression of STIM1. Accordingly, overexpression of TRPC1 in PC12 cells increased neurite outgrowth while that of TRPC5 suppressed it. Clearly, suppression of neurite outgrowth by TRPC5 requires the channel function of TRPC5. Strikingly however, multiple lines of evidence show that the TRPC1-induced neurite outgrowth was independent of TRPC1-mediated Ca^{2+} influx. Thus, TRPC1 and TRPC5 similarly increased Ca^{2+} influx but only TRPC1 induced neurite outgrowth, the constitutively STIM1(D76A) mutant that activates Ca^{2+} influx by TRPC and Orai channels did not increase neurite outgrowth, and a channel-dead pore mutant of TRPC1 increased neurite

outgrowth to the same extent as WT TRPC1. Regulation of neurite outgrowth by TRPC1 thus seems independent of Ca^{2+} influx and TRPC1-promoted neurite outgrowth depends on the surface expression of TRPC1. Therefore, the possibility remains that TRPC1 merely acts as a scaffold at the cell surface to assemble a signaling complex to stimulate neurite outgrowth (Heo et al. 2012).

Golli proteins, products of the myelin basic protein gene (MBP), function as a new type of modulator of intracellular Ca^{2+} levels in oligodendrocyte progenitor cells (OPCs). They affect a number of Ca^{2+}-dependent functions, such as OPC migration and process extension. Pharmacologically induced Ca^{2+} release from intracellular stores evokes a significant extracellular Ca^{2+} entry after store depletion in OPCs, and Golli promoted activation of Ca^{2+} influx by SOCCs in cultured OPCs as well as in tissue slices. Strikingly, using a small interfering RNA knockdown approach, it was shown that TRPC1 is involved in SOCC in OPCs and is modulated by golli. Golli is physically associated with TRPC1 at OPC processes and TRPC1 expression is essential for the effects of golli on OPC proliferation. Thus, Ca^{2+} uptake through TRPC1 is an essential component in the mechanism of OPC proliferation (Paez et al. 2011).

It is also know that bone morphogenic proteins (BMPs) are involved in axon pathfinding. Indeed, a BMP7 gradient causes bidirectional turning responses from nerve growth cones. This effect is due to activation of the kinase LIM (LIMK) and the phosphatase Slingshot (SSH). Both enzymes regulate actin dynamics by modulating the actin-depolymerizing factor (ADF)/cofilin-mediated actin dynamics. This interaction requires the expression of TRPC1. It was suggested that TRPC1 mediated Ca^{2+} signals thus support, through calcineurin phosphatase, SSH activation and growth cone repulsion (Wen et al. 2007).

Another important player in the developing brain is Wnt5a. It has been shown in vivo that Wnt5a gradients surround the corpus callosum and guide callosal axons by Wnt5a induced repulsion, which also involves Ryk receptors. Application of pharmacological inhibitors to acute brain slices revealed a signalling pathway involving Ca^{2+}release through IP_3 receptors and calcium entry, presumably through TRPCs. Expression of Ryk siRNA revealed that knock-down of the Ryk receptor reduced outgrowth rates of postcrossing but not precrossing axons by 50 % and caused axon misrouting. In the corpus callosum CaMKII inhibition reduced the outgrowth rate of postcrossing (but not precrossing) axons and caused severe guidance errors, which resulted from reduced CaMKII-dependent repulsion downstream of Wnt/calcium signalling (Hutchins et al. 2010). Wnt5a is thought to propel cortical axons down the corticospinal tract and through the corpus callosum by repulsive mechanisms. In cultured dissociated early postnatal cortical neurons from hamsters, exposure to a gradient of Wnt5a is a model for studying the mechanism of Wnt5a effects. Turning assays indicated that cortical axons were repelled away from a point source of Wnt5a. Surprisingly, during the 1-h turning assay, axons exposed to Wnt5a also increased their growth rates by almost 50 %. Ryk receptors but not Frizzled (Fz) receptors were required for Wnt5a-promoted axon outgrowth, whereas both Ryk and Fz receptors were required for repulsive growth-cone turning. Both Ryk and Fz receptors mediated calcium signalling, which is required

for axon outgrowth and repulsive turning. Treatments with pharmacological inhibitors revealed that distinct Ca^{2+} signalling mechanisms were involved in Wnt5a-dependent axon outgrowth versus repulsive guidance. Ca^{2+} release from intracellular stores through inositol 1,4,5-trisphosphate receptors was required for Wnt5a-induced axon outgrowth but not for repulsive turning. In contrast, Ca^{2+} entry through TRPCs was required for both repulsive growth-cone turning and Wnt5a-increased axon outgrowth. Taken together, these results indicate that a guidance cue can induce increased rates of axon outgrowth simultaneously with repulsive guidance and may provide an understanding of how cortical axons may be repelled down the spinal cord in vivo (Hutchins et al. 2010; Li et al. 2010).

As mentioned above, the action of many extracellular guidance cues on axon pathfinding requires Ca^{2+} influx at the growth cone (Hong et al. 2000; Nishiyama et al. 2003; Henley and Poo 2004; Henley et al. 2004), but how activation of guidance cue receptors leads to opening of plasmalemmal ion channels remains largely unknown. Recent findings reveal that $PI(3,4,5)P_3$ elevation polarizes to the growth cone's leading edge and can serve as an early regulator during chemotactic guidance (Henle et al. 2011). A gradient of a chemoattractant triggered rapid asymmetric $PI(3,4,5)P_3$ accumulation at the growth cone's leading edge, as detected by the translocation of a GFP-tagged binding domain of Akt, in Xenopus laevis spinal neurons. Growth cone chemoattraction requires in this setting $PI(3,4,5)P_3$ production and Akt activation, and genetic perturbation of polarized Akt activity disrupted axon pathfinding in vitro and in vivo. Furthermore, patch-clamp recording from growth cones revealed that exogenous $PI(3,4,5)P_3$ rapidly activated cation currents, with properties reminiscent of TRPC channels, and asymmetrically applied $PI(3,4,5)P_3$ was sufficient to induce chemoattractive growth cone turning in a manner that required downstream Ca^{2+} signalling. Which TRPC channels are specifically involved remains unclear from this work.

Immunophilins, including FK506-binding proteins (FKBPs), are protein chaperones with peptidyl-prolyl isomerase (PPIase) activity. FKBPs are most highly expressed in the nervous system, where their physiological function remains however unclear. Interestingly, FKBP12 and FKBP52 catalyze cis/trans isomerization of regions of the TRPC1 protein, which is implicated in controlling channel opening. FKBP52, on the other hand, mediates stimulus-dependent TRPC1 gating through isomerization, which is required for chemotropic turning of neuronal growth cones to netrin-1 and myelin-associated glycoprotein and for netrin-1/DCC-dependent midline axon guidance of commissural interneurons in the developing spinal cord. FKBP12 mediates opening of TRPC1 is not required for growth cone responses to netrin-1. This study demonstrates a novel physiological function of proline isomerases in chemotropic nerve guidance through TRPC1 gating and may have significant implication in clinical applications of immunophilin-related therapeutic drugs (Shim et al. 2009).

TRPV1 is expressed in the neurites and in the filopodia of central neurons. Several data indicate that it regulates growth cone morphology and growth cone movement. Activation of TRPV1 results in growth cone retraction and formation of varicosities along the neuritis (Goswami and Hucho 2007). In relation with this, it is

interesting to consider that MYCBP2 is upregulated in the cerebellum and hippocampus, during the major synaptogenic period in these structures. MYCBP2 has been demonstrated to influence neuronal outgrowth and synaptogenesis by regulating the p38 MAPK-signaling pathways. Surprisingly, in the peripheral nervous system, the loss of MYCBP2 inhibits the internalization of TRPV1. Since both TRPV1 and MYCBP2 are involved in the neuronal growth in brain, this effect of MYBPC2 on TRPV1 might be a part of the mechanism regulating neuronal growth in hippocampus and cerebellum (Holland and Scholich 2011).

TRPV1 could be also involved in CNS regeneration after lesions, i.e. in the leech CNS: exposure to TRPV1 agonists after a nerve cut enhances neurite outgrowth, while capsazepine exposure produces this opposite effect (Meriaux et al. 2011).

Using siRNA interference to control TRPV4 expression in DRG neurons cultures, Jang et al. (2012) showed that TRPV4 can mediate neurite outgrowth via the regulation of neurtrophic factors. This regulation of neurite outgrowth could also occur in brain structures where TRPV4 is largely expressed. More than this, this study suggests than aberrant activity of TRPV4 could lead to some pathologies due to neuritogenesis defects.

Another vanilloid TRP channel, TRPV2, is also involved in growth cone guidance probably via sensing of membrane stretch during development (Shibasaki et al. 2010).

TRPM3 is activated by pregnolone sulfate (PS), a neurosteroid which is retrogradly released in cerebellum and in hippocampus. Interestingly, during development, PS release potentiates and refines the glutamatergic synapses in brain. Pharmacological experiments using a TRPM3 antagonist has demonstrated an inhibition of the PS induced glutamatergic synapse potentiation (Zamudio-Bulcock et al. 2011; Zamudio-Bulcock and Valenzuela 2011). Although there is no direct evidence, since the *trmp3* KO mice have not been analysed in these studies, it might be suggested that TRPM3 acts a modulator of glutamatergic transmission in brain and therefore might play a role in synaptic contact establishment.

2.2 A Role for TRP Channels in Synaptic Plasticity and Behaviour

TRPC are widely expressed in the brain and play several roles in development and normal neuronal function. Members of the TRPC family are generally coupled to activation of Gq coupled receptors. Activation of phospholipase C leads to production of IP3 and diacylglycerol (DAG). The latter is described as a specific activator of TRPC3, TRPC6 and TRPC7. TRPC1 and TRPC4 are reported to be store-operated, i.e. activated by depletion of IP_3 sensitive stores, or receptor operated and finally TRPC5 is activated by increases of intracellular [Ca^{2+}]. Thus it can be anticipated that TRPC channels are players when Gq coupled neuronal receptors are stimulated. This class of receptors includes metabotropic muscarinic, glutamate

and GABA receptors. With this in mind, it is not surprising that TRPC channels have been implicated in processes such as spine formation and modulation of synaptic transmission through membrane depolarization (Tai et al. 2009).

For instance, it is known that group I metabotropic glutamate receptors (mGluRs) play an essential role in cognitive function. Group 1 mGluR activation induced in CA1 pyramidal neurons intracellular Ca^{2+} waves and a biphasic electrical response composed of a transient Ca^{2+} -dependent SK channel-mediated hyperpolarization and a (possibly TRPC-mediated) sustained depolarization. The generation and magnitude of the SK channel-mediated hyperpolarization depended solely on the rise in intracellular Ca^{2+} concentration whereas the TRPC channel-mediated depolarization required both a small rise in $[Ca^{2+}]_i$ and mGluR activation. Surprisingly in this study, TRPC-mediated current were suppressed by forskolin-induced rises in cAMP. Thus, SK- and TRPC-mediated currents robustly modulate pyramidal neuron excitability by decreasing and increasing their firing frequency. Apparently, cAMP levels provide an additional level of regulation by modulating TRPC-mediated sustained depolarization that might stabilize periods of sustained firing (El-Hassar et al. 2011). The mGluR1 receptor is particularly important for synaptic signalling and plasticity in the cerebellum. Unlike ionotropic glutamate receptors that mediate rapid synaptic transmission, mGluR1s produce in cerebellar Purkinje cells a complex postsynaptic response consisting of two distinct signal components, namely a local dendritic calcium signal and a slow excitatory postsynaptic potential. The basic mechanisms underlying these synaptic responses were clarified in recent years. Dendritic calcium signal results from IP_3 receptor-mediated calcium release from internal stores. mGluR1-mediated slow excitatory postsynaptic potentials are mediated by the transient receptor potential channel TRPC3. This surprising finding established TRPC3 as a novel postsynaptic channel for glutamatergic synaptic transmission (Hartmann et al. 2011).

It is a common feature that neurons sum their input by spatial and temporal integration. Temporally, presynaptic firing rates are converted to dendritic membrane depolarizations by postsynaptic receptors and ion channels. In several regions of the brain, including higher association areas, the majority of firing rates are low. For rates below 20 Hz, the ionotropic receptors alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and N-methyl-d-aspartate (NMDA) receptor will not produce effective temporal summation. Interestingly, TRP channels activated by metabotropic glutamate receptors would be more effective, owing to their slow kinetics. Using a computational model of the TRP channel and its intracellular activation pathway, it was suggested that synaptic input frequencies down to 3–4 Hz and inputs consisting of as few as three to five pulses can be effectively summed. Temporal summation characteristics of TRP channels may be important at distal dendritic arbors, where spatial summation is limited by the number of concurrently active synapses. It may be particularly important in regions characterized by low and irregular rates (Petersson et al. 2011).

Finally, activation of muscarinic receptors on pyramidal cells of the cerebral cortex induces the appearance of a slow afterdepolarization that can sustain autonomous spiking after a brief excitatory stimulus. This phenomenon has been

hypothesized to allow for the transient storage of memory traces in neuronal networks. Muscarinic receptors have the ability to induce the inward aftercurrent underlying the slow afterdepolarization which is inhibited by expression of a Gq-11 dominant negative mutant and which is also markedly reduced in a phospholipase C ß1 (PLCβ1) knock-out mouse. These results indicate that the Gq-11/PLCß1 cascade plays a key role in the ability of muscarinic receptors to signal the inward current. Muscarinic afterdepolarizations might be mediated by a calcium-activated nonselective cation current. Surprisingly, it was found that expression of a TRPC dominant negative protein inhibits, and overexpression of wild-type TRPC5 or TRPC6 enhances, the amplitude of the muscarinic receptor-induced inward aftercurrent. Furthermore, coexpression of TRPC5 and T-type calcium channels is sufficient to reconstitute a muscarinic receptor-activated inward current in human embryonic kidney HEK-293 cells. These results indicate that TRPC channels might mediate the muscarinic receptor-induced slow afterdepolarization seen in pyramidal cells of the cerebral cortex and might suggest a possible role for TRPC channels in mnemonic processes (Yan et al. 2009).

TRPC6 is reportedly localized post-synaptically in excitatory synapses and promotes their formation via a Ca^{2+}/calmodulin-dependent kinase IV (CaMKIV) – cAMP-response-element binding protein (CREB)-dependent pathway. Overexpression of TRPC6 increases the number of spines in hippocampal neurons and TRPC6 knockdown with RNAi decreases the number. Transgenic mice overexpressing *trpc6* showed enhancement in spine formation, and a better spatial learning and memory in Morris water maze. These results reveal a previously unknown role of TRPC6 in synaptic and behavioral plasticity (Zhou et al. 2008). These results were confirmed in a second study (Tai et al. 2008). Interestingly, it was shown that the peak expression of TRPC6 in rat hippocampus was between postnatal day 7 and 14, a period known to be important for maximal dendritic growth. Mechanistically, these authors suggest that Ca^{2+} influx through the TRPC6 channel leads to CaMKIV and CREB. Overexpression of TRPC6 increased phosphorylation of both factors and promoted dendritic growth in hippocampal cultures. Downregulation of TRPC6 suppressed phosphorylation of both CaMKIV and CREB and impaired dendritic growth. Expressing a dominant-negative form of CaMKIV or CREB blocked the TRPC6-induced dendritic growth. Furthermore, inhibition of Ca^{2+} influx suppressed the TRPC6 effect on dendritic growth. In transgenic mice overexpressing *Trpc6*, the phosphorylation of CaMKIV and CREB was enhanced and the dendritic growth was also increased. Thus it seems that TRPC6 plays an important role during the development of the central nervous system (CNS) and has a profound impact on learning and memory through the regulation of spine formation (Tai et al. 2008).

In the cerebellum, Purkinje cell TRPC3 channels underlie the slow excitatory postsynaptic potential (EPSP) observed following parallel fibre stimulation. TRPC3 channel opening requires stimulation of metabotropic glutamate receptor 1 (mGluR1), activation of which can also lead to the induction of long term depression (LTD), which underlies cerebellar motor learning. LTD induction requires protein kinase C (PKC) and protein kinase G (PKG) activation, and whilst PKC phosphorylation targets are well established, virtually nothing is known about PKG

targets in LTD. TRPC3 channels are inhibited following phosphorylation by PKC and PKG in expression systems, we examined whether native TRPC3 channels in Purkinje cells are a target for PKG or PKC, thereby contributing to cerebellar LTD. In Purkinje cells, activation of TRPC3-dependent currents is not inhibited by conventional PKC or PKG to any significant extent and that inhibition of these kinases does not significantly impact on TRPC3-mediated currents, TRPC3-dependent currents may differ significantly in their regulation from those overexpressed in expression systems (Nelson and Glitsch 2012).

TRPV1 is largely expressed in brain and plays a surprisingly important 'non-sensory role' in brain. The expression of other Vanilloid TRP proteins has been reported in different brain structures (Kauer and Gibson 2009) and role is strikingly versatile and is involved in the general "excitability" of the cortex (Mori et al. 2012). Indeed, TRPV1 activation induces long-term depression at CA1 interneurons synapses (Gibson et al. 2008). Activation of TRPV1 by capsaicin and capsazepin led to the depression of the communication at interneuron synapses. This capsaicin induced LTD was absent in the *trpv1* KO mouse. This synaptic depression apparently is mediated via a presynaptic activation of calcineurin, a phosphatase known to decrease neurotransmission probably linked in DRG to TRPV1 (Wu et al. 2005). This inhibition of the excitatory transmission via TRPV1 activation has also been reported in the dentate granule cells. Chávez et al. (2010) showed that this depression of the synaptic communication was due to an internalization of the AMPA receptor in a calcineurin dependent manner.

Surprisingly, application of capsaicin also enhances the long-term potentiation of pyramidal neurons in the CA1 of hippocampus. Bennion et al. (2011) proposed that this modulation of synaptic plasticity by TRPV1 is mediated by its effects on the inhibitory GABAergic system (Bennion et al. 2011). The enhancement of LTP in CA1 neurons would be then the consequence of the depression of the synaptic communication of the inhibitory interneurons in the CA1 region previously reported by Gibson et al. (2008). The influence on synaptic plasticity is also important in the Nucleus Accumbens (NAc) which plays a key role in goal-directed behaviours and reward dependent learning and in amygdala. In NAc, as in dentate gyrus, TRPV1 can trigger LTD via the endocytosis of the AMPA receptors. Nevertheless, on the opposite of the modulation of the synaptic plasticity in hippocampus, in the NAc, the endocannabinoids act post synaptically through TRPV1 (Grueter et al. 2010). Remarkably and although the mechanism remains unclear, capsaicin application in amygdala increases the amplitude of the LTP, suggesting a role for TRPV1 in the modulation of synaptic plasticity in this structure.

This TRPV1 mediated synaptic plasticity in brain might explain some properties of Docosahexaenoic acid (DHA). DHA is known to enhance cognitive functions (Morley and Banks 1998). DHA supplementation in primary hippocampal neuron cultures regulates TRPV1 and TRPV2 expression in a dose dependent manner without altering TRPV3 or TRPV4 expression. This suggests that DHA positive effects on memory could be mediated by modulation of the endovanilloid receptors expression.

In accordance with this modulation of hippocampus synaptic plasticity, TRPV1 also presents a role in the memory consolidation. In vivo injection of capsazepine disrupted memory consolidation following a strong training protocol. This might highlight a possible synergic role of the endocannabinoid and endovanilloid system in memory consolidation (Genro et al. 2012).

TRPV1 could also control the anxiety-like behavior through its expression in the medial prefrontal cortex. Injection of capsaicin increases anxiogenic response in mice whereas capsazepine injection significantly exhibits an anxiolytic effect (Manna and Umathe 2011). This was confirmed by injections of capsazepine in prefrontal cortex of rats (Aguiar et al. 2009). Moreover, anandamide release has opposite effect on the anxiety behaviour: cannabinoid receptor type 1 (CB1) activation inhibits whereas TRPV1 activation enhances anxiety-like behaviour. The blockade of TRPV1 might be a functional tool to treat anxiety while preventing the risks associated with the long-term use of benzodiazepines (Moreira et al. 2011). Interestingly, another study reported that the *trpv1* KO mice exhibit less stress or anxiety than WT mice (Marsch et al. 2007).

Although there is no direct evidence for an involvement of TRPV1 in the obsessive compulsive disorder (OCD), TRPV1 might be considered as a potential therapeutic target in such a depression syndrome. Indeed, Umathe et al. (2012) have reported that a TRPV1 antagonist produced a persistent inhibition of the OCD while capsaicin or anandamide produced the opposite effect. Inhibition of TRPV1 might be an effective tool in the treatment of OCD.

Finally however, some caution should be taken concerning the role of TRPV1 in brain. In 2011, Cavanaugh et al. created a *trpv1* reporter mouse and actually showed that the expression of TRPV1 in brain is much more restricted than first reported. No expression, neither functional activity of TRPV1 could be detected in hippocampus, amygdala or cerebellum. This study puts previous studies involving TRPV1 in physiology of brain in a different perspective. Indeed, it should be noted also that previous studies showing an implication of TRPV1 in hippocampus synaptic plasticity via capsaicin application, but never recorded TRPV1 direct activation by calcium imaging or whole cell recording. These discrepancies could have several explanations. First, Chávez et al. (2010) and Grueter et al. (2010) suggested the role of TRPV1 in the synaptic plasticity in response to endocannabinoids. This endocannabinoid-induced plasticity could also be triggered by other TRP channels. Indeed Watanabe and colleagues showed that anandamine could activate TRPV4 via epoxyeicosatrienoic acid (Watanabe et al. 2003) and TRPV4 is known to be expressed in hippocampus (Shibasaki et al. 2007). Another possibility could be that TRPV1 triggers LTD via a non-conducting function (Kaczmarek 2006). In such conditions, some non-functional alternative splicing forms of TRPV1 could be expressed in hippocampus and respond to capsaicin without any calcium influx. Indeed, the authors reported a TRPV1 expression (Cavanaugh et al. 2011) in hippocampus interneurons, but did not report any capsaicin response in calcium imaging, suggesting a eventual non functional form of TRPV1.

TRPM channels have also been implicated in neuronal plasticity. TRPM2 is a Ca^{2+} permeable cation channel activated by oxidative stress and is involved in cell death. However, it may be also a modulator of hippocampal synaptic plasticity. Indeed, Olah et al. (2009) have described TRPM2 as a regulator of voltage-dependent Ca^{2+} channels and the NMDA receptors via a rise in the intracellular calcium concentration, and its depolarizing effect. The study of hippocampus slices in a *trpm2* KO context showed that the LTD is selectively impaired because of inhibition of the kinase GSK3beta, confirming TRPM2 as a key player in hippocampal synaptic plasticity (Xie et al. 2012). Additionally, TRPM2 is linked to neuronal cell death after oxidative stress induced by glutathione (GSH). GSH inhibits TRPM2 channels through a thiol-independent mechanism, which plays an important role in aging and neurological diseases associated with depletion of GSH (Belrose et al. 2012).

Finally, it has been shown that TRPA1 is involved in the glycinergic neurotransmission generating IPSPs in the rat medullary dorsal horn (Substantia gelatinosa) and also as a presynaptic channel in the nucleus supraopticus regulating glutamate release (Yokoyama et al. 2011; Cho et al. 2012).

2.3 TRP Channels as Players in Neuronal Activity

Apart from the role of TRP channels in specific processes such as memory formation and neuronal development, an ever increasing number of studies links TRPC channels with specific neuronal receptor activity. Basically TRPC channels can provide a Ca^{2+} influx pathway, which couples to intracellular functions, or TRPC channels can support a depolarization, which would influence action potential triggering and bursting behavior. As such, TRPC channels have been linked with metabotropic glutamate, GABA and acetylcholine receptors (see above, and e.g. Berg et al. 2007), serotonin 2C and leptin receptors in pro-opiomelanocortin neurons and kisspeptin receptors in hypothalamic neurons (Qiu et al. 2011; Sohn et al. 2011; Williams et al. 2011).

In the mammalian central nervous system, slow synaptic excitation involves the activation of metabotropic glutamate receptors (mGluRs). TRPC3, but not TRPC1, is needed for mGluR-dependent synaptic signaling in mouse cerebellar Purkinje cells. TRPC3 is the most abundantly expressed TRPC subunit in Purkinje cells. In mutant mice lacking TRPC3, both slow synaptic potentials and mGluR-mediated inward currents are completely absent, while the synaptically mediated Ca^{2+} release signals from intracellular stores are unchanged. Importantly, *trpc3* knockout mice exhibit an impaired walking behavior. Taken together, these results establish TRPC3 as a new type of postsynaptic channel that mediates mGluR-dependent synaptic transmission in cerebellar Purkinje cells and is crucial for motor coordination (Hartmann et al. 2008; Hartmann and Konnerth 2008).

Cholecystokinin (CCK) is one of the most abundant neuropeptides in the brain where it interacts with two G protein-coupled receptors (CCK-1 and CCK-2).

Activation of both CCK receptors increases the activity of phospholipase C (PLC) resulting in increases in intracellular Ca^{2+} release and activation of protein kinase C (PKC). High density of CCK receptors has been detected in the superficial layers of the entorhinal cortex (EC). Effects of CCK on neuronal excitability of layer III pyramidal neurons in the EC include a remarkable increase of the firing frequency of action potentials, which are mediated via activation of CCK-2 receptors and required the functions of G proteins and PLC. In a recent study, CCK-mediated facilitation of neuronal excitability appeared independent of IP_3 receptors and PKC, but relying on the activation of a cationic channel to generate membrane depolarization. This cationic channel shows a pharmacological profile which has been described for TRPC channels (but albeit relatively unselective): inhibition by 2-aminoethyldiphenyl borate (2-APB) and flufenamic acid (FFA) and potentiation by Gd^{3+} and 100 μM La^{3+}. Furthermore, CCK-induced enhancement of neuronal excitability was significantly inhibited by intracellular application of the antibody to TRPC5 suggesting the involvement of TRPC5 channels (Wang et al. 2011).

Another interesting growth hormone whose receptor has been linked with TRPC channels is brain-derived neurotrophic factor (BDNF) (Jia et al. 2007; Sossin and Barker 2007). BDNF is believed to be an important regulator of striatal neuron survival, differentiation, and plasticity. Reduction of BDNF delivery to the striatum has been implicated in Huntington's disease. With respect to TRP channels, an interesting study suggested that they might contribute to intracellular signaling pathways, which lead to short-term induction of striatal gene expression by BDNF. Indeed, gene expression responses to BDNF can be abolished by inhibitors of TrkB (K252a) and calcium (chelator BAPTA-AM) and the (non-selective) transient receptor potential cation channel [TRPC] antagonist SKF-96365 (Gokce et al. 2009). BDNF also induces synaptic potentiation at both neuromuscular junctions (NMJs) and synapses of the CNS through a Ca^{2+} dependent pathway. Pharmacological inhibition or morpholino-mediated knockdown of Xenopus TRPC1 (XTRPC1) can significantly attenuate the BDNF-induced potentiation of the frequency of spontaneous synaptic responses at the NMJ. XTRPC1 was required specifically in postsynaptic myocytes for BDNF-induced Ca^{2+} elevation and full synaptic potentiation at the NMJ, suggesting a previously underappreciated postsynaptic function of Ca^{2+} signalling in neurotrophin-induced synaptic plasticity (McGurk et al. 2011).

Persistent neuronal activity lasting seconds to minutes has been proposed to allow for the transient storage of memory traces in entorhinal cortex and thus could play a major role in working memory. Nonsynaptic plateau potentials, induced by acetylcholine, account for persistent firing in many cortical and subcortical structures. The expression of these intrinsic properties in cortical neurons involves the recruitment of a non-selective cation conductance of unknown origin. In layer V of rat medial entorhinal cortex, muscarinic receptor-evoked plateau potentials and persistent firing induced by carbachol require PLC, decrease of $PI(4,5)P_2$, and a permissive $[Ca^{2+}]_i$. Plateau potentials and persistent activity were suppressed by the generic nonselective cation channel blockers FFA (100 μM) and 2-APB (100 μM), as well as by the TRPC channel blocker SKF-96365 (50 μM) and are not affected by the TRPV channel blocker ruthenium red (40 μM). The TRPC3/6/7 activator OAG

did not induce or enhance persistent firing evoked by carbachol. Voltage clamp recordings revealed a carbachol-activated, nonselective cationic current with a heteromeric TRPC-like phenotype, including outward rectification and a reversal potential around 0 mV. Moreover, plateau potentials and persistent firing were inhibited by intracellular application of the peptide EQVTTRL that disrupts interactions between the C-terminal domain of TRPC4/5 subunits and associated PDZ proteins of the NHERF family and which has been reported to be important for TRPC4/C5 channel function (Harteneck et al. 2003), suggesting that TRPC4-5 mediated currents significantly contribute persistent depolarisation of neurons and thus controls the firing and mnemonic properties of projection neurons in the entorhinal cortex (Zhang et al. 2011b).

As mentioned above, TRPC6 is expressed in several types of neurons, including cerebrospinal-fluid contacting neurons (Wu et al. 2011), cortical neurons (Tu et al. 2009b), and in the substantia nigra of normal rat brain (Giampa et al. 2007).

Interestingly, Hyperforin, one of the main bioactive compounds of the medicinal plant Hypericum perforatum (St. John's wort), activates TRPC6 without affecting the other TRPC channels (Tu et al. 2009a). A recent studies describes its impact on the BDNF receptor TrkB and on adult hippocampal neurogenesis, since they appear central to the mechanisms of action of antidepressants. Chronic hyperforin treatment on cortical neurons in culture and on the brain of adult mice led to increased expression of TRPC6 channels and TrkB via SKF-96365-sensitive channels controlling a downstream signaling cascade involving Ca^{2+}, protein kinase A, CREB and p-CREB. Hyperforin augmented the expression of TrkB in the cortex but not in the hippocampus where neurogenesis remained unchanged (Gibon et al. 2012).

St. John's Wort (SJW) has been used medicinally for over 5,000 years and first gained attention as the constituent of SJW responsible for its antidepressant effects. Since then, several of its neurobiological effects have been described, including neurotransmitter re-uptake inhibition, the ability to increase intracellular sodium and calcium levels, TRPC6 activation, NMDA receptor antagonism as well as antioxidant and anti-inflammatory properties. Until recently, its pharmacological actions outside of depression had not been investigated. Hyperforin has been shown to have cognitive enhancing and memory facilitating properties. Importantly, it has been shown to have neuroprotective effects against Alzheimer's disease (AD) neuropathology, including the ability to disassemble amyloid-beta (Aβ) aggregates in vitro, decrease astrogliosis and microglia activation, as well as improve spatial memory in vivo (Griffith et al. 2010).

The analysis of *Trpc6*$^{-/-}$ mice clearly shows that TRPC6 activity affects behaviour. *Trpc6*$^{-/-}$ mice showed no significant differences in anxiety in a marble burying test, but demonstrated reduced exploration in the square open field and the elevated star maze (Beis et al. 2011).

Using electromyography and transcranial magnetic stimulation, Mori et al. (2012) described for the first time that some single nucleotide polymorphisms of *trpv1* in human can regulate cortical excitability probably by modulation of glutamate release at synapses. In the striatum, TRPV1 regulates the release of the excitatory messenger glutamate. Capsaicin application enhances the frequency of

glutamate-mediated spontaneous (sEPSCs) and miniature (mEPSC) excitatory postsynaptic currents (Musella et al. 2008, 2010). It also modulates GABA transmission, an inhibitory pathway, via endocannabinoids (eCBs). The effect of capsaicin application both on glutamate and GABA transmission is lacking in the *trpv1* KO mice.

Therefore, TRPV1 modulation offers alternative therapeutic routes in disorders of striatal neurotransmission (Musella et al. 2008, 2010). Moreover other studies suggest that this regulation of synapse activity might occur in several other structures such as the pineal gland (Reuss et al. 2010).

Some brain neurons present a specific firing behaviour, called burst firing. This spiking behaviour is characterized by a sustained firing activity. Such a burst firing activity is involved in different brain processes like reward circuit, short-term memory in an emotional and experience dependent learning context, respiratory rhythms regulation. Ca^{2+} activated non-selective (CAN) currents are proposed to be key players of sustained firing activity mechanisms (Rubin et al. 2009). TRPM4 and TRPM5 are considered as the channel underlying CAN and could contribute in this reasoning to many brain processes (Launay et al. 2002; Hofmann et al. 2003). So far, the most investigated process of burst firing behaviour in which TRPM4 plays a role is described in the pre-Bötzinger Complex (preBötC) neurons (Pace et al. 2007). The preBötC is involved in the respiratory rythmogenesis (Feldman and Del Negro 2006). These neurons are characterized by an oscillating activity and by the synchronization of their burst firing. Only 20 % of these neurons present a pacemaker activity, meaning that most of the neurons generate inspiratory drive potentials by evoking post-synaptic currents that depend on intrinsic membrane properties (Del Negro et al. 2005). CAN currents have been proposed to be responsible for amplifying glutamatergic synaptic drive by transforming the glutamatergic synaptic inputs to membrane depolarization (Pace et al. 2007; Mironov 2008; Mironov and Skorova 2011). Pace et al. showed that calcium influx was able to induce some plateau potentials, and external sodium substitution and flufenamic acid exposure attenuated those plateau potentials. They also proposed CAN activation by glutamatergic inputs could direct (via NMDA-R calcium influx) or indirect (via mGluR induced IP_3 dependent calcium release, or AMPA-R activation of voltage gated calcium channels). Crowder et al. (2007) detected by RT-PCR TRPM4 and TRPM5 expression in preBötC neurons and showed that excess of PIP_2 augmented the inspiratory drive potential and the effect was modulated by flufenamic acid (FFA) application (Crowder et al. 2007). Thus, TRPM4 current could be activated by calcium waves in the soma and generate inspiratory bursts by boosting glutamatergic synaptic inputs. More recently, a novel pathway of activation of TRPM4 has been suggested in this system: the Epac/cAMP pathway. Epac agonist application on preBötC neurons sensitized calcium mobilization from IP3 internal calcium stores that stimulated TRPM4 and potentiated bursts of action potentials (Mironov and Skorova 2011). It remains unclear however, whether TRPM4 activity itself is regulated by this mechanism.

This mechanism of activation via glutamatergic synaptic inputs and the role of TRPM4/5 in burst firing activity might be conserved also in other brain structures.

Mrejeru et al., have described a similar mechanism in dopaminergic (DA) neurons of substantia nigra (Mrejeru et al. 2011). Those neurons present two different behaviours, tonic firing and bursts of action potentials. They showed by electrophysiology that NMDA currents recruit a CAN current capable of generating a plateau potential. This CAN current can be blocked by flufenamic acid and 9-phenanthrol application. Since mRNA expression of TRPM2 and TRPM4 has been detected by RT-PCR (TRPM5 could not be detected), they hypothesized TRPM4 to be the channel involved in the burst firing behavior. Although TRPM4 current has not been directly recorded in dopaminergic neurons, and the specificity of flufenamic acid and 9-phenanthrol on brain slices has not been determined, Mrejeru et al. provide the first evidences that TRPM channels (TRPM2 and TRPM4) are expressed in substancia nigra neurons and could be a part of the reward circuit by boosting NMDA currents during burst firing.

The neurons of the lateral nucleus of amygdala also display such a sustained firing activity. The graded increase in firing is linked to a CAN current and is blocked by flufenamic acid application (Egorov et al. 2002). The Allen Brain Atlas shows TRPM4/5 mRNA expression in amygdala, leading to the conclusion that either one or the two channels are involved in a burst firing activity in the lateral nucleus and then are part of the mechanism for sustaining information about novel items in a short term memory in a context of emotional and experience dependent learning.

Although no direct evidence of endogenous TRPM4 or TRPM5 currents in neurons are now available, a similar process of sustained firing activity dependent on CAN channels exists in diverse structures such as the motoneurons of the nucleus ambiguous, the layer II neurons of the entorhinal cortex (Egorov et al. 2002), the sensory neurons of the olfactory bulbs (Pressler and Strowbridge 2006), indicating possibly a new role for TRPM4 and TRPM5 in firing behavior in brain physiology. However, see also data mentioned above that imply a more prominent role of TRPC4/C5 channels in this process (Wang et al. 2011; Zhang et al. 2011b). In the absence of a specific pharmacology, TRP specific knockout mice or knockdown strategies are clearly needed to clarify this issue.

Finally, an intriguing role for TRPA1 in astrocytes has been shown. Astrocytes contribute to the formation and function of synapses and are found throughout the brain, where they show intracellular store-mediated Ca^{2+} signals. Recently, using a membrane-tethered, genetically encoded calcium indicator (Lck-GCaMP3), it was reported that Ca^{2+} fluxes mediated by spontaneously open TRPA1 channels gave rise to frequent and highly localized 'spotty' Ca^{2+} microdomains near the membrane that contributed appreciably to resting Ca^{2+} levels in astrocytes. Work in cultured astrocytes and in brain slices showed that inhibiting these Ca^{2+} signals with a TRPA1 specific blocker, leads to decreased astrocyte resting Ca^{2+} concentrations, and decreased interneuron inhibitory synapse efficacy. It was shown that influx through TRPA1, reduces the activity of a GABA transporter in astrocytes, GAT-3, which leads to elevated extracellular GABA levels, and reduced miniature inhibitory post-synaptic currents (mIPSC's) specifically in interneurons, but not in pyramidal neurons. This work highlights the housekeeping role of astrocytes in

neuronal networks, and specifically the role of intracellular Ca^{2+} levels and TRPA1 therein (Clarke and Attwell 2011; Shigetomi et al. 2012).

3 TRP Channels Cause Neurological Diseases

3.1 TRP Channels Could Play a Role in Disease Mechanisms

Considering their function as Ca^{2+} influx channels, and considering the critical role of intracellular [Ca^{2+}] dynamics in neuronal differentiation, functional signalling and survival it is clear that dysfunctional TRP channels can be expected to have a profound effect on the neuron's health status.

In neurons, excessive Ca^{2+} entry occurs via over-activation of glutamate receptors (NMDA, AMPA, KA) or of a range of channels and transporters (TRPM2, TRPM7, NCX, ASICs, CaV1.2, and hemichannels). Potentially toxic cytoplasmic calcium concentrations can also occur due to release from internal stores, either through physical damage to mitochondria and the endoplasmic reticulum, or a malfunction of receptors and channels present in their membranes. Such increases of cytoplasmic calcium concentrations can trigger a range of downstream neurotoxic cascades, including the uncoupling mitochondrial electron transfer from ATP synthesis, and the activation and overstimulation of enzymes such as calpains and other proteases, protein kinases, nitric oxide synthase (NOS), calcineurin and endonucleases. Alterations in Ca^{2+} homeostasis have been suggested in the onset/progression of neurological diseases, such as Parkinson's, Alzheimer's, bipolar disorder, hereditary ataxia and Huntington's or with neurological aspects of aging (Amaral et al. 2007; Amaral and Pozzo-Miller 2007a, b; Adachi et al. 2008; Poduslo et al. 2008, 2009; Roedding et al. 2009; Cucchiaroni et al. 2010; Becker et al. 2011).

TRP channels are also important regulators of membrane potential. They will support slow depolarization of the cell and shape burst firing patterns of neurons or support persistent activity of neurons. In this sense it can be anticipated that gain-of-function mutations of TRP channels will contribute to prolonged burst firing patterns and vice versa. Disease states which are associated with this in relation to TRP channels include ataxia and epilepsy (Adachi et al. 2008; Becker et al. 2009, 2011; Tai et al. 2009). Epilepsy is caused mainly by perturbances of the balance of excitation and inhibition within the central system. Because TRPV1 activation modulates activity dependent synaptic efficacy, TRPV1 blockade is now considered as a potential antiepilepsy treatment (Fu et al. 2009). Basically, all the TRPM could be key players in epilepsy. Indeed, the balance in ion homeostasis is important for the neuronal network activity. TRP channels could fine-tune this neuronal activity, so any perturbance of TRP physiology might be considered as an epileptogenic event (Stawicki et al. 2011). An epileptic seizure is composed of recurrent bursts of intense firing. For instance, Schiller Y (2004) recorded a Ca^{2+} activated

cation (CAN) current in neocortex slices treated with bicuculline to induce seizure (Schiller 2004). This current was unaffected by changing chloride concentrations but was sensitive to intracellular calcium changes and was blocked by flufenamic acid application. This CAN current is activated by calcium influx through NDMA receptors and voltage gated calcium channels. This is the first direct evidence that CAN current is involved in a pathological process. Indeed this current could support sustained seizure like events (Schiller 2004). Interestingly the mechanism seems to be similar to what has been described in the preBötC and substancia nigra neurons. Since TRPM4 and TRPM5 have been shown to function as CAN channels, further investigation in *trpm4* and *trpm5* KO mice could improve the understanding of the pathophysiological process leading to epileptic seizure.

TRPV channels, in cooperation with the endocannabinoid system, influence GABAergic and glutamatergic synapses and play a modulatory function on dopamine transmission. Through these mechanisms TRPV and endocannabinoids have an important influence on various neurobiological processes (e.g., control of movement, motivation/reward) and, particularly, on different pathologies affecting these processes such as basal ganglia disorders, schizophrenia (Fernandez-Ruiz et al. 2010), and drug addiction.

TRPM4 is thought to be underlying the boosting of NMDA current in DA neurons (Mrejeru et al. 2011). Since those neurons are vulnerable to neurodegeneration, this CAN current boost mechanism may also explain the high sensitivity of DA neurons for excitotoxicity. In this case, TRPM4 could be considered as a potential drug target in Parkinson disease (PD). But TRPM4 is not the only TRPM that may be involved in PD. TRPM2 current has been recorded in DA neurons and the injection of Rotenone, used as a model of PD, induces a current that can be specifically inhibited by TRPM2 blockers. The ROS production induced by the rotenone injection is probably the key player in the activation of TRPM2 in this model (Freestone et al. 2009). Moreover, there is wide agreement that oxidative stress induced TRPM2 activation could lead to cell death. This highlights a possible relation between TRPM2 and the neurodegenerative part of PD (Belrose et al. 2012) described. Human genetic studies in western countries also revealed that some single nucleotides polymorphisms in *trpm2* and *trpm7* could be associated with risk factors for certain form of Parkinsonian Dementia Complex (Hermosura et al. 2005, 2008; Hermosura and Garruto 2007). Nevertheless, a Japanese study could not find any correlation between *trpm7* SNP and PD (Hara et al. 2010). This tends to suggest that, mainly TRPM2 should be considered as a risk factor for neurodegenerative diseases as well as a potential therapeutic target.

3.2 TRPs Channels in Brain Injury and Stroke

TRPM7 is a potential target for neuroprotection after brain injury. Suppressing the expression of TRPM7 in hippocampal CA1 neurons causes resistance to ischemic cell death, preserved cell function and prevented ischemia-induced deficits in

memory (Sun et al. 2009). Depletion of intracellular Mg^{2+}, a symptom of traumatic brain injury and a reduction of extracellular Ca^{2+} are both associated with poor neurological outcome and are both conditions which activated TRPM7 thereby possibly increasing the Ca^{2+} load of neuronal cells. This leads to secondary injury processes and to cell death following brain injury, including stroke (Cook et al. 2009) TRPM7 has been implicated in ischemic brain damage. TRPM7 gene variation might play a role in the risk of ischemic stroke (Romero et al. 2009).

3.3 TRPs, Schizophrenia and Bipolar Disorders

TRP channels play a role in the pathogenesis of schizophrenia. TRPV1, in cooperation with the endocannabinoid system, influences GABAergic and glutamatergic synapses and play a modulatory function on dopamine transmission. Through these mechanisms, TRPV1 and endocannabinoids have an important influence on various neurobiological processes (e.g., control of movement, motivation/reward) and, particularly, on different pathologies affecting these processes like basal ganglia disorders, schizophrenia, and drug addiction (Fernandez-Ruiz et al. 2010).

Natural compounds, used in traditional medicine as anti-depressants, target TRP channels, e.g. *Incensole* acetate which is released by the burning of resin from the *Boswellia* plant has been used for religious and cultural ceremonies for millennia. It activates TRPV3, which is expressed in the brain and causes anxiolytic-like, antidepressive-like behavioral effects and protects against brain ischemia (Moussaieff et al. 2008, 2012). As mentioned above already, St. John's Wort has been used medicinally for over 5,000 years. Recently, Hyperforin, an antidepressive compound obtained from St. John's Wort, has been identified as effective activator of TRPC6 (Leuner et al. 2007). It causes cognitive enhancing, memory facilitating properties and has probably neuroprotective effects (Griffith et al. 2010). TRPM2, which is highly expressed in the striatum (caudate nucleus and putamen) is supposed to play a key role in bipolar disorders (Aita et al. 1999; Uemura et al. 2005; Xu et al. 2006, 2009; Roedding et al. 2012). Recent case-control studies implicate TRPM2 conferring risk for bipolar disorder (BD) and genetic variants of TRPM2 have been identified to be coupled with BD supporting a role for this channel in the pathogenesis of this disorder (Xu et al. 2009) (see for a review Chahl 2007).

3.4 Lessons from KO Mice

In the absence of a clear and selective pharmacology of TRP channels, TRP deficient mice remain the gold standard for delineating their functional role in neurons, and their possible contribution to disease states. Another possibility is the use of inbred mice with acquired mutations, which display a neurological

phenotype which can be delineated to a mutation in a specific gene. An interesting example for this approach is TRPC3.

In *Trpc3*$^{-/-}$ mice it has been shown that slow synaptic potentials, which are associated with metabotropic glutamate receptor mediated activation of an inward cation current are absent in cerebellar purkinje cells. This is associated with impaired walking behavior and suggests that defects in TRPC3 could contribute to impaired motor control and coordination also in human patients. Interestingly, shortly thereafter a mouse line was identified from a large-scale phenotype-driven mutagenesis, the Moonwalker mouse, which displays severe motor and coordination defects, including impaired gait and balance. Genome sequencing revealed that these mice have mutation in the *trpc3* gene, which allegedly makes the channel more active. Thus, a gain of function and a loss of function of the same channel leads to similar defects in mice. Intriguingly, the gain-of-function mutant in the Moonwalker mice is associated with increased Purkinje cell loss and altered dendritic development, as displayed by decreased dendritic length and arborisation. Thus, one could unify these data by appreciating the loss of a depolarizing current in the KO mice, which leads to defect in mGluR signaling, and realizing that the gain of function mutant will disturb the normal Ca^{2+} and Na^{+} homeostasis at the developing dendrites which will lead to developmental abnormalities (Trebak 2010).

Interestingly, in another mouse model of cerebellar ataxia, the staggerer mouse, there was also a link with defective mGlu-TRPC3 signalling. Staggerer mutant mice have a functional loss of a transcription factor, Retinoid-related Orphan Receptor alpha (RORalpha), which is abundantly expressed in Purkinje cells (PCs) of the cerebellum. Homozygous staggerer (sg/sg) mice show cerebellar hypoplasia and congenital ataxia. Sg/sg mice serve as an important extreme mouse model of the hereditary spinocerebellar ataxia type 1 (SCA1), since it has been shown that RORalpha dysfunction is strongly correlated with SCA1 pathogenesis. The prominent synaptic dysfunction in these mice is that sg/sg mice lack metabotropic glutamate receptor (mGluR)-mediated slow EPSCs completely. Western blot analysis in the sg/sg cerebellum revealed expression of mGluR1 and TRPC3, both of which underlie mGluR-mediated slow currents in WT PCs. Immunohistochemical data demonstrated marked mislocalization of mGluR1 on sg/sg PCs. These results suggest that disruption of mGluR signalling at PF-PC synapses is one of the major synaptic defects in sg/sg mice and may manifest itself in SCA1 pathology and cerebellar motor control in general (Mitsumura et al. 2011).

3.5 Lessons from Human Disease

Until now, only one TRP channel has been linked causally with a human neuronal disease. Indeed, mutations in the TRPML1 gene are responsible for the development of the devastating lysosomal storage disease disorder Mucolipidosis type IV. Lysosomal storage diseases (LSDs) are caused by inability of cells to process the material captured during endocytosis (Kiselyov et al. 2010, 2011).

TRPML1, TRPML2 and TRPML3 belong to the mucolipin family of the TRP superfamily of ion channels. The founding member of this family, TRPML1 was cloned during the search for the genetic determinants of the lysosomal storage disease mucolipidosis type IV (MLIV). Mucolipins are predominantly expressed within the endocytic pathway where they appear to regulate membrane traffic and/or degradation of lysosomal storage vesicles. The physiology of TRPML proteins raises some of the most interesting questions of the modern cell biology. Their traffic and localization is a multi-step process involving a system of adaptor proteins, while their ion channel activity possibly exemplifies the rare cases of regulation of endocytic traffic and hydrolysis by ion channels (Puertollano and Kiselyov 2009).

Mucolipidosis type IV arises from mutations in TRPML1 (Bargal et al. 2000, 2001; Bassi et al. 2000; Slaugenhaupt 2002). The two other members, TRPML2 and TRPML3 multimerize with TRPML1, are involved in TRPML1 distribution and trafficking. TRPML1 functions as a Ca^{2+} and iron release channel in lysosomes (Dong et al. 2010; Shen et al. 2012). The pathogenic mechanism by which loss of TRPML1 leads to abnormal cellular storage and neuronal cell death is however still poorly understood. Yeast two-hybrid and co-immunoprecipitation experiments identified interactions between TRPML1 and Hsc70 as well as TRPML1 and Hsp40. Hsc70 and Hsp40 are members of a molecular chaperone complex required for protein transport into the lysosome during chaperone-mediated autophagy (CMA). Fibroblasts from MLIV patients show a defect in CMA in response to serum withdrawal. This defect in CMA was subsequently confirmed in purified lysosomes isolated from control and MLIV fibroblasts. The amount of lysosomal-associated membrane protein type 2A (LAMP-2A) is reduced in lysosomal membranes of MLIV fibroblasts. As a result of decreased CMA, MLIV fibroblasts have increased levels of oxidized proteins compared to control fibroblasts. Mechanistically, TRPML1 may act as a docking site for intralysosomal Hsc70 allowing it to more efficiently pull in substrates for CMA. It is also possible that TRPML1 channel activity may be required for CMA (Venugopal et al. 2009). More specifically, it was suggested that TRP-ML1 modulates postendocytic delivery to lysosomes by regulating interactions between late endosomes and lysosomes (Miedel et al. 2008).

Lysosomal lipid accumulation, defects in membrane trafficking and altered Ca^{2+} homoeostasis are common features in many lysosomal storage diseases. Interestingly, in fibroblasts from patients with another lysosomal storage disorder, Nieman Pick syndrome (NP), it was shown that sphingomyelins accumulate in lysosomes. Sphingomyelins (SMs) are plasma membrane lipids that undergo sphingomyelinase (SMase)-mediated hydrolysis in the lysosomes of normal cells. Patch-clamp analyses revealed that TRPML1 channel activity is inhibited by SMs, but potentiated by SMases. In NP-type C cells, increasing TRPML1's expression or activity was sufficient to correct the trafficking defects and reduce lysosome storage and cholesterol accumulation. Thus, it was proposed that abnormal accumulation of luminal lipids causes secondary lysosome storage by blocking TRPML1- and

Ca^{2+}-dependent lysosomal trafficking, which might be a common feature in lysosomal storage disorders (Shen et al. 2012).

Finally, a Drosophila model with a defective *Trpml* gene recapitulates the key disease features, including abnormal intracellular accumulation of macromolecules, motor defects and neurodegeneration. The basis for the buildup of macromolecules was defective autophagy, which resulted in oxidative stress and impaired synaptic transmission. Late-apoptotic cells accumulated in *trpml* mutant brains suggesting diminished cell clearance. The accumulation of late apoptotic cells and motor deficits could be rescued by expression of $trpml^{+}$ in neurons, glia or hematopoietic cells. Thus, from this model it was concluded that the neurodegeneration and motor defects result primarily from decreased clearance of apoptotic cells, and it was suggested that bone marrow transplantation may limit the progression of MLIV, hematopoietic cells in humans are involved in clearance of apoptotic cells (Venkatachalam et al. 2008).

4 Conclusion

TRP channels are relatively new membrane proteins that are involved in a plethora of cell functions and are mainly appreciated as sensory ion channels. This review maps TRP channels as important players in the function of our brain including the forming of hard-wired connections in our developing brain by growth cone guidance, regulation of synaptogenesis, spine forming and modulation of synaptic plasticity. This new view on the function of TRP channels in our central nervous system has already identified some of these channels as potential pharmaceutical targets and has led to a new understanding of several brain diseases. However, we have just entered a new era of neurophysiology and we anxiously await exciting discoveries in a rapidly expanding field of brain research.

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The Channel Physiology of the Skin

Attila Oláh, Attila Gábor Szöllősi, and Tamás Bíró

Abstract During embryonic development, the skin, the largest organ of the human body, and nervous system are both derived from the neuroectoderm. Consequently, several key factors and mechanisms that influence and control central or peripheral nervous system activities are also present and hence involved in various regulatory mechanisms of the skin. Apparently, this is the case for the ion and non-ion selective channels as well. Therefore, in this review, we shall focus on delineating the regulatory roles of the channels in skin physiology and pathophysiology. First, we introduce key cutaneous functions and major characteristics of the channels in question. Then, we systematically detail the involvement of a multitude of channels in such skin processes (e.g. skin barrier formation, maintenance, and repair, immune mechanisms, exocrine secretion) which are mostly defined by cutaneous non-neuronal cell populations. Finally, we close by summarizing data suggesting that selected channels are also involved in skin diseases such as e.g. atopic dermatitis, psoriasis, non-melanoma cancers and malignant melanoma, genetic and autoimmune diseases, etc., as well as in skin ageing.

List of Abbreviations

5-HT	5-Hydroxytryptamine
ACh	Acethylcholine
ACTH	Corticotropin
AD	Atopic dermatitis
AML	Antimicrobial lipid
AMP	Antimicrobial peptide

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AMPA(R)	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (receptor)
AQP	Aquaporin
ATP	Adenosine-5′- triphosphate
BCC	Basal cell carcinoma
BK	Large conductance Ca^{2+}-activated K^+-channel
Ca_v	L-type voltage-gated Ca^{2+}-channels
$[Ca^{2+}]_e$	Extracellular Ca^{2+}-concentration
$[Ca^{2+}]_i$	Intracellular Ca^{2+}-concentration
CCL	Chemokine ligand
CGRP	Calcitonin gene-related peptide
ChAT	Choline-acetyltransferase
CNG channels	Cyclic nucleotide-gated channels
COX	Cyclooxygenase
CRH	Corticotropin releasing hormone
CVI	Chronic venous insufficiency
Cx	Connexin
CXCL	CXC chemokine ligand
CXCR	CXC chemokine ligand receptor
DD	Darier's disease (keratosis follicularis)
DMBA	Dimethylbenz[a]anthracene
EGF	Epidermal growth factor
ENaC	Amiloride-sensitive Na^+ channels
GABA	Gamma-aminobutyric acid
HS	Hidradenitis suppurativa (acne inversa)
ICAM-1	Intercellular adhesion molecule-1
IK1	Intermediate conductance K_{Ca}
IL	Interleukin
I-RTX	5′-Iodoresiniferatoxin
K_{2P}	Two-pore K^+-channels
K_{Ca}	Ca^{2+}-activated K^+-channels
K_{ir}	Inward rectifier K^+-channels
LPS	Bacterial lipopolysaccharide
mAChR	Muscarinic acethylcholine receptor
mGluR	Metabotropic glutamate receptor
MMP-1	Matrix metalloproteinase-1
MUFA	Monounsaturated fatty acid
nAChR	Nicotinic acethylcholine receptor
Na_v	Voltage-gated Na^+-channels
NGF	Nerve growth factor
NHEK	Normal human epidermal keratincyte
NK-1 (receptor)	Neurokinin-1 (receptor)
NMDA(R)	N-methyl-D-aspartate (receptor)

NMF(s)	Natural moisturizing factor(s)
NO	Nitric oxide
PCNA	Proliferating cell nuclear antigen
PG	Prostaglandin
PPP	Palmoplantar pustulosis
SCC	Squamous cell carcinoma
SLURP-1	Secreted mammalian Ly-6/uPAR-related protein 1
SP	Substance P
str.	Stratum (layer)
TLR	Toll-like receptor
TNFα	Tumor necrosis factor alpha
TRH	Thyreotropin releasing hormone
TRP	Transient receptor potential
TRPA	"Ankyrin" subfamily of the TRP channels
TRPC	"Canonical" or "classical" subfamily of the TRP channels
TRPM	"Melastatin" subfamily of the TRP channels
TRPML	"Mucolipin" subfamily of the TRP channels
TRPP	"Polycystin" subfamily of the TRP channels
TRPV	"Vanilloid" subfamily of the TRP channels
TSH	Thyreotropin
TSW	Avène thermal spring water

1 The Skin and Its Key Functions

The skin is the largest barrier of the human body which protects the internal organs from various effects of the external environment, such as temperature changes, mechanic impacts, UV radiation and harmful pathogens. However, the skin is also our largest neuro-immuno-endocrine organ as it actively participates in the regulation of the body's water content, body temperature and possesses a multitude of sensory, endocrine, and immune functions. Below, we introduce key aspects of cutaneous physiology (for details see Bukowsky 2010; Draelos and Pugliese 2011).

1.1 The Functional Anatomy of the Skin

The skin is the largest organ of the integumentary system (the organ system that protects the body from damage) and is composed of multiple layers and cell types.

Epidermis: The outermost layer of the skin is made of keratinocytes (providing the waterproofing and serving as key components of the "active" skin barrier); Merkel cells operating as mechanoreceptors; melanocytes which define skin color

by the complex melanogenesis; and Langerhans cells which are professional antigen-presenting cells of the skin immune system. In addition, afferent nerve endings for the sensation of touch, pressure, temperature as well as pain and itch also reach the epidermis.

Dermis: The middle layer of the skin is a dense connective tissue composed of extracellular matrix components (collagens and elastic and reticular fibers) produced mainly by dermal fibroblasts. It is supplied by blood and lymphatic vessels and is densely innervated by both sensory afferent as well as motor efferent (which participate e.g. in vasoregulation) nerve fibers establishing a complex neuronal network. Of further importance, the pilosebaceous unit (hair follicles and sebaceous glands) and other appendages (sweat glands) are also located in this compartment.

Hypodermis (or subcutis): The lowermost layer of the skin is formed by adipocytes, fibroblasts, and macrophages. Similar to the dermis, it is also supplied by blood vessels and nerves.

1.2 Key Functions of the Skin

The various cell types of the skin layers form complex, multicellular communication networks, the proper function of which establishes the physiological skin homeostasis. These homeostatic mechanisms can be classified to three groups, i.e. barrier functions, neuroendocrine functions, and other functions (Fig. 1).

1.2.1 Barrier Functions

Possibly the most important function of the skin is the formation of the barrier (extensively reviewed in Elias and Feingold 2006). For a long time, it was believed that it is a "passive" function that originates from the unique structural features and the special anatomical properties of the skin. However, in the last few decades, it became increasingly accepted that the different types of cutaneous cells possess very important functions in generating a coordinated, "active" protection, thus forming a true first line of defense against the harmful impacts of the external environment such as e.g. physical environmental challenges (UV, temperature), microbial invasions, allergens, chemical irritants, etc.

The barrier exhibits a complex nature; hence, we can distinguish among different levels of protection (Fig. 2). Yet, the different levels constantly communicate and coordinate their actions to be able to act according to the following "needs":

- "Keep the barrier intact"
- "Moisturize: attract and keep the water"
- "Should the barrier be destroyed, regenerate and repair it"
- "Let the valuable things penetrate the skin, both upward and downward"
- "Do not let the bad things invade the skin and the body"
- "Should the bad things penetrate, fight and destroy them"

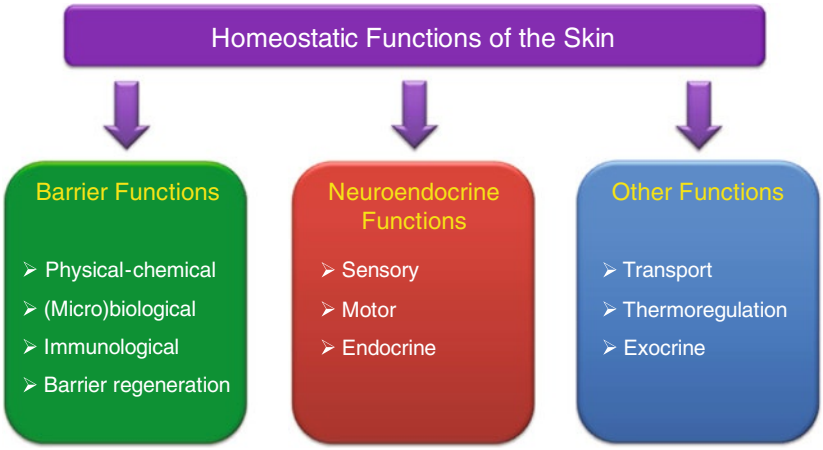

Fig. 1 *Key functions of the skin*. The multiple homeostatic functions of the skin can be classified into the groups of barrier, neuroendocrine, and other functions. For further details, see text under Sect. 1.2

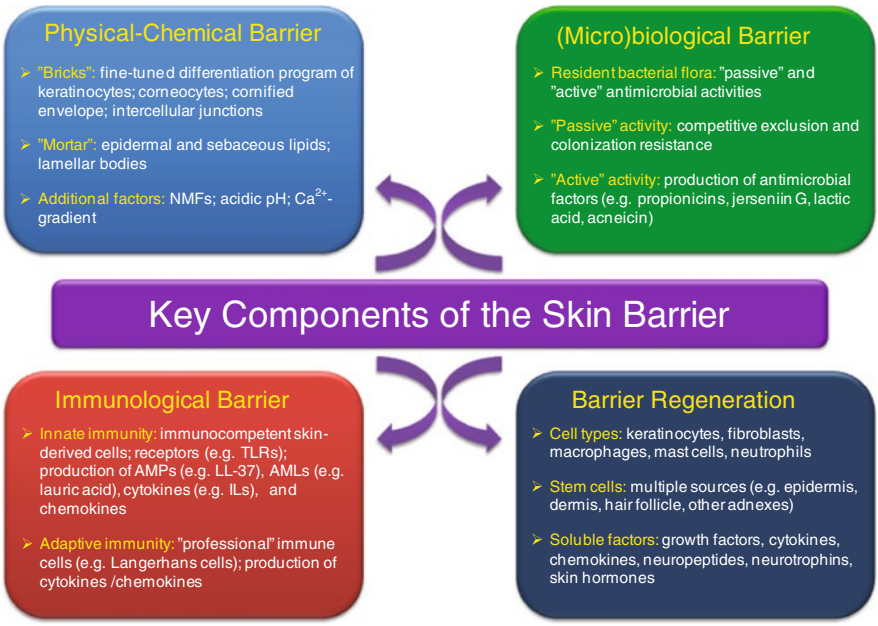

Fig. 2 *Key components of the complex skin barrier*. The highly complex skin barrier provides multiple levels of protection for the organism. These include the physical-chemical barrier, the (micro)biological barrier, and the immunological barrier as well as the life-long regeneration of these components. For further details, see text under Sect. 1.2.1

The Physical-Chemical Barrier

The key components of the outermost physical/mechanical barrier are the keratinocytes of the epidermis. During the course of their life-long, apoptosis-driven, physiological differentiation program, as they move "upward" from the deepest basal layer through the spinous (str. spinosum) and granular (str. granulosum) layers, their permeability to Ca^{2+} increases and the resulted elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) activates peptidases and convert pro-filaggrin into filaggrin. Filaggrin then aggregates various cytokeratins and other intermediate filaments in the superficial cells which, after they have become anucleated (corneocytes), generate the solid mechanical/physical shield, i.e. the str. corneum, which is considered as the "real physical barrier" (Madison 2003; Proksch et al. 2008; Jensen and Proksch 2009; Rawlings 2010).

In the str. corneum, each terminally differentiated corneocyte is surrounded by a protein shell called the cornified envelope. This highly insoluble structure – which is a product of (again) Ca^{2+}-dependent processes involving e.g. keratinocyte-specific transglutaminases – is composed of mainly loricrin and involucrin which form extensive links between each other and other filamentous structures of the cells (such as the above filaggrin and cytokeratins). In addition, a further stabilization of the corneocyte barrier is provided by corneodesmosomes, gap junctions, and other intercellular junctions formed by junctional proteins such as e.g. desmogleins, cadherins, envoplakin, etc. Importantly, the produced filaggrin (and possibly other proteins) will eventually be degraded in the corneocytes. The resulted amino acids will then be used to synthesize natural moisturizing factors (NMFs) which, due to their hygroscopic (water-holding) features, provide the proper hydration of the epidermis and hence, as a "mechanical shaping factor", establish another key component of the physical barrier (Madison 2003; Proksch et al. 2008; Jensen and Proksch 2009; Rawlings 2010).

As a "morphological metaphor", corneocytes can therefore be imaged as "bricks in the wall" to form the physical/mechanical barrier. It is common knowledge, however, that "bricks cannot be stabilized without a proper mortar"; in the skin, the mortar is formed by lipids of the epidermis. Indeed, in the lower spinous and granular layers, lipid-containing lamellar bodies are formed in the keratinocytes. During the maturation of keratinocytes towards the str. corneum, various (again, Ca^{2+}-dependent) enzymes degrade the outer envelope of the lamellar bodies thereby releasing (via exocytosis) their content to the interstitial space at the border of the str. granulosum and corneum. This process results in the establishment of the physical/chemical "mortar", i.e. the continuous intercellular lipid layers of the epidermis. It should also be noted that involucrin and other cell-cell junction proteins of the corneocytes also serve as substrates for the covalent attachment of ceramide derivates resulting in the corneocyte-bound lipid envelope which binds both to the cornified (protein) envelope and also to the intercellular lipid lamellae. Therefore, the constantly produced lipids (i.e. cholesterol, ceramides, and free fatty acids) – which are further supplemented by the high lipid content of the sebum, produced and released (to the skin surface) by the sebaceous glands – not only stabilize the "bricks", but also

provide additional waterproofing and physical protection to the skin (Elias and Feingold 2006; Proksch et al. 2008; Rawlings 2010).

Of great importance, the epidermal lipids also contribute to other "chemical" cutaneous homeostatic mechanisms such as e.g. setting the acidic pH. Furthermore, epidermal keratinocytes and sebocytes actively secrete additional factors exhibiting antimicrobial properties. These include (1) antimicrobial peptides (AMPs) such as e.g. the small cationic molecules defensins (which insert to bacterial walls and hence form "lethal" pores), LL-37 cathelicidin, cathepsins, etc.; and (2) antimicrobial lipids (AML) such as saturated (e.g., lauric acid, $C_{12:0}$) and unsaturated (e.g., monounsaturated MUFA sapienic acid, $C_{16:1\Delta6}$) fatty acids (Gallo and Huttner 1998; Bardan et al. 2004; Braff and Gallo 2006; Niyonsaba et al. 2009; Tóth et al. 2011b). The AMPs and AMLs not only strengthen the chemical defense of the skin but, as members of the innate immunity, contribute to the complex inflammatory/immune processes organized by the skin immune system (see also below).

The (Micro)biological Barrier

Similar to other barriers seen in various body parts, the skin also has a rich resident, commensal bacterial flora including e.g. *Propionibacterium acnes* and *Staphylococcus epidermidis* (Gallo and Nakatsuji 2011; Kranich et al. 2011; Littman and Pamer 2011). Traditionally, it was suggested, that these microbes have a relatively passive function; they populate their niches and "use up" the available food sources hence making it more difficult (if not impossible) for the infection and colonization of pathogenic microbes (this process is referred to as competitive exclusion) (Rioux and Fedorak 2006). However, the "commensal" relationship (i.e. beneficial for the bacteria yet mostly neutral for the skin) has recently been revisited and a rather "symbiotic" (i.e. mutually beneficial) association has been suggested. Indeed, it was recently shown that bacteria of the normal skin flora (including e.g. *Propionibacterium acnes*) secrete factors (e.g. propionicins, jenseniin G, acneicin, lactic acid) that possess bacteriostatic or even antibacterial properties against certain pathogenic strains (e.g. some Gram-negative bacteria, yeasts and molds) (Faye et al. 2000; Miescher et al. 2000; Cogen et al. 2008). In addition, the skin commensal flora also seems to exert a continuous and dynamic action on the skin immune system; indeed, resident bacteria were shown to modulate AMP production of keratinocytes as well as cytokine production of other cutaneous immunocompetent cells (see also below) (Gallo and Nakatsuji 2011). Finally, it should also be noted that the constant physiological desquamation of the "dead" corneocytes not only strengthens the physical and biological barriers but also makes it difficult for the pathogenic microorganisms to establish permanent colonies.

The Immunological Barrier

Various immunocompetent cells and humoral factors establish the skin immune system (reviewed in Bos and Kapsenberg 1993; Kupper and Fuhlbrigge 2004). As immune cells, resident and infiltrating phagocytic cells, natural killer cells, mast

cells, professional antigen-presenting cells (i.e. epidermal Langerhans cell, dermal dendritic cell) as well as T and B lymphocytes are localized in various skin compartments. In addition, a plethora of cytokines, chemokines, and other inflammatory mediators, as well as the aforementioned AMPs and AMLs, are synthesized in and hence released from practically all cell types of the skin. Therefore, upon infections, allergen exposure or barrier rupture, these innate and adaptive immunity components are co-activated to induce an orchestrated inflammatory and immune response (reviewed in Girardi 2007; Nestle et al. 2009; Takeuchi and Akira 2010).

Of further importance, keratinocytes and sebaceous gland-derived sebocytes – which, as shown above, play key roles in the establishment of the physical-chemical barrier – were introduced as additional sentinels of the skin immune system. This immune role is attributed not only to their production of AMPs and AMLs and the antimicrobial sebum (see above), but also to their capability to recognize external pathogens via the functional expression of all sorts of pathogen recognition receptors, including various members of Toll-like receptor family (TLRs), i.e. TLR1-6 and 9 (Pivarcsi et al. 2003; Miller 2008; Terhorst et al. 2010; Tóth et al. 2011b). Activation of these receptors by various pathogenic microbes, via the release of numerous pro-inflammatory agents, leads to the initiation of active defense mechanisms, and as a result, adaptive and innate immune events are launched (Pivarcsi et al. 2004; Kurokawa et al. 2009).

The Barrier Regeneration

As was shown above, the proper formation, maintenance, and function of the physical-chemical epidermal barrier depends on the constant proliferation–differentiation turnover of epidermal keratinocytes (and, via sebum production, of sebocytes). Upon disruption of the epidermal barrier – which, experimentally, can be performed by e.g. chemical agents (acetone, detergents), UV exposure, or mechanical tape stripping, which removes the corneocytes (Pinkus 1951) – the aforementioned processes are accelerated due to the active contribution of engaged keratinocytes and sebocytes in response to various agents released from the damaged cells (Proksch et al. 2008; Rawlings 2010).

However, skin injuries very often reach the deeper skin layers resulting in a much more complex response which can be exemplified by the wound healing processes (Epstein 1999). Indeed, during the multiple phases of wound healing (e.g. coagulation, inflammation, proliferation, remodeling) numerous cutaneous cell types (including infiltrating macrophages and polymorphonuclear neutrophils, microvascular endothelial cells, dermal fibroblasts, epidermal keratinocytes) are activated and their cell-specific proliferation–migration–differentiation programs are initiated (Enoch and Leaper 2005; Reinke and Sorg 2012). Of further importance, wound healing is not possible without the active contribution of intracutaneous stem cells located in various cutaneous compartments including e.g. the epidermis, sebaceous and sweat glands, and, possibly most importantly, in the hair follicles (Tiede et al. 2007; Lau et al. 2009). It should also be noted that

cellular regeneration programs and stem cell activities are orchestrated by a multitude of locally generated (by the above cell types), soluble mediators (e.g. growth and trophic factors, cytokines, chemokines, neuropeptides, neurotrophins, hormones) and concomitant changes in the expressions of cell surface molecules (e.g. receptors, adhesion molecules, integrins) recognizing these agents (Werner and Grose 2003; Gurtner et al. 2008; Koh and DiPietro 2011).

Therefore, the delicate balance of cell/organ proliferation, survival, death, differentiation, and mediator production of practically all non-neuronal cell populations of the skin collectively establish the "life-long" regeneration and rejuvenation of the tissue and hence enables the skin barrier homeostasis (Gurtner et al. 2008; Reinke and Sorg 2012).

Related Cutaneous Diseases

In light of the central role of barrier functions in skin biology, it is not surprising at all that impairment of the aforementioned balance results in pathological barrier formation and maintenance which eventually lead to the development of skin diseases. These conditions include e.g. irritant and allergic contact dermatitis, burns, ulcers, etc. On the other hand, several skin immune abnormalities may secondarily impair the epidermal skin barrier such as seen e.g. in Mycosis fungoides and in the autoimmune pemphigus vulgaris. However, the consequences of the very often co-existing impaired skin barrier and cutaneous inflammatory/immune responses may establish positive feed-back loops. These autocatalytic mechanisms, in turn, result in the development of such high-prevalence, chronic inflammatory "barrier diseases" as the atopic dermatitis (AD) and psoriasis (reviewed in Proksch et al. 2008; Boguniewicz and Leung 2011).

Finally, skin tumors should also be mentioned. As in many organs, defective differentiation and/or uncontrolled proliferation of cutaneous cells may lead to the development of tumors. In the skin, non-melanoma skin cancers, i.e. basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), and malignant melanomas establish the major groups of tumors with increasingly growing incidence (Samarasinghe and Madan 2012; Tremante et al. 2012). It is also noteworthy that stem cells which otherwise play key roles in wound healing are also implicated in skin tumor formation. Since wound repair and tumorigenesis both depend on intracutaneous communication networks of skin cells, the proper control of the inter- and intracellular signaling pathways are of key importance in successfully preventing tumor formation (Arwert et al. 2012).

1.2.2 Neuroendocrine Functions

Since this review will mainly focus on the roles of ion and non-ion selective channels expressed by non-neuronal cells, below, we only briefly summarize the neuroendocrine and other functions of the skin.

Sensory functions: Besides establishing the complex cutaneous barrier against constant environmental challenges, the skin simultaneously operates as the largest sensory organ of the vertebrate body (reviewed in Roosterman et al. 2006; Slominski et al. 2008). Indeed, all skin compartments are densely innervated by sensory afferent fibers specialized for the sensation and then neuronal processing of mechanical signals (touch, pressure, vibration), osmotic and thermal (heat, cold) challenges, chemical agents as well as noxious and pruritogenic stimuli inducing pain and itch, respectively (reviewed in Ansel et al. 1997; Paus et al. 2006a; Roosterman et al. 2006; Fuchs and Horsley 2008). However, the activation of these sensory neurons not only induce the classical, ortho-dromic transmission of the signals (i.e. generation and propagation of action potentials) to the central nervous system, but may also result in the anti-dromic release of certain neuropeptides (such as substance P [SP] and calcitonin gene-related peptide [CGRP]) (Ansel et al. 1997; Luger 2002). As the "efferent" functions of the sensory afferents, these neuropeptides may act on cutaneous non-neuronal cell types and exert local immuno-endocrine, vasoregulatory, and trophic actions. In addition, sensory stimuli as well as the released neuropeptides may induce the liberation of a plethora of mediators from non-neuronal cells which, vice versa, may act on the sensory nerve endings. Of further importance, the local, intracutaneous accumulation of these mediators may also act on other non-neuronal cell types of the skin and hence may alter their proliferation–differentiation status (Ansel et al. 1997; Luger 2002; Paus et al. 2006a, b; Peters et al. 2007; Fuchs and Horsley 2008). Therefore, the established multi-directional, multi-cellular communication networks not only participate in the aforementioned formation and maintenance of the complex physical and immunological cutaneous barriers but also significantly modulate skin sensation processes ("sensory roles" of the non-neuronal cells) (Bíró et al. 2007; Denda et al. 2007a; Denda and Tsutsumi 2011; Lee and Caterina 2005; Fernandes et al. 2012).

Motor functions: The skin is also supplied by "truly" efferent fibers which belong to the somatomotor group. These sympathetic and parasympathetic nerves control e.g. cutaneous vasoregulation (dilation or constriction of blood vessels), piloerection, skin metabolic activities, exocrine functions, etc. (see also below) (Hodges and Johnson 2009).

Endocrine functions: The skin is also our largest endocrine organ (Roosterman et al. 2006; Slominski et al. 2008). Indeed, the skin not only responds to the actions of circulating hormones but various cutaneous cells and tissues themselves produce a wide-array of hormones. Intriguingly, two peripheral equivalents of central hypothalamic – pituitary – target organ axes, i.e. Corticotropin Releasing Hormone (CRH) – Corticotropin (ACTH) – Cortisol; Thyrotropin Releasing Hornone (TRH) – Thyrotropin (TSH) – Thyroxine, are functionally expressed in the skin (Arck et al. 2006; Slominski et al. 2008; Bodó et al. 2010; Poeggeler et al. 2010; Ramot et al. 2011; Knuever et al. 2012). These, mostly locally released and acting hormones, on the one hand, provide additional humoral components to the multicellular networks regulating multiple skin functions. On the other hand, these hormones also act as active members of the intracutaneous "stress response system" which, via systemic neuro-endocrine mechanisms, keeps continuous contact with its central counterpart, thereby establishing the "brain-skin connection" (Arck et al. 2006;

Paus et al. 2006b). In addition to the above hormones, certain skin cells express the full enzymatic machinery to synthesize e.g. vitamin D, testosterone, and estrogens which mostly control local events of growth, differentiation, and metabolism of non-neuronal skin cells (Schmuth et al. 2007; Zouboulis et al. 2007; Slominski et al. 2008; Tóth et al. 2011a).

1.2.3 Other Functions

Transport functions: The proper barrier enables the up- and downward transport of respiratory gases, nutrients as well as topically applied products (pharmaceuticals, cosmeceuticals) between skin layers (Lademann et al. 2011).

Thermoregulatory functions: The skin plays multiple roles in thermoregulation. With the subcuticular adipose tissue (which is cca. 50 % of body fat), the skin is the major thermal insulator of the body. In animals, insulation is further supported by neuronal piloerection. In addition, the aforementioned neuronal and humoral vasoregulatory mechanisms (vasodilation, vasoconstriction) regulate the large cutaneous blood supply and thereby precisely control direct heat losing mechanisms (i.e. radiation, convection and conduction). Finally, evaporation (both insensible via skin pores and sensible via sweating) and its control by neuronal and humoral actions are also related to the skin (Johnson 2010; Nakamura 2011; Pitoni et al. 2011).

Exocrine functions: Skin appendages produce and release (to the skin surface) of sweat and sebum which exocrine products, as mentioned above, participate e.g. in thermoregulation, physical-chemical barrier formation, antimicrobial activity, etc.

2 A Short Introduction of Ion and Non-ion Selective Channels

The channels are pore proteins found in various (surface, intracellular) membranes of the cells. They are specialized for the passive transport of certain molecules between the cellular compartments separated by the membranes in which they are located.

Below we summarize the major channel groups and shortly introduce their key characteristics, with special emphasis on those which have regulatory roles in skin physiology. For the functional classification of channel proteins, we used the International Union of Basic and Clinical Pharmacology (IUPHAR) database. For details and references, please visit the IUPHAR website (http://www.iuphar-db.org) and corresponding textbooks of Physiology and Pharmacology.

2.1 Ion Channels

Via these membrane pores, certain ions are transported (selectively or non-selectively) along their electrochemical gradients. The classification of the ion channels is mainly based on their gating characteristics (i.e. the energy form of

the stimulus that opens or, rarely, closes the given channel) and other properties. Yet, it should be emphasized that certain channels exhibit "mixed" gating features; e.g. we will mention such channels whose opening could be equally regulated by binding of the respective ligands, certain voltages, and other factors.

2.1.1 Voltage-Gated Ion Channels

Like most of the ion channels, voltage-gated pores – whose gating properties are mainly regulated by alterations in the membrane potentials – were originally described on excitable cells (i.e. various neurons and muscle types) as key molecules involved in the generation of action potentials. However, it also became apparent that, besides this electrogenic role, they additionally participate in a multitude of other cellular functions not only on excitable but also on non-excitable cells. These mechanisms (as will be detailed below) involve, among others, secretion of various mediators, regulation of intracellular ionic homeostasis, cellular growth and differentiation, immune response, etc. With respect to the skin, voltage-gated Na^+ channels (Catterall et al. 2012a), Ca^{2+} channels (Catterall et al. 2012b), K^+ channels (Gutman et al. 2012a) as well as Ca^{2+} activated K^+ channels (Gutman et al. 2012b), two-pore domain K^+ channels (Plant et al. 2012), and cyclic nucleotide-gated (CNG) non-selective cationic channels (Biel et al. 2012) are of greatest importance.

2.1.2 Ligand-Gated Ion Channels

The common feature of these channels is that they are gated by binding of (more or less) specific and/or selective ligands to the respective binding sites. Actually, they function as ionotropic receptors for neurotransmitters, neuromodulators, hormones, and other mediators participating in autocrine, paracrine and endocrine intercellular communication mechanisms. Similar to other ion channels, these receptors were first described on neurons and only lately on non-neuronal cells of the body. These channels "signal" mostly via modulating the intracellular ionic homeostasis of their host cells which, in turn, initiates various downstream signal transduction pathways including alterations of activities of e.g. kinase systems, enzymes and factors involved in the regulation of gene expression, cellular metabolic enzymes, etc.

Within this group, below, we review the cutaneous impact of the following ligand-gated channels:

Ionotropic cholinergic receptors: Nicotinic (nAChR) and muscarinic (mAChR) cholinergic receptors are specialized for mediating the cellular actions of acetylcholine (ACh), a key neurotransmitter and mediator. Among them nAChRs function as ligand-gated channels whereas mAChRs are seven-transmembrane (7-TM) G-protein-coupled receptors. Human nAChRs are composed of different subunits, i.e. α1–α10, β1–β4, γ, δ, and ε which can be combined to pharmacologically distinct, homo- or heteropentameric, non-selective cationic channels (Millar et al. 2012).

Ionotropic glutamate receptors: Glutamate may act on metabotropic 7-TM (mGluR) or various ionotropic receptors. Within the latter group, the following non-selective cationic channels can be distinguished: N-methyl-D-aspartate receptors (NMDAR) exhibiting high permeability for Ca^{2+}; α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR); and kainate receptors (Peters et al. 2012).

Ionotropic purinergic receptors: Extracellular ATP may exert its cellular actions by binding to P2Y 7-TM metabotropic and P2X ionotropic purinergic receptors. So far, seven P2X receptors are identified; all of them function as non-selective, mostly Ca^{2+}-permeable cationic channels (Khakh et al. 2001; Evans et al. 2012).

Ionotropic 5-hydroxytryptamine receptors: Among the multiple 5-hydroxytryptamine (5-HT) receptors, only 5-HT3 receptors operate as ligand-gated, cation-selective, pentameric ion channels (Lummis et al. 2012).

Ionotropic gamma-aminobutyric acid receptors: Gamma-aminobutyric acid (GABA) signals via ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors. $GABA_A$ receptors are Cl^- selective, heteropentameric channels derived from seven main receptor subunits (α, β, γ, δ, ε, π and θ) (Olsen and Sieghart 2008; Olsen et al. 2012).

Glycine receptors: Similar to $GABA_A$ receptors, glycine receptors also function as pentameric Cl^- channels (Lynch 2012).

2.1.3 Transient Receptor Potential Ion Channels

Although the IUPHAR database classifies transient receptor potential (TRP) ion channels among the voltage-gated ones (Clapham et al. 2012), due to their "mixed" gating properties and, moreover, to their key roles in cutaneous physiology (see below), we decided to detail their characteristics under a separate subheading.

TRP ion channels exhibit intriguing "mixed" gating properties as they function as broadly expressed polymodal "cellular sensors" (Clapham 2003). Indeed, they can be equally activated and/or modulated by e.g. alterations in temperature and pH, osmolarity, ionic concentrations, endogenous mediators, external chemical irritants, membrane potential changes, etc. (Ramsey et al. 2006; Damann et al. 2008; Vriens et al. 2008, 2009). In addition, as we will see below, TRP channels not only act as "sensors", but also as key "effectors" of various physiological (and often pathophysiological) processes such as e.g. cellular homeostasis of different ions, secretory mechanisms, sensory functions of the nervous system, inflammation, proliferation, differentiation, cell survival, etc. (Nilius and Owsianik 2010; Denda and Tsutsumi 2011; Moran et al. 2011; Fernandes et al. 2012).

Up to date, 28 mammalian members have been identified which can be further classified into the subfamilies of the canonical (or classical, TRPC), the vanilloid (TRPV), the melastatin (TRPM), the mucolipin (TRPML), the polycystin (TRPP), and the ankyrin (TRPA) groups (Clapham et al. 2012). As detailed below, multiple TRPs participate in the regulation of skin functions.

2.1.4 Other Ion Channels

Within this group, we introduce the amiloride-sensitive, epithelial Na^+ channels (ENaC) which belong to the ENaC/degenerin ion channel family of genetically related glycoproteins. ENaC can be formed by different combinations of four homologous subunits, named ENaCα, β, δ, and γ (de la Rosa et al. 2000; Kellenberger and Schild 2002). The key unique feature of ENaC channels that they are mostly (if not exclusively) expressed on non-neuronal cells.

2.2 Non-ion Selective Channels

These membrane pores (which are, very often, also permeable for ions) enable the transport of various other molecules. Several members of the below families are involved in cutaneous functions.

2.2.1 Aquaporins

Aquaporins are a family of integral transmembrane proteins that facilitate osmotic fluid transport in numerous human tissues. They are involved in transepithelial and transcellular water movement, although more recent results point to their possible role in gas transport as well. Thirteen mammalian aquaporins have been identified to date, which can be classified into two groups; (1) aquaporin molecules that only transport water (AQP-1, AQP-2, AQP-4, AQP-5 and AQP-8) and (2) aqua-glyceroporin molecules that also transport glycerol and other small molecules such as lactic acid (AQP-3, AQP-7, AQP-9 and AQP-10) (Hara-Chikuma and Verkman 2008a).

2.2.2 Connexins

Connexins (Cx) are transmembrane proteins that homo- or heterosextamerize on the plasma membrane to form the hemi-channel connexons. Connexons on adjoining cells associate to form gap junctional channels, and allow the direct passage and exchange of ions, secondary messenger molecules (cAMP, IP_3), energy sources (ATP, GTP), reducing/oxidizing agents (glutathione) and nutrients (glucose, amino acids) between cells. Therefore, gap junctions are key molecules of cell–cell communications (Proksch et al. 2008; Xu and Nicholson 2012).

2.2.3 Pannexins

Pannexins (Panx) are mammalian orthologs of the invertebrate gap junction proteins innexins (Panchin et al. 2000). However, pannexins do not take part in the formation of gap junctions; rather they form single membrane channels in

cellular communication with the environment (Sosinsky et al. 2011). To date, three Panxs have been described: Panx1 appears to be ubiquitously expressed whereas Panx2 was mostly found in the adult brain. Panx3 expression was identified in osteoblasts, synovial fibroblasts, whole joints of mouse paws, and cartilage from the inner ear (Baranova et al. 2004) as well as in cartilage, the heart, and, of great importance, human skin (Penuela et al. 2007). Panx1 has been implicated in numerous cellular functions such as immune response, tumorigenesis, apoptosis, and ischemic cell death. In addition, Panx2 and Panx3 have been shown to take part in the differentiation and development of tissues which express these channels (reviewed in Penuela et al. 2012a).

3 Roles of Channels in Skin Physiology and in Certain Dermatoses

In this chapter, we provide an extensive review on the roles of various ion and non-ion selective channels in the regulation of certain functions of the skin. Since the involvement of a multitude of (mostly voltage-gated and TRP) ion channels in sensory neuron-coupled functions (such as e.g. thermosensation, pain, itch) are extensively detailed in numerous comprehensive reviews, below, we focus on defining the roles of the channels on non-neuronal cells (summarized in Table 1). In addition, we present data on the potential impact of these molecules in certain skin diseases (summarized in Table 2).

3.1 Roles of Channels in Epidermal Physical-Chemical Barrier Functions and Barrier Recovery

As we introduced above (under Sect. 1.2.1), the formation, maintenance, and recovery of the epidermal physical-chemical barrier are mainly determined by the proper, $[Ca^{2+}]_i$-dependent differentiation program of the epidermal keratinocytes resulting in the lipid-embedded layers of corneocytes. Therefore, in this chapter, we introduce roles of channels (which regulate intracellular ion homeostasis) in controlling growth, differentiation, and survival of keratinocytes. Moreover, we present findings of animal experiments aimed at defining rate of recovery after barrier insults. Finally, since sebaceous gland-derived sebum production is an additional factor of the chemical epidermal barrier, we also detail the related channel physiology of sebocytes.

3.1.1 Voltage-Gated Channels

Voltage-Gated Ca^{2+}-Channels

The main subunit of the L-type voltage-gated Ca^{2+} channels, $Ca_v\alpha_{1C}$, was identified in mouse and human epidermis in situ (Denda et al. 2006). In addition, functional

Table 1 Highly selected skin-related functions of certain designated channels

Channels	Putative function(s)	Reference(s)
Ca_v channels	Promotion of keratinocyte differentiation; delay of barrier recovery	Denda et al. 2003b, 2006
nAChRs	Promotion of keratinocyte differentiation and wound healing; delay of barrier recovery	Grando et al. 1996; Arredondo et al. 2003; Denda et al. 2003a; Kurzen et al. 2005; Chernyavsky et al. 2007; Kurzen et al. 2007; Radek et al. 2010; Curtis and Radek 2012
P2X7	Promotion of keratinocyte differentiation and antitumor effects	Slater et al. 2003; Inoue et al. 2005; Deli et al. 2007
NMDAR	Promotion of keratinocyte differentiation, delay of barrier recovery	Denda et al. 2003a; Fuziwara et al. 2003; Fischer et al. 2004a, b
$GABA_A$	Promotion of barrier recovery and wound healing	Denda et al. 2002b, 2003a; Han et al. 2007; Ito et al. 2007
Glycine receptor	Promotion of barrier recovery	Denda et al. 2003a
TRPV1	Delay of barrier recovery and wound healing	Bodó et al. 2005; Bíró et al. 2006; Denda et al. 2007b; Tóth et al. 2011a; Yun et al. 2011
	Antitumor effects	Bode et al. 2009
TRPV3	Promotion of barrier recovery and wound healing	Denda et al. 2007b; Cheng et al. 2010; Miyamoto et al. 2011
TRPV4	Promotion of barrier recovery	Denda et al. 2007b; Kida et al. 2011; Sokabe et al. 2010; Sokabe and Tominaga 2010
TRPA1	Promotion of barrier recovery	Denda et al. 2010b
TRPC1, 4, 5, 6, and 7	Promotion of keratinocyte differentiation and possible antitumor effects	Cai et al. 2006; Beck et al. 2008; Müller et al. 2008; Woelfle et al. 2010; Shanmugam et al. 2012
TRPM1, 2 and 8	Antitumor effects	Deeds et al. 2000; Duncan et al. 2001; Miller et al. 2004; Zhiqi et al. 2004; Orfanelli et al. 2008; Slominski 2008; Yamamura et al. 2008a; Lu et al. 2010
AQP3	Promotion of barrier recovery	Hara et al. 2002; Hara and Verkman 2003

Ca_v channels were found on cell cultures of normal human epidermal keratinocytes (NHEKs) (Denda et al. 2003b, 2006). Of great importance, in hairless mice, topical application of Ca_v channel antagonists (nifedipine and R-(+)-BAY K8644) to mechanically injured skin (tape stripping) accelerated barrier recovery whereas treatment with a Ca_v channel agonist (S-(−)-BAY K8644) delayed barrier repair (Denda et al. 2006). Likewise, topical application of Ca^{2+} on the skin after str. corneum barrier disruption delayed the recovery of the barrier which effect was prevented by the co-administration of the Ca_v channel antagonists nifedipine and verapamil (Lee et al. 1991). In good agreement with these findings, Ca_v channels were shown to mediate the effects of adrenergic β_2 receptor agonists to inhibit barrier repair (Denda et al. 2003b, 2006).

Table 2 Putative roles of key channels in the pathogenesis of selected human skin diseases and skin-related processes

Diseases or skin-related conditions	Putative pathomechanism(s) and Related channel(s)	Reference(s)
Psoriasis	Chronic use of Ca_v channels is associated with the disease	Cohen et al. 2001
	Upregulation of non-functional CNG channels	McKenzie et al. 2003
	Altered expression profile of 5-HT3	Lundeberg et al. 2002; Nordlind et al. 2006
	Downregulation of NMDAR1	Fischer et al. 2004b
	Upregulation of GABA ligand and $GABA_A$ receptor	Nigam et al. 2010
	Downregulation of TRPC1, 4, 5, 6, and 7 and the coupled Ca^{2+}-influx	Leuner et al. 2011
	Upregulation of AQP9	Suárez-Fariñas et al. 2011
	Upregulation of Cx26 which can be suppressed by anti-psoriasis therapy	Lucke et al. 1999; Shaker and Abdel-Halim 2012
	Irregular nAChR expression pattern	Curtis and Radek 2012
Atopic dermatitis	Altered P2X7 expression profile	Pastore et al. 2007
	Irregular nAChR subtype expression pattern	Curtis and Radek 2012
	Downregulation of NMDAR1	Kang et al. 2009
	Decreased GABA-ergic signaling	Hokazono et al. 2010
	Activation of TRPV1 and 3 results in AD-like syndromes in mice	Asakawa et al. 2006; Xiao et al. 2008; Yun et al. 2011
	Upregulation of AQP3	Olsson et al. 2006; Nakahigashi et al. 2011
Non-melanoma skin cancers	Upregulation of $K_v3.4$; inhibition of $K_v3.4$ suppressed tumor cell growth	Chang et al. 2003
	NMDAR1 expression inversely correlates with the degree of malignancy	Nahm et al. 2004; Kang et al. 2009
	Downregulation of P2X7-coupled signaling; altered P2X receptor expression pattern	Burnstock 2006;Greig et al. 2006; Gorodeski 2009; Burnstock et al. 2012
	Decreased TRPV1 and TRPC6-coupled signaling; TRPV1-KO mice exhibit increased tumorigenesis	Bode et al. 2009; Woelfle et al. 2010
	Downregulation of TRPC1 and 4	Beck et al. 2008
	Upregulation of AQP3; AQP3-KO mice exhibit decreased tumorigenesis	Hara-Chikuma and Verkman 2008c
Malignant melanoma	Increased activity of $K_{Ca}3.1$, K_{ir} and TASK-3	Lepple-Wienhues et al. 1996; Schmidt et al. 2010; Kosztka et al. 2011
	Upregulation of P2X7	Slater et al. 2003
	TRPM1 expression inversely correlates with the degree of metastatic potential; Upregulation of antisense TRPM2; Downregulation of TRPM8; Activation of TRPM8 inhibit tumor cell growth	Deeds et al. 2000; Duncan et al. 2001; Tsavaler et al. 2001; Miller et al. 2004; Zhiqi et al. 2004; Orfanelli et al. 2008; Lu et al. 2010

(continued)

Table 2 (continued)

Diseases or skin-related conditions	Putative pathomechanism(s) and Related channel(s)	Reference(s)
	Upregulation of Panx1 in mouse melanoma; Silencing of Panx1 inhibits tumor growth	Penuela et al. 2012b
Olmsted syndrome	The main pathogenetic cause is the gain-of-function mutation of TRPV3	Lin et al. 2012
Skin ageing	Altered K^+-channel expression profile (fibroblasts)	Zironi et al. 2010
	Altered nAChR expression profile (fibroblasts)	Arredondo et al. 2003
	Upregulation of P2X7 (keratinocytes)	Inoue et al. 2005
	Upregulation of TRPV1 and the coupled signaling (keratinocytes)	Lee et al. 2009a; Lee et al. 2012
	Downregulation of AQP3 (keratinocytes)	Li et al. 2010

Interestingly, in a retrospective case–control study, chronic (>2 years) intake of Ca_v channel blockers (nifedipine, felodipine, and amlodipine) was found to be significantly associated with both the exacerbation as well as the precipitation of new-onset psoriasis (Cohen et al. 2001), a skin disease with altered keratinocyte functions and impaired epidermal skin barrier (reviewed in Proksch et al. 2008; Boguniewicz and Leung 2011).

Collectively, these findings suggest that proper Ca_v channel activation is a key factor in the $[Ca^{2+}]_i$-dependent events of keratinocyte differentiation and hence epidermal mechanical barrier formation. However, the above results with the application of Ca_v channel agonists/antagonist and of Ca^{2+} to mechanically injured skin implies that extreme accumulation of Ca^{2+} in the keratinocytes may lead to impaired keratinocyte differentiation and hence barrier recovery (see also under Sect. 3.1.5).

Ca^{2+}-Activated K^+-Channels

Various Ca^{2+}-activated K^+-channels (K_{Ca}) were implicated in the regulation of growth and differentiation of epidermal keratinocytes. Indeed, K_{Ca} channels were identified on both human and mouse epidermis in situ and also on NHEKs. The activation of the 70 pS conductance K_{Ca} channel was shown to be indispensible for the effect of elevated extracellular Ca^{2+}-concentration ($[Ca^{2+}]_e$) to induce keratinocyte differentiation (Mauro et al. 1997). In addition, on cultured human immortalized HaCaT keratinocytes, a large-conductance (170 pS) K_{Ca} channel (BK) (IUPHAR nomenclature: $K_{Ca}1.1$) was detected and was implicated in the establishment of resting membrane potential; therefore, these channels may also control Ca^+-influx and differentiation (Nguyen and Markwardt 2002). Furthermore, $[Ca^{2+}]_e$ or vitamin D induced differentiation of NHEKs were shown to upregulate mRNA levels of the intermediate-conductance K_{Ca} (IK1) (IUPHAR nomenclature:

$K_{Ca}3.1$) channel (Manaves et al. 2004) which were suggested to play a central role in linking changes in membrane potential to the growth and differentiation of HaCaT keratinocytes (Koegel and Alzheimer 2001).

3.1.2 Ligand-Gated channels

nAChRs

Practically all cell types of the skin express nAChRs which control a plethora of cutaneous functions. These were detailed in comprehensive reviews (Kurzen et al. 2004; Grando et al. 2006; Curtis and Radek 2012); therefore, below, we only highlight the most important nAChR-coupled functions.

Keratinocytes, as one of the major extra-neuronal sources, were shown to produce and release ACh (Grando et al. 1993), similar to a multitude of cutaneous cells which express the ACh-synthesizing enzyme, choline-acetyltransferase (ChAT) (Wessler et al. 2003). In addition, an upward (i.e. towards the str. corneum) concentration gradient of free ACh was detected in the epidermis (Nguyen et al. 2001), in parallel with the also upward epidermal Ca^{2+} gradient (Hennings et al. 1980; Lansdown 2002). Of further importance, in situ expressions of multiple nAChR subunits were identified in the human epidermis; α3, α5, α9, and β2 subunits were localized mainly to the basal layers whereas α7, α10, β1, and β4 subunits were found in the str. spinosum and granulosum (Nguyen et al. 2001; Kurzen et al. 2004).

That the above extra-neuronal nAChR-coupled cholinergic system is indeed functional in keratinocytes was shown in numerous studies. In NHEKs, nicotine increased Ca^{2+} influx and increased cellular differentiation (upregulation of expression of keratin 10, transglutaminase type I, involucrin, and filaggrin as well as induction of cornified envelop formation) (Grando et al. 1996). As a possible mechanism of action of nicotine, in organotypic keratinocyte cultures, inhibition of α9 subunit was shown to markedly inhibit epidermal differentiation, suppress expressions of proteins involved in epidermal cell-cell contacts, and induce lipid accumulation in the basal layers suggesting barrier disruption (Kurzen et al. 2005, 2007). In line with these findings, lower levels of cell adhesion molecules (cadherins, catenins) were detected in epidermis of α9 (as well as α3) knockout mice (Nguyen et al. 2004). Consequently, stimulation of nAChRs resulted in epidermal thickening and higher lipid content of the corneal layer (Kurzen et al. 2005, 2007).

Furthermore, as was shown in cultured keratinocytes and knockout animals, elimination of α7 receptor activities or levels also inhibited differentiation (suppression of levels of filaggrin, loricrin, and cytokeratins). In addition, decreased levels of apoptosis markers (caspase-3), but increased expressions of proliferation markers (Ki-67, proliferating cell nuclear antigen [PCNA]) were detected in epidermis of α7 knockout mice (Arredondo et al. 2002). It is concluded therefore that cutaneous ACh signaling, most probably by inducing Ca^{2+} influx to keratinocytes via multiple nAChR channels, plays a key role in inducing terminal epidermal differentiation and hence barrier formation.

However, topical application of the nAChR agonist nicotine to the skin of hairless mice delayed the barrier repair after tape stripping (Denda et al. 2003a). Furthermore, topical administration of nicotine to mouse skin also resulted in a marked suppression of AMP production (Radek et al. 2010; Curtis and Radek 2012) which, in turn, may lead to barrier impairment (see also below). The possible explanation(s) for these quite "unexpected" findings will be provided under Sect. 3.1.5.

Ionotropic Glutamate Receptors

Among these ion channels, certain NMDARs and AMPARs are expressed in epidermal keratinocytes. Indeed, in human skin, the NMDAR1 subunit (IUPHAR nomenclature: GluN1) was found in all layers of the epidermis; the greatest expression was located to the granular layer (Fischer et al. 2004a, b). NMDAR1 was also identified on cultured NHEKs and HaCaT keratinocytes (Morhenn et al. 1994; Fischer et al. 2004a), especially at the site of cell-cell contacts (Nahm et al. 2004). NMDAR1 expressed by cultured keratinocytes is functional since the application of NMDA resulted in elevation of $[Ca^{2+}]_i$ which was suppressed by MK-801, an NMDAR inhibitor (Fuziwara et al. 2003; Nahm et al. 2004). Moreover, it appears that the physiological NMDAR-coupled signaling mechanisms are indispensible for proper growth and differentiation of keratinocytes. Indeed, treatment of NHEKs with MK-801 markedly suppressed the expression of differentiation markers cytokeratin 10 and filaggrin (Fischer et al. 2004a, b).

Interestingly, in hairless mice, topical application of glutamate (Denda et al. 2003a), aspartate (non-specific glutamate receptor agonists), and NMDA (Fuziwara et al. 2003), unlike AMPA, delayed the barrier recovery after disruption with tape stripping which effect was effectively abrogated by the co-administration of MK-801 and D-AP5 (another NMDAR antagonist). Of further importance, topical administration of NMDAR antagonists alone accelerated the barrier repair (Fuziwara et al. 2003). Since epidermal keratinocytes are able to synthesize and release glutamate (Fischer et al. 2009) and, furthermore, barrier injury markedly increased the release of glutamate from mouse skin (Fuziwara et al. 2003), it is proposed that the ionotropic glutamatergic signaling of keratinocytes plays a key role in the processes of barrier damage. This idea is further strengthened by presenting that NMDAR antagonists specifically inhibited the actions of oleic acid to pathologically increase transepidermal water loss (indicator of barrier impairment) and to induce keratinocyte hyperproliferation in mice. Furthermore, in cultured NHEKs, NMDAR inhibitors likewise inhibited the effects of oleic acid to elevate $[Ca^{2+}]_i$ and to stimulate production of IL1α (Katsuta et al. 2009) which cytokine, along with ATP, is regarded as a "mediator" of barrier disruption (Wood et al. 1996). The complex role of NMDAR-coupled mechanisms in barrier formation and repair will be discussed under Sect. 3.1.5.

P2X Receptors

Multiple ionotropic P2X receptors were identified in the skin and were implicated in various skin functions. Since a recent paper (Burnstock et al. 2012) reviewed characteristics of the cutaneous purinergic system, we highlight only major components of it.

Several P2X receptors were detected in human epidermis and cultured NHEKs. The expression of mRNA specific for P2X2, P2X3, P2X5, and P2X7 receptors were increased in differentiated cells. Since P2X agonists elevated $[Ca^{2+}]_i$, it is proposed that multiple P2X receptors might be involved in the regulation of epidermal differentiation (Inoue et al. 2005).

Indeed, in normal rat epidermis, P2X5 receptors were found to be highly expressed in proliferating and differentiating epidermal keratinocytes in basal and suprabasal layers whereas P2X7 receptors were associated with terminally differentiated keratinocytes in the str. corneum. In addition, expressions of P2X5 receptors were found to be increased in the regenerating epidermis (Greig et al. 2003).

Of further importance, similar to the above ACh and glutamate induced mechanisms, ATP was also shown to delay barrier recovery in hairless mice via the stimulation of another purinergic receptor, P2X3, also functionally expressed by epidermal keratinocytes. Consequently, inhibitors of P2X3 receptors accelerated skin barrier repair and prevented epidermal hyperplasia induced by skin barrier disruption (Denda et al. 2002a). The significance of these data will be discussed under Sect. 3.1.5.

5-HT3 Receptors

5-HT3 receptors were localized to basal epidermal keratinocytes in human skin in situ (Lundeberg et al. 2002; Nordlind et al. 2006), yet, as of today, we lack information about the functional role of these receptors in epidermal biology. However, as shown below (under Sect. 3.6.2), altered expression patterns were observed in psoriatic (but not in AD) skin.

$GABA_A$ Receptors

As we detailed above, the modulation of $[Ca^{2+}]_i$ homeostasis of epidermal keratinocytes via various ion channels is a key factor in regulating the physical epidermal barrier. It appears, however, that the control of Cl^- influx to keratinocytes establishes an additional mechanism. Indeed, $GABA_A$ receptors were identified in mouse epidermis (Denda et al. 2002b). In addition, in the aforementioned hairless mouse model, topical application of GABA accelerated barrier repair and prevented epidermal hyperplasia via the stimulation of epidermal $GABA_A$ receptors (Denda et al. 2002b, 2003a). In line with these findings, GABA induced Cl^- influx to NHEKs which was blocked by the $GABA_A$ receptor antagonist bicucullin. Since

GABA can be synthesized by human keratinocytes and dermal fibroblasts (Canellakis et al. 1983; Ito et al. 2007) and hence can be released upon skin barrier injury, it can be postulated that cutaneous non-neuronal GABA-ergic signaling acts as a key autocrine regulator of epidermal barrier homeostasis – just as described for locally produced and released ACh, glutamate, and ATP and their ionotropic receptor-coupled signal transduction mechanisms (for details, see also Sect. 3.1.5).

Glycine Receptors

Glycine receptors, another group of ligand-gated Cl^- channels, are also involved in barrier regeneration. In hairless mice, topical application of glycine, similar to the effect of $GABA_A$ receptor stimulation, accelerated the barrier repair after tape stripping which effect was completely prevented by the glycine receptor antagonist strychnine (Denda et al. 2003a).

3.1.3 TRP Channels

Numerous TRP channels exhibit permeability for Ca^{2+}, hence significantly modulate cellular Ca^{2+} homeostasis (Holzer 1991; Szallasi and Blumberg 1999; Caterina and Julius 2001; Clapham 2003; Dhaka et al. 2006; Nilius and Mahieu 2006; Ramsey et al. 2006; Nilius et al. 2007; Vriens et al. 2009). As detailed above, alterations in the $[Ca^{2+}]_i$ markedly affect proliferation and differentiation programs as well as of survival and mediator production of various skin cells (Hennings et al. 1980; Lansdown 2002; Proksch et al. 2008; Tóth et al. 2009b). Therefore, besides the well-appreciated contribution to sensory neuron-coupled sensory processes (e.g. pain, itch) detailed in numerous comprehensive reviews, the functional expression of Ca^{2+}-permeable TRP channels on several non-neuronal skin cell types implicate their roles in controlling cutaneous growth and differentiation.

TRPV1

TRPV1, the heat-sensitive (>43 °C) "capsaicin receptor", was originally described on nociceptive sensory neurons (Caterina et al. 1997, 2000) and was implicated in a multitude of sensory-neuron coupled processes including sensation of e.g. pain, itch, warm, chemical agents, etc. Moreover, TRPV1 was shown to be involved in neurogenic inflammation and inflammation-related thermal hyperalgesia (reviewed in Szallasi and Blumberg 1999; Caterina and Julius 2001; Clapham 2003; Dhaka et al. 2006; Vriens et al. 2008, 2009). However, besides sensory neurons, an emerging body of evidence indicates that TRPV1 is widely expressed on several non-neuronal cell-types, including those of the skin. Indeed, expression of TRPV1 was demonstrated on epidermal and hair follicle keratinocytes, mast cells, Langerhans cells, sebocytes and endothelial cells (Bíró et al. 1998a, b; Birder et al. 2001;

Denda et al. 2001; Inoue et al. 2002; Southall et al. 2003; Amantini et al. 2004; Bodó et al. 2004, 2005; Stander et al. 2004; Basu and Srivastava 2005; Tóth et al. 2009a, b, 2011a).

Functional TRPV1 channels were identified on cultured keratinocytes as well, where their stimulation by either capsaicin or heat induced membrane currents and the influx of Ca^{2+} resulting in the concomitant elevation of $[Ca^{2+}]_i$. These cellular actions were effectively inhibited by capsazepine, a TRPV1 antagonist suggesting the specific involvement of the channel (Inoue et al. 2002; Southall et al. 2003; Bodó et al. 2004, 2005; Radtke et al. 2011). Furthermore, just as has been described on numerous extra-cutaneous cell types (Sanchez et al. 2006; Prevarskaya et al. 2007), activation of TRPV1 (most probably via the resulting Ca^{2+}-influx) on NHEKs decreased proliferation and increased apoptosis (Tóth et al. 2011a) suggesting that these effects may all contribute to altered barrier functions. Indeed, activation of TRPV1 delayed the barrier recovery after tape stripping which effect was blocked by the topical application of capsazepine (Denda et al. 2007b). Likewise, oral administration of another TRPV1 antagonist, PAC-14028, also accelerated barrier recovery after mechanical and dermatitis-associated barrier injuries (Yun et al. 2011).

Currently, we lack information on the possible roles of TRP channels in the production of those structural lipids, which constitute the major portion of the epidermal chemical barrier. However, TRPV1 channels (and, as suggested by our preliminary observations, TRPV3 and TRPV4 as well) (Oláh et al. 2009, 2010; Ambrus et al. 2011) are involved in the regulation of lipid-rich sebum production of the sebaceous glands. Indeed, TRPV1 was identified in the human sebaceous gland in situ (Bodó et al. 2004; Stander et al. 2004; Roosterman et al. 2006; Zouboulis et al. 2008; Tóth et al. 2009b). In addition, stimulation of TRPV1 expressed on human sebaceous gland-derived immortalized SZ95 sebocytes (Zouboulis et al. 1999) by capsaicin inhibited basal and arachidonic acid-induced lipid synthesis and suppressed expressions of multiple genes involved in cellular lipid homeostasis (Tóth et al. 2009b). These data collectively argue for that TRPV1 inhibits the formation and the recovery of the physical-chemical skin barrier.

TRPV3 and TRPV4

TRPV3 is most abundantly expressed on epidermal keratinocytes; yet, it was also found on sensory neurons in co-expression with TRPV1 (Peier et al. 2002b; Smith et al. 2002; Xu et al. 2002; Eid and Cortright 2009). TRPV4 was originally described as an osmoreceptor expressed in various tissues including sensory neurons (Liedtke et al. 2000; Strotmann et al. 2000; Wissenbach et al. 2000; Delany et al. 2001) and keratinocytes (Suzuki et al. 2003). Both TRPV3 and TRPV4 are activated by physiological, innocuous warm temperature ranges (>33 °C for TRPV3 and cca. >30 °C for TRPV4) (Guler et al. 2002; Peier et al. 2002b; Smith et al. 2002; Watanabe et al. 2002; Xu et al. 2002; Benham et al. 2003; Eid and Cortright 2009) and their genetic deletion results in altered sensation of thermal stimuli (Todaka et al. 2004; Lee et al. 2005; Moqrich et al. 2005).

TRPV3 and TRPV4 are implicated in the regulation of the physical-chemical epidermal barrier. Indeed, TRPV3 was found to form a functional complex with the receptor of epidermal growth factor (EGF), which is indispensable for the physiological formation of the barrier. Moreover, deletion of TRPV3 resulted in impaired epidermal barrier formation (e.g. thinner cornified envelope, decreased transglutaminase activity) (Cheng et al. 2010). In addition, temperature ranges activating TRPV3 and TRPV4 as well as agonists of TRPV4 (but, interestingly, not of TRPV3) accelerated barrier recovery after tape striping (Denda et al. 2007b). The barrier promoting role of TRPV4 was also verified by employing temperature challenges and specific agonists on cultured NHEKs and human skin cultures (Kida et al. 2011).

Of further importance, TRPV4 was found to co-localize and interact with junctional proteins (β-catenin and E-cadherin) which further suggest its role in the formation of the epidermal barrier (Kida et al. 2011). In support of this proposal, in TRPV4 KO mice, leaky cell-cell junctions and delayed actin rearrangement and stratification were observed which were associated with reduced $[Ca^{2+}]_i$ levels and suppressed Rho activation (Sokabe et al. 2010; Sokabe and Tominaga 2010).

TRPV6

The Ca^{2+}-selective TRPV6, a non-thermosensitive member of the TRPV family, was also shown to promote epidermal differentiation and, most probably, barrier formation. Indeed, silencing of TRPV6 impaired keratinocyte differentiation (decreased expression of cytokeratin 10, involucrin and transglutaminase 1; impaired formation of intercellular contacts and stratification) induced by the elevation of $[Ca^{2+}]_e$ (Lehen'kyi et al. 2007). Moreover, TRPV6-mediated Ca^{2+}-influx was shown to be involved in mediating the differentiation-stimulatory effects of vitamin D3 (Bouillon et al. 2006; Lehen'kyi et al. 2007). Interestingly, treatment of NHEKs with Avène Thermal Spring water (TSW), which was shown to be beneficial in various human dermatoses, increased TRPV6 channel expression and initiated a TRPV6-mediated Ca^{2+}-entry that resulted in differentiation (increased expression of involucrin and cytokeratins 1 and 10) (Lehen'kyi et al. 2011). In accordance with these findings, the skin of TRPV6 KO mice displays fewer and thinner layers of str. corneum, decreased total Ca^{2+}-content, and loss of the normal Ca^{2+}-gradient in the skin (Bianco et al. 2007).

TRPC Channels

Various TRPC channels (TRPC1, TRPC4-7) were found to be expressed in keratinocytes (Bezzerides et al. 2004; Cai et al. 2005; Fatherazi et al. 2007), where their expression levels showed marked dependence on differentiation status of the cells (Cai et al. 2005, 2006; Fatherazi et al. 2007). Among them, TRPC1 (Cai et al. 2006; Beck et al. 2008), TRPC4 (Beck et al. 2008) and TRPC6 (Müller et al. 2008) were shown to promote the differentiation of epidermal

keratinocytes. Indeed, silencing of TRPC1 or TRPC4 prevented $[Ca^{2+}]_e$-induced differentiation (Beck et al. 2008). Moreover, TRPC6 activation by hyperforin induced NHEK differentiation and inhibition of cell proliferation (Müller et al. 2008). Likewise, TRPC6 was shown to mediate (at least in part) the epidermal differentiation-promoting effects of triterpenes, which inhibit cancer cell growth of various cell types (reviewed in Shanmugam et al. 2012). Triterpenes increased Ca^{2+}-influx and upregulated various differentiation markers in a TRPC6-dependent manner, and also elevated the expression of TRPC6 in keratinocytes and in human skin explants (Woelfle et al. 2010).

TRPA1

Like many other TRP channels, TRPA1 was first identified on sensory neurons (Story et al. 2003; Kobayashi et al. 2005). The channel can be activated by noxious cold (<17 °C) and other agents (e.g. mustard oil, allyl isothiocyanate, cinnamaldehyde, formalin, and nicotine) (Bandell et al. 2004; Jordt et al. 2004; McNamara et al. 2007; Karashima et al. 2009; Talavera et al. 2009). Similar to its closest "functional relative", i.e. TRPV1, TRPA1 was also shown to be involved in numerous sensory neuron-coupled processes such as e.g. thermosensation, pain, itch, neurogenic inflammation, etc. (Dhaka et al. 2006; Nilius and Mahieu 2006; Ramsey et al. 2006; Nilius et al. 2007).

Importantly, TRPA1 expression was also reported on epidermal keratinocytes. Exposure of NHEKs to low temperature (13–15 °C) or to TRPA1 agonists (allyl isothiocyanate or cinnamaldehyde) induced elevation of $[Ca^{2+}]_e$, which was prevented by the co-application of the TRPA1 antagonist HC030031; interestingly, these effects were more prominent on undifferentiated cells (Tsutsumi et al. 2010). Moreover, treatment of NHEKs with icilin (activator of both TRPA1 and TRPM8, another cold-sensitive channel, see below) caused alterations in the expressions of adhesion and extracellular matrix components as well as molecules regulating cell cycle, apoptosis, and differentiation (Atoyan et al. 2009; Bíró and Kovács 2009).

These data suggest that TRPA1 on keratinocytes may regulate the epidermal barrier. Indeed, following tape stripping to mice, topical application of the above TRPA1 agonists accelerated barrier recovery, which effect was prevented by pretreatment with HC030031. Interestingly, HC030031 alone delayed the barrier recovery which argues for the "constitutive" role of TRPA1 in epidermal barrier homeostasis. Local cooling of the skin (10–15 °C for 1 min) evoked similar effects, most probably via accelerated secretion of (barrier-forming) lamellar bodies at the interface of stratum granulosum and corneum; this action was also inhibited by the TRPA1 antagonist (Denda et al. 2010b).

TRPM Channels

TRPM8 is another cold sensitive (<25 °C) channel, originally found on a specific subset of sensory neurons which usually do not express TRPV1. The channel is considered as a major sensor of environmental cold stimuli and it can also be

activated by menthol, eucalyptol or the synthetic "supercooling" agent icilin (McKemy et al. 2002; Peier et al. 2002a; Bautista et al. 2007; Colburn et al. 2007).

Importantly, topical application of menthol or the TRPM8 agonist WS12 to mice potentiated the barrier recovery after tape stripping, which effect was blocked by the TRPM8 specific antagonist BTCT (Denda et al. 2010a). Since TRPM8 was identified on epidermal keratinocytes (Denda et al. 2010a), these results argue for that (similar to the other cold receptor, TRPA1) TRPM8 is also involved in skin homeostasis.

3.1.4 Non-ion Selective Channels

Aquaporins

Numerous AQP were shown to play key roles in various cutaneous functions. AQP3, the key aquaglyceroporin, regulates hydration of the skin, a major determinant of the physical properties of the epidermis (see reviewed in Hara-Chikuma and Verkman 2008a; Qin et al. 2011). AQP3 is abundantly expressed and functionally localized in cultured keratinocytes (Sugiyama et al. 2001) and to the basal and spinous layers (but, importantly, not in the str. corneum) of human and rat epidermis in situ (Frigeri et al. 1995; Matsuzaki et al. 1999; Sougrat et al. 2002). In addition, AQP3 levels (as well as water and glycerol contents) were higher in proliferating mouse keratinocytes but were reduced upon $[Ca^{2+}]_e$ or vitamin D3 induced differentiation (Zheng and Bollag 2003; Hara-Chikuma et al. 2009).

In perfect agreement with these findings, AQP3-deficient mice exhibit a characteristic skin phenotype such as dry, rough and aged skin appearance, reduced glycerol content and hydration of the epidermis (Ma et al. 2002), impaired elasticity, and delayed barrier recovery after tape stripping (Hara et al. 2002). Interestingly, expressions of differentiation markers and the differentiation process of keratinocytes as well as epidermal structure and lipid, amino acid, and ionic contents were not different from those of the wildtype animals (Hara et al. 2002; Hara-Chikuma et al. 2009). Of further importance, the cutaneous malfunctions of AQP3-deficient mice could be corrected by oral administration of glycerol which points to an intrinsic defect in water-holding capacity of the skin due to the lack of glycerol transport (Hara and Verkman 2003).

It is noteworthy that AQP3 was also identified in sebaceous glands (Frigeri et al. 1995). Since epidermal glycerol, mostly located to the str. corneum, is also derived from sebaceous glands (Fluhr et al. 2003), further studies are invited to determine the relative contribution of AQP3 localized to sebaceous glands in the regulation of the glycerol homeostasis of the skin.

It should also be mentioned that other AQPs (e.g. AQP1, 9, and 10) were also identified in human and murine keratinocytes and epidermis (Sugiyama et al. 2001; Boury-Jamot et al. 2006; Rojek et al. 2007). Yet, their functional role is not known.

Connexins

In animal models (and in different human skin conditions, see below), the central role of certain gap-junction-forming connexins in the establishment of the epidermal barrier was suggested (reviewed in Proksch et al. 2008). Indeed, mice lacking the C-terminal region of Cx43, the most abundantly expressed connexin form in the human epidermis, show a highly defective epidermal barrier, most probably due to suppressed filaggrin expression and hence impaired terminal differentiation of the epidermal keratinocytes (Maass et al. 2004). On the other hand, downregulation of another connexin, Cx26, is required for barrier acquisition during development. Indeed, epidermal overexpression of Cx26 (which is hardly detectable in the healthy, adult epidermis) resulted in the development of psoriasiform hyperproliferation and infiltration of immune cells. Moreover, overexpression of Cx26 induced ATP release from keratinocytes which, in turn, delayed epidermal barrier recovery (Djalilian et al. 2006).

Pannexins

Expressions of Panx1 and 3 have been described both in human (Penuela et al. 2007) and murine (Celetti et al. 2010) epidermal keratinocytes. Interestingly, the expression pattern of Panx1 changed in embryonic and newborn skin, with a higher expression found after birth (Panx3 expression showed no such alteration). Functionally, when overexpressed in organotypic rat keratinocytes, both Panxs decreased cell proliferation, whereas Panx1 also disrupted the architecture of organotypic epidermis and dysregulated the expression and cellular localization of cytokeratin 14 (Celetti et al. 2010). Taken together, these findings suggest that certain Panxs (especially Panx1) are important factors in keratinocyte differentiation.

3.1.5 The Complex "Channel" Regulation of the Epidermal Barrier: Controversies, Explanations, Theories

As detailed above, the delicate regulation of $[Ca^{2+}]_i$ and the coupled Ca^{2+}-dependent processes is the key event in controlling the physiological growth and differentiation of keratinocytes. Correspondingly, numerous Ca^{2+}-permeable channels were shown to promote the (terminal) differentiation of keratinocytes. These include voltage-gated Ca_v channels; K_{Ca} K^+-channels; nAChRs subtypes α3, α7, and α9; NMDA glutamate receptors; P2X5 and P2X7 purinergic receptors; and TRPC1, C4, C6 as well as TRPV6 (and possibly TRPV3, TRPA1, and TRPM8) TRP channels.

However, although the formation and maintenance of the epidermal physical-chemical barrier is based on the proper keratinocyte differentiation program, the activation of some of these channels (Ca_v channels, nAChRs, NMDARs, purinergic

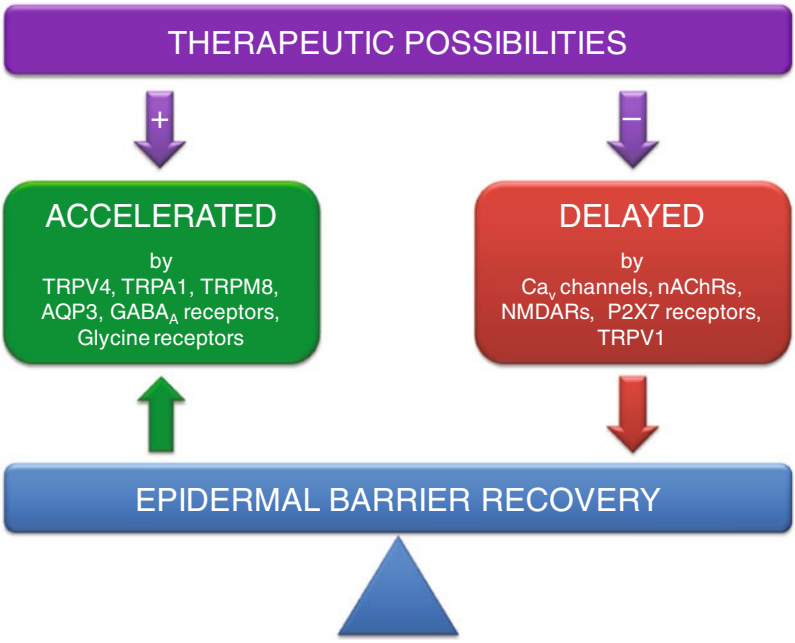

Fig. 3 *The complex "channel" regulation of the epidermal barrier recovery*. Numerous channels play central, yet partly antagonistic, roles in the regulation of the recovery processes following the mechanical disruption of the epidermal barrier. Therefore, targeted modulations of channel activities represent exciting, novel future therapeutic possibilities. For further details, see text under Sect. 3.1

receptors) was shown to delay, whereas stimulation of others (TRPV4, TRPA1, TRPM8, and possibly TRPV3 and V6) accelerated the recovery of the barrier after mechanical disruption (Fig. 3).

As possible explanations for these quite unexpected findings, the followings could be listed:

- As we presented above, keratinocytes are able to synthesize and release ACh, ATP, and glutamate. Moreover, an "upward" Ca^{2+}-gradient was described in the epidermis. Therefore, these locally produced, autocrine/paracrine mediators constitutively promote the differentiation of keratinocytes, via the activation of their respective ionotropic, Ca^{2+}-permeable receptors/channels and the concomitant increase in $[Ca^{2+}]_i$.
- However, during mechanical disruption of the epidermis, the release of these endogenous agents from keratinocytes is markedly increased and the high-level activation of their receptors may result in an excessive Ca^{2+} influx which impairs keratinocyte differentiation. It appears, therefore, that the intracutaneous "ACh-ATP-glutamate-Ca^{2+} tone" is indispensible for the maintenance of the healthy barrier; however, when this "fine-tuned tone" is pathologically augmented, these agents may start functioning as mediators of the barrier injury itself.

- The validity of this theory is supported by experimental data obtained after topical administration of these agents as well as their agonists and antagonists to mechanically injured barrier (tape stripping) in mice. Indeed, topical agonists (by further increasing the already highly augmented "tone") delayed barrier recovery whereas antagonists (by normalizing the "tone") accelerated the rate of recovery.
- However, experimental data about the role of TRPV4, TRPA1, and TRPM8 channels do not really fit to this theory. Namely, as detailed before, the activation of these (likewise) Ca^{2+}-permeable channels also stimulated epidermal differentiation. Interestingly, topical application of their agonists after tape stripping – in contrast to the effects of ionotropic cholinergic, glutamatergic, and purinergic stimulations – accelerated barrier repair.
- With respect to the promoting role of TRPV4 it was suggested that, due to its sensitivity to not only to moderate heat but also to osmotic challenges, it may act as part of the "keratinocytes sensory system" that recognizes water flux (Sokabe and Tominaga 2010). A possible support for this hypothesis could be that AQP3 water (and glycerol) channels are actively participating in the barrier regeneration processes by increasing water flux and water content within the epidermis. Nevertheless, the direct or indirect connection between TRPV4 and AQP3 has not yet been revealed.
- Also, further studies are needed to clarify the (most probably distinct) signaling pathways which are initiated after induction of TRPA1 or TRPM8 activities by agonists or by cooling of the skin surface.

Finally, it should be mentioned that the induction of Cl^--influx to keratinocytes by topical activation of GABA-ergic and glycinergic signaling mechanisms also accelerated barrier regeneration. As possible mechanisms of action, it can be postulated that the Cl^--influx results in hyperpolarization of the cell membrane which (as a pro-proliferating factor) speeds up the turn-over of keratinocytes to "heal" the barrier. In addition, the Cl^--mediated hyperpolarization may counterbalance the effect of the augmented "ACh-ATP-glutamate-Ca^{2+} tone" thereby preventing the excessive Ca^{2+} influx and its damaging consequences.

Nevertheless, since modulation of the activities of these, rather complex, mechanisms may represent novel therapeutic approaches; further studies are urgently invited to (1) dissect the exact mechanistic details of their modes of action; and (2) explore their impact in such high-prevalence "barrier diseases" as AD or psoriasis.

3.2 Roles of Channels in Wound Healing

Like in the formation and regeneration of the epidermal physical-chemical barrier, multiple channels participate in the complex multi-cellular events of wound healing.

3.2.1 Voltage-Gated Channels

Voltage-Gated and Ca^{2+}-Activated K^+-Channels

All three members of the K_{Ca} channel family – i.e. large-conductance BK, intermediate-conductance IK, and small-conductance K_{Ca} – were identified on cultured human dermal fibroblasts. Moreover, activation of BK or IK K_{Ca} channels decreased the proliferation of fibroblasts and induced apoptotic changes by mitochondrial membrane potential disruption (but without the involvement of the caspase-dependent apoptotic pathways) (Yun et al. 2010). In addition, nitric oxide (NO), which plays an important promoting role in wound healing (Shi et al. 2003), was shown to stimulate BK K_{Ca} channel activity via the engagement of protein kinase A and G coupled signaling pathways in human dermal fibroblasts (Lim et al. 2005; Roh et al. 2007) and via increasing cyclic-GMP in human hair follicle-derived dermal papilla fibroblasts (Nameda et al. 1996). It appears, therefore, that K_{Ca} are involved in fibroblast-driven cutaneous wound healing.

Other voltage-gated K^+-channels (fast-inactivating A-type K^+-channels; inward rectifier (K_{ir}) K^+-channels; cell-to-cell contact-associated K^+-channels) as well as Na^+ channels (tetrodotoxin-sensitive Na^+-channels) were also identified on human dermal fibroblasts; yet their functional roles were not revealed (Estacion 1991).

3.2.2 Ligand-Gated Channels

nAChRs

Keratinocyte migration events, such as chemokinesis and chemotaxis, are key processes of epithelial re-epithelialization during wound healing (Epstein 1999, Enoch and Leaper 2005; Reinke and Sorg 2012). Since locally produced ACh, which could be released during injury of the skin, may act as both a chemokine and a chemoattractant for cell migration (reviewed in Grando et al. 2006), involvement of non-neuronal nicotinergic signaling in wound healing is also proposed. Indeed, α3β2 nAChR channels were shown to play central roles in mediating ACh-dependent chemokinesis whereas α7 nAChRs were found to be involved in chemotaxis (Chernyavsky et al. 2004). In addition, α7 channels (and a complex intracellular signaling pathway involving Ras/Raf-1/MEK1/ERK-mediated upregulation of integrins) were described to control directional migration of keratinocytes (Chernyavsky et al. 2005). Finally, α9 nAChRs seem to be indispensable for the initial phase of epithelialization as the coupled signaling controls the dynamics and strength of cell-cell cohesion as well as the disassembly and reassembly of focal adhesions (Chernyavsky et al. 2007).

Another key cell type of wound healing is the dermal fibroblast (see above, Sect. 1.2.1) which also possess a functional cholinergic system. Indeed, α3, α5, α7, β2, and β4 nAChRs were described in cultured human dermal fibroblast. Among these, as revealed by gene silencing, mainly α3 nAChR channels were implicated in

mediating the effects of nicotine to significantly modulate cell growth, cycling, and survival (upregulation of p21, cyclin D1, PCNA, Ki-67, caspase 3 and bcl-2 mRNA transcripts) as well as production of extracellular matrix components (upregulation of collagen type Iα_1, elastin, and matrix metalloproteinase-1, MMP-1). Therefore, the cholinergic system may play a key role in controlling proper dermal fibroblast functions involved in tissue remodeling and wound healing (Arredondo et al. 2003).

$GABA_A$ Receptors

Of further importance, non-neuronal GABA-ergic mechanisms seem to not only stimulate epidermal barrier recovery, but also cutaneous wound healing. In a rat excisional open wound model, topical GABA treatment, most probably via activation of $GABA_A$ receptors, was shown to effectively accelerate the healing process (especially its early phase) by stimulating keratinocyte reepithelialization and fibroblast organization as well as by upregulating fibroblast growth factor and platelet-derived growth factor, implying extracellular matrix synthesis and remodeling of the skin (Han et al. 2007). Further supporting its promoting role, GABA was shown to stimulate the synthesis of hyaluronic acid, a key component of the extracellular matrix, and to enhance the survival rate of the human dermal fibroblasts against oxidative stress (Ito et al. 2007).

3.2.3 TRP Channels

TRPV1

Although the expression of functional TRPV1 channels was described on human dermal fibroblasts (Kim et al. 2006), the role of the channels in fibroblast-specific functions was not assessed. However, activation of TRPV1 by capsaicin in organ-cultured human scalp hair follicles (HF) inhibited hair shaft elongation and induced premature follicular regression (catagen transformation) (Bodó et al. 2005). In line with these data, stimulation of TRPV1 in HF-derived cultured outer root sheath (ORS) keratinocytes, which showed the greatest TRPV1 expression in the HF (Bodó et al. 2004, 2005; Stander et al. 2004), resulted in suppression of proliferation and the onset of apoptosis. The growth inhibitory role of TRPV1 was further verified in TRPV1 knockout mice; i.e. a remarkable delay in the onset of the apoptosis-driven catagen retardation was observed when compared to the HF cycle of wildtype animals (Bíró et al. 2006).

As mentioned above, the HF (and especially the ORS compartment) is a rich source of stem cells activated during wound healing and tissue regeneration in general. Therefore, these data collectively suggest that TRPV1-coupled signaling rather inhibits wound healing, just as seen for the formation and regeneration of the epidermal barrier (see above).

TRPV3

As will be discussed below (see under Sect. 3.4.2), activation of TRPV3 on keratinocytes by heat or various agonists results in the release of various mediators. Among these, NO, released upon TRPV3 stimulation of keratinocytes, was shown to promote keratinocyte migration in vitro and, as expected, wound healing in vivo (Miyamoto et al. 2011).

Furthermore, using the above HF organ-culture and ORS cell culture models, we have recently shown that activation of TRPV3, identical to the above action of TRPV1, inhibited hair shaft elongation and cellular proliferation and induced apoptosis (Borbíró et al. 2011). Interestingly, TRPV3 KO mice exhibited only subtle and irregular hair abnormalities (wavy hair coat and curly whiskers) (Moqrich et al. 2005). However, a gain-of-function (Gly573Ser) mutation of the *trpv3* gene resulted in a spontaneous hairless phenotype in DS-*Nh* mice (Yoshioka et al. 2009) as well as in hairless WBN/Kob-Ht rats (Asakawa et al. 2006; Imura et al. 2007). These results collective argue for the negative regulatory role of TRPV3 in the HF.

3.2.4 Non-ion Selective Channels

Aquaporins

In addition to impaired epidermal differentiation and barrier functions, a delayed wound healing process was also seen in AQP3-deficient mice (Hara et al. 2002; Hara-Chikuma and Verkman 2008a), which suggests the role of AQP3 in promoting epidermal cell migration and proliferation. Indeed, by using AQP3-knockdown (small interfering RNA) NHEK cultures and AQP3-knockout mouse keratinocyte cultures (Hara-Chikuma and Verkman 2008c), it was proposed that the water, transported via AQP3, is likely to play a role in epidermal hydration and hence migration controlled by changes in the hydrostatic pressure. On the other hand, the transported glycerol may equally act as an energy source for ATP production; a precursor for fat and phospholipid synthesis (such as phosphatidylglycerol, a phospholipase D product, which is known to regulate keratinocyte proliferation and differentiation) (Qin et al. 2011); and an osmotically active agent (Hara-Chikuma and Verkman 2008b).

Connexins

As shown above, Cx26 promotes proliferation (and inhibits differentiation) of keratinocytes. In line with these data, expression of Cx26 was found to be increased during the epidermal regeneration phase of wound healing (Brandner et al. 2004), with an additional suppression of the level of the pro-differentiating Cx43 (Wang et al. 2007).

3.3 Roles of Channels in Cutaneous Immune Functions and in the "Formation" of the Immunological Barrier

The skin possesses its own immune system which involves numerous cellular and humoral components of the innate and adaptive immunity. Immuno-competent cells express various ion channels which, as shown below, play significant roles in the regulation of cutaneous inflammatory and immune responses.

3.3.1 Voltage-Gated Channels

Although expressions of genes encoding various voltage-gated K^+-channels ($K_{ir}2.1$, $K_{ir}2.4$, and BK K_{Ca} α and β4 channel subunits), Na^+-channels ($Na_v1.8$ and $Na_v1.9$ channels as well as the auxiliary subunit $Na_v\beta1.1$), and Ca^{2+}-channels (the auxiliary subunit $Ca_v\alpha_2\delta_2$) were identified on human skin-derived mast cells (Bradding et al. 2003), their functional role is not known. Likewise, we lack data on whether the functional voltage-gated channels expressed by keratinocytes and fibroblasts may participate in the immune responses of these cells.

3.3.2 Ligand-Gated Channels

nAChRs

Nicotinic AChR-coupled signaling was suggested to be involved in cutaneous inflammatory responses. As we mentioned above, topical administration of nicotine to mouse skin resulted in a marked suppression of AMP production which effect was reversed by the nAChR antagonist α-bungarotoxin (Radek et al. 2010). Likewise, stimulated production of AMPs (LL-37 cathelicidin, β-defensin) in NHEKs was suppressed by ACh which effect was reversed by α-bungarotoxin. Of further importance, Chga knockout mice, which exhibit unopposed nAChR activation due to genetic deletion of the endogenous nAChR inhibitor, catestatin (Mahapatra et al. 2005), showed increased susceptibility to bacterial infections. These data propose that the proper intracutaneous cholinergic ACh-nAChR signaling not only regulates skin barrier formation and wound healing (see above, Sects. 3.1.2 and 3.2.2), but also the innate host defense of the skin. Moreover, since activity of the neuronal and the non-neuronal, cholinergic systems is markedly increased during chronic stress, the pathological augmentation of the above mechanism may contribute to the highly elevated susceptibility to infection following prolonged stress (Radek et al. 2010; Curtis and Radek 2012).

P2X Receptors

Certain P2X receptors were also implicated in skin immune functions and inflammation. Indeed, in human skin vascular endothelial cells, among the several

ionotropic purinergic receptors expressed by these cells, P2X4 was described in mediating the effect of ATP to increase Ca^{2+}-influx and to induce the release of the pro-inflammatory and vasoactive NO and prostaglandin PGI_2 (Yamamoto et al. 2000). In addition, among P2X receptors, the human dermal microvascular endothelial cell-1 (HMEC-1) cell line was shown to strongly express P2X4, P2X5, and P2X7 receptors and weakly express P2X1 and P2X3 receptors (Seiffert et al. 2006). Administration of ATPγS, a hydrolysis-resistant purinergic agonist, to HMEC-1 cells increased the release of numerous pro-inflammatory mediators (IL-6, IL-8, monocyte chemoattractant protein-1, growth-regulated oncogene-α) and upregulated the expression of intercellular adhesion molecule-1 (ICAM-1); these events were effectively prevented by various purinergic antagonists.

Of further importance, intradermal administration of ATPγS in mice resulted in an enhanced contact hypersensitivity response and the induction of delayed-type hypersensitivity. Moreover, in cultured mouse Langerhans cells, ATPγS (in the presence of bacterial lipopolysaccharide [LPS] and granulocyte-macrophage colony-stimulating factor) enhanced the antigen-presenting functions of the cells (Granstein et al. 2005). In perfect line with these data, mice lacking the P2X7 receptor were shown to be resistant to contact hypersensitivity. Dendritic cells from P2X7-deficient mice failed to induce sensitization to contact allergens and did not release IL-1β, a key molecule in the sensitization process, in response to LPS and ATP (Weber et al. 2010).

Expression of functional P2X7 receptors was also demonstrated both on human and mouse epidermal Langerhans cells (Georgiou et al. 2005; Tran et al. 2010). Activation of P2X7 on human Langerhans cells induced downstream signaling events, i.e. shedding of the low-affinity receptor for IgE (CD23), which effect was impaired in Langerhans cells obtained from subjects homozygous for the loss-of-function polymorphism in the P2X7 receptor (Georgiou et al. 2005).

On cultured NHEKs, extracellular ATP displayed a complex regulation of interferon-γ stimulated chemokine expression, with upregulation of chemokine ligand 2 (CCL2), CCL5 and CXC chemokine ligand 8 (CXCL8), and suppression of the receptor CXC chemokine receptor 3 (CXCR3), CXCL9, CXCL10, and CXCL11. It is suggested that P2X7 receptors are involved in this complex process (Pastore et al. 2007). Of further importance, P2X7 receptors expressed by human keratinocytes were also implicated as key components of the signaling pathway (P2X7-SFK-Akt-CREB/ATF1) activated by LL-37 cathelicidin, a multifunctional immunomodulatory AMP, to augment the production of immune mediators in response to microbial compounds (Nijnik et al. 2012).

Stimulation of functional P2X7 receptors was also found to induce the release of the pro-inflammatory cytokine IL-6 on human skin fibroblasts (Solini et al. 1999). In addition, augmented ATP release and enhanced P2X7 receptor-mediated cellular responses (including microvesiculation, enhanced fibronectin and IL-6 secretion, accelerated apoptosis) were demonstrated on dermal fibroblasts of type 2 diabetic subjects (Solini et al. 2004).

Collectively, it is proposed that ATP, when released after trauma, infection or exposure to contact allergens, may act as an endogenous adjuvant to enhance the immune response, most probably via P2X7-coupled signaling found on

immunocompetent keratinocytes, Langerhans cells, microvascular endothelial cells, and fibroblasts. Interference with P2X7 receptors may therefore be a promising strategy for the prevention of allergic contact dermatitis and possibly other inflammatory skin conditions.

$GABA_A$ Receptors

In NC/Nga mice, a murine model of AD, oral administration GABA reduced the development of AD-like skin lesions, most probably by suppressing serum immunoglobulin E and splenocyte IL-4 production (Hokazono et al. 2010). Although it cannot be excluded that the above beneficial effects were due to the aforementioned effects of GABA to promote barrier formation and repair (which processes are highly impaired in AD), these results also propose the anti-inflammatory functions of the non-neuronal GABA-ergic signaling of the skin.

3.3.3 TRP Channels

As mentioned above (Sect. 1.2.2), activation of sensory afferents in the skin results in the release of various neuropeptides (SP, CGRP) which – via the stimulation of immunocompetent cells of the skin (e.g. keratinocytes, sebocytes, mast cells, etc.) and the concomitant induction of liberation of various inflammatory mediators (cytokines, chemokines, vasoactive agents) from these cells – induces neurogenic inflammation (Ansel et al. 1997; Luger 2002; Paus et al. 2006a, b; Peters et al. 2007; Fuchs and Horsley 2008). With respect to TRP channels, TRPV1 and TRPA1 were implicated in this process. However, the identification of various functional TRPs on non-neuronal cell types of the skin suggests that these molecules are also involved in non-neurogenic skin inflammation.

TRPV1

As we have detailed above (Sect. 3.1.3), the TRPV1 inhibitor PAC-14028, when applied orally, accelerated barrier recovery after tape stripping. However, PAC-14028 seems to be beneficial against experimentally induced AD as well (Yun et al. 2011). Indeed, in a mouse model of AD (induced by *Dermatophagoides farina* and oxazolone), the orally administered TRPV1 antagonist was able to efficiently prevent the dermatitis-associated barrier damages (by suppressing of trans-epidermal water loss, inducing reconstruction of epidermal lipid layers, and normalizing of altered expressions of epidermal differentiation markers) and, at the same time, improved the AD-like symptoms (clinical severity, skin score, serum IgE levels, mast cell degranulation status, etc.).

In good agreement with these in vivo data, TRPV1 activation on cultured human keratinocytes by capsaicin resulted in the induction of cyclooxygenase-2 (COX-2) and the release of pro-inflammatory IL-8 and PGE_2 (Southall et al. 2003).

Importantly, stimulation of TRPV1 by heat on NHEKs not only altered proliferation and cellular survival, but also induced MMP-1 production (Li et al. 2007; Lee et al. 2008). Likewise, TRPV1-coupled Ca^{2+}-dependent signaling was shown to be involved in mediating the effects of UV irradiation to upregulate MMP-1 in cultured keratinocytes (Lee et al. 2009b). Furthermore, in a mouse model, the TRPV1 inhibitor 5′-iodoresiniferatoxin (I-RTX), when applied topically, was shown to effectively prevent the UV-induced reactions (skin thickening, inflammation, upregulation of MMPs, COX-2, and pro-inflammatory cytokines such as IL-1β, IL-2, IL-4, tumor necrosis factor-α, TNFα) (Lee et al. 2011).

Finally, it should be mentioned that activation of TRPV1 by capsaicin on cultured HF-derived ORS keratinocytes (besides inducing cellular arrest and apoptosis, see above under Sect. 3.2.3) stimulated the synthesis of the pro-inflammatory IL-1β and transforming growth factor-β_2 (Bodó et al. 2005). These results collectively argue for the pro-inflammatory role of TRPV1 in non-neurogenic cutaneous inflammation.

TRPV3

As we have detailed above (Sect. 3.2.3), the gain-of-function (Gly573Ser) mutation of the *trpv3* gene resulted in a hairless phenotype in mice and rats. However, of great importance, this mutation is also accompanied by a spontaneously developing AD-like dermatitis (Asakawa et al. 2006; Xiao et al. 2008). Moreover, keratinocyte-targeted transgenic overexpression of the mutant $TRPV3^{Gly573Ser}$ channels in mice also led to the development of AD-like cutaneous (dermatitis, hyperkeratosis, itch, infiltration of mast cells and CD4+ lymphocytes, increased skin nerve growth factor [NGF] levels) and systemic (increased serum levels of IgE and pro-inflammatory cytokines) symptoms (Yoshioka et al. 2009). As a further support for its pro-inflammatory role, TRPV3 stimulation in cultured keratinocytes by agonists (eugenol, 2-aminoethoxydiphenyl borate) or heat was shown to induce the release of the pro-inflammatory IL-1α and PGE_2 (Xu et al. 2006; Huang et al. 2008).

TRPA1

TRPA1, similar to TRPV1 and TRPV3, also seems to act as a pro-inflammatory channel. Stimulation of TRPA1 on NHEKs induced the synthesis of the pro-inflammatory IL-1α and IL-1β (Atoyan et al. 2009). Moreover, as expected, topical application of the TRPA1 agonist cinnamaldehyde induced skin inflammation. Interestingly, however, whereas the edema component was prevented by aprepitant, an antagonist of the tachykinin NK1 receptor recognizing SP released from sensory afferents upon TRPA1 stimulation, it was not affected by HC030031, a TRPA1 antagonist. On the contrary, the cinnamaldehyde-induced leukocyte infiltration was effectively suppressed by the TRPA1 inhibitor whilst the NK1 antagonist was ineffective (Silva et al. 2011).

These intriguing data suggest that the TRPA1-coupled signaling on sensory neurons and non-neuronal skin cells, when co-activated e.g. by topical or intracutaneous administrations of agonists, act in concert to equally induce neurogenic and non-neurogenic skin inflammation. We propose that this is the case for TRPV1 and possibly for TRPV3 as well.

3.3.4 Non-ion Selective Channels

Aquaporins

The aquaglyceroporin AQP7 was identified on mouse dermal and epidermal dendritic cells. In dendritic cells isolated from AQP7 deficient mice, significantly decreased antigen uptake and reduced chemokine-dependent cell migration were identified in comparison to wild-type cells. Moreover, AQP7-deficient mice exhibited a suppressed accumulation of antigen-retaining dendritic cells in the lymph node after antigen application to the skin. These results suggest that AQP7 in skin dendritic cells is primarily involved in antigen uptake and in the subsequent migration of the cells which suggest their role in the initiation of the concomitant immune responses (Hara-Chikuma et al. 2012). AQP3 and AQP9 were also found in monocyte-derived Langerhans cells but their role is still unclear (Boury-Jamot et al. 2006).

In addition, TNFα coupled signaling (involving p38 and Erk kinase cascades) was shown to suppress AQP3 expression in cultured keratinocytes which effect may contribute to the pro-inflammatory effects of this cytokine (Horie et al. 2009).

3.4 "Sensory Roles" of Epidermal Keratinocytes

As we detailed above (Sect. 1.2.2), various stimuli that reach the skin may not only activate sensory afferent fibers, but also non-neuronal skin-derived cells. Among these cells, direct activation of epidermal keratinocytes, which establish the very first line of defense, results in the release of various mediators. These agents, in turn, act on the sensory afferents and induce their excitation. Therefore, keratinocytes and, via the established multi-cellular neuronal – non-neuronal cell networks, possibly other skin-derived cells significantly contribute to skin sensory physiology.

3.4.1 Voltage-Gated Ion Channels

Voltage-Gated Na^+-Channels

Various voltage-gated Na^+-channels were identified on epidermal keratinocytes. $Na_v1.1$, $Na_v1.6$, and $Na_v1.8$, expressed on rat cultured keratinocytes, were shown to contribute to the release of ATP from these cells (Zhao et al. 2008).

It was suggested that the release ATP, in turn, may stimulate nociceptive sensory afferents (located in a close vicinity of epidermal keratinocytes in the epidermis) (Ansel et al. 1997) and hence may initiate pain. In addition, in situ epidermal expressions of $Na_v1.5$, $Na_v1.6$, and $Na_v1.7$ were identified on histological skin sections from healthy human subjects. Interestingly, levels of these channels were shown to be markedly increased in skin samples obtained from patients with various pain syndromes (complex regional pain syndrome type 1 and post-herpetic neuralgia), with additional appearances of $Na_v1.1$, $Na_v1.2$, and $Na_v1.8$. Although it is not known whether or not these channels contribute to the regulation of keratinocyte growth control, the "sensory roles" of the increased Na^+-channel expression in the pathogenesis of the above pain syndromes is suggested (Zhao et al. 2008).

Two-Pore K^+ Channels (K_{2P})

Six two-pore K^+ channels (TASK-1, TASK-2, TASK-3, TREK-1, TREK-2 and TRAAK) were described in human epidermal HaCaT keratinocytes as well as in rat skin. Since K^+ currents were induced by different activators of these channels (arachidonic acid and heat), these results suggest that K_{2P} channels could act as thermosensors in human keratinocytes (Kang et al. 2007).

3.4.2 TRP Channels

TRPV3

Similar to the above, TRPV3 (and most probably TRPV4) expressed by keratinocytes may also provide thermo-sensory functions to these cells. Namely, moderate heat-activation of TRPV3 expressed by keratinocytes resulted in the release of ATP which, in turn, may stimulate sensory neurons (Chung et al. 2003, 2004; Lee et al. 2005; Mandadi et al. 2009). Likewise, overexpression of TRPV3 in keratinocytes was shown to modulate sensory processes by the TRPV3-mediated release of PGE2 (Huang et al. 2008). Finally, NO, which is released from keratinocytes upon TRPV3 stimulation, not only promotes keratinocyte migration and wound healing (see above, Sect. 3.2.3), but also regulates thermosensory behavior, most probably by acting on and hence stimulating thermosensitive sensory afferents (Miyamoto et al. 2011).

3.4.3 Other Ion Channels

Amiloride-Sensitive Na^+ Channels

Amiloride-sensitive epithelial Na^+ channels (ENaCδ) were also found in human epidermis. In cultured NHEKs, acidic stress, activator of these channels, evoked

ATP release which was inhibited by amiloride. Interestingly, ENaCβ and γ were also identified in human keratinocytes; yet, their physiological functions are not known. These data suggest that ENaCδ expressed by keratinocytes may be involved in pH sensing of the skin (Yamamura et al. 2008b).

3.5 Other Skin Functions

Here, we mostly detail the roles of various channels in the control of sweat production and skin metabolism.

3.5.1 Voltage-Gated Channels

Ca^{2+}-Activated K^+-Channels

K_{Ca} channels also play key roles in regulating exocrine gland functions of the skin. Indeed, BK K_{Ca} channels were identified on primary cultures of human (Henderson and Cuthbert 1991a) and equine (Huang et al. 1999) eccrine sweat gland cells as well as on exocrine gland cells in frog skin (Andersen et al. 1995; Sørensen et al. 2001). In human cell cultures (especially in the younger, dividing ones), these BK K_{Ca} channels, located on the basolateral membrane of the cell, were implicated in the Ca^{2+}-dependent secretory and absorptive events seen in the intact sweat gland.

In cultured human eccrine sweat gland cells, intermediate-conductance IK K_{Ca} channels were also identified. Interestingly, estradiol rapidly activated these channels in an estrogen receptor-independent manner. In addition, estradiol was shown to induce the translocation of IK K_{Ca} both to the apical and basolateral cell membranes in a calmodulin-dependent manner. This mechanism was suggested as a new mode of estrogen action in human sweat gland epithelial cells (Muchekehu and Harvey 2009).

3.5.2 Ligand-Gated Channels

nAChRs

It is a common knowledge in physiology that sweating can be induced by efferent neuronal cholinergic stimulation, mediated by the binding of the released ACh to mAChRs expressed by the sweat glands. Likewise, sweating can be induced by intradermal injection of cholinomimetic compounds, which can be efficiently prevented by the mAChR antagonist atropine (Longmore et al. 1985; Smith et al. 1992). However, application of ACh to primary human sweat gland-derived epithelial cells was shown to also induce Ca^{2+} influx which may argue for the existence of functional nAChR channels (Lei et al. 2008). Indeed, various nAChR channels

(including α3 and α7) were described in the ductal and acinar compartments of sweat glands. Moreover, the enzymatic apparatus for the synthesis and degradation of ACh is also expressed by sweat glands (Kurzen et al. 2004; Hagforsen 2007). Therefore, further studies are invited to define the relative contribution of nAChRs and mAChRs to sweating induced by neuronal and non-neuronal ACh.

3.5.3 Non-Ion Selective Channels

Aquaporins

AQP5 was found to be expressed in secretory cells and ductal parts of sweat glands in humans, rats and mice (Nejsum et al. 2002; Song et al. 2002). Using various methods, Song et al. concluded that AQP5 deletion in mice did not affect intensity and composition of sweat secretion (Song et al. 2002). However, others have shown that genetic depletion of AQP5 in mice greatly decreased the response of sweat glands to pilocarpine, a known inducer a sweat production (Nejsum et al. 2002). In light of these data, further studies are needed to unambiguously define the role of AQP5 in human sweat secretion.

AQP7 is also expressed by subcutaneous adipocytes and seems to be involved in cutaneous fat metabolism. Indeed, in AQP7 knockout mice, a progressive adipocyte hypertrophy was observed which effect was most probably due to the reduced AQP7-facilitated plasma membrane glycerol exit from adipocytes (Hara-Chikuma et al. 2005; Hibuse et al. 2005).

3.6 Skin Diseases

So far, we have presented a plethora of evidence about the active contribution of numerous channels in various skin functions. Therefore, it is not surprising at all that multiple channels are reportedly associated with multiple skin conditions (summarized in Table 2). However, it should be mentioned that most of the below data only indirectly link the given channel to the given disease, and that only very few "real", pathogenetic correlations could be identified. Therefore, further studies are invited to explore the causative roles of the identified alterations in the expressions/functions of the channels.

Below, we start by listing the available literature data in relation to the most prevalent "barrier diseases", AD and psoriasis. Then we continue with describing the roles of the channels in various skin tumors and in other dermatoses. Finally, although skin ageing per se cannot be considered as a disease, the related, quite exciting findings prompted us to close this section with mentioning the possible involvement of certain channels in the ageing process.

3.6.1 AD

Ligand-Gated Channels

The expression level of ChAT (which is a key enzyme of ACh biosynthesis) was found to be highly elevated in the epidermis (14-fold) and in the upper dermis (3-fold) of AD patients when compared to healthy skin compartments (Wessler et al. 2003). Moreover, irregular nAChR subtype expression patterns were described in AD lesions (Curtis and Radek 2012). Likewise, in lesional skin of AD (and psoriasis) patients, intense P2X7 reactivity was confined to the cell membrane of the basal layer, with an additional, diffuse P2Y1 metabotropic purinergic receptor immunostaining throughout the epidermis (Pastore et al. 2007). Unfortunately, the pathogenetic roles of these phenomena are not clarified. Also, the human clinical relevance of those intriguing findings (detailed under Sect. 3.3.2.3) that orally administered GABA was beneficial against experimentally induced AD in mice should also be carefully investigated.

TRP Channels

As we have shown (Sect. 3.3.3), TRPV1 and TRPV3 activities promoted the development of AD-like dermatitis in mice. However, further studies are required to define the roles of these (and possibly other) TRP channels in the pathogenesis of human AD.

Non-ion Selective Channels

Elevated AQP3 expression was found in AD skin (Olsson et al. 2006; Nakahigashi et al. 2011). In addition, CCL17, which is highly expressed in AD, was found to be a strong inducer of AQP3 expression and enhanced keratinocyte proliferation. In a mouse model of AD, the induced epidermal hyperplasia, a characteristic symptom of the disease, was reduced in AQP3-deficient mice, with a decreased number of proliferating keratinocytes (Nakahigashi et al. 2011). These results suggest the possible involvement of AQP3 in the development of AD.

It should be mentioned that although altered levels of AQP3 were also described in the closely related epidermal spongiosis associated with eczema (Boury-Jamot et al. 2006) and erythema toxicum neonatorum (Marchini et al. 2003), the functional role of AQP3 in these diseases is not yet known.

3.6.2 Psoriasis Vulgaris

Voltage-Gated Channels

Keratinocytes and skin from psoriatic individuals were found to express higher levels of mRNA encoding the non-functional splice-variant of cyclic

nucleotid-gated (CNG), Ca^{2+}-permeable, non-selective cationic channels. Since overexpression of the splice variant by transfection of HEK293 in culture leads to loss of protein expression for the functional CNG channels (McKenzie et al. 2003); and, furthermore, since Ca^{2+} influx to human keratinocytes may occur, among others, via CNG channels, these data may suggest the potential role of this CNG isoform shift in psoriasis.

Ligand-Gated Channels

As was shown above, NMDAR-coupled signaling was implicated in the proper growth and differentiation of keratinocytes. In support of this proposal, in parakeratotic skin lesions of psoriasis patients, a significant reduction in the expression of NMDAR1 in the upper epidermis was identified (Fischer et al. 2004b). This alteration was suggested to result in a suppressed Ca^{2+} influx to the diseased keratinocytes leading to impaired differentiation and barrier formation, hallmarks of the disease.

As mentioned above, 5-HT3 receptor was localized to basal epidermal keratinocytes in human skin in situ. This expression pattern was not altered in skin samples of AD patients or in non-involved psoriatic skin; however, 5-HT3 receptor was identified in the acrosyringium, but not in basal keratinocytes, in involved psoriatic skin (Lundeberg et al. 2002; Nordlind et al. 2006). Therefore, it can be hypothesized (and to be investigated in future trials) that epidermal 5-HT3 receptors may contribute to the development of psoriasis.

Expressions of GABA ligand and $GABA_A$ receptor were found to be increased in inflammatory cells located in lesional psoriatic skin when compared to non-lesional skin parts. GABA ligand was mostly expressed in macrophages whereas $GABA_A$ receptor was localized in macrophages, neutrophils and lymphocytes. Moreover, a positive correlation was identified between the inflammatory cell GABA release and $GABA_A$ receptor expression, and the severity of pruritus, a characteristic symptom of the disease (Nigam et al. 2010).

TRP Channels

Decreased expressions of the pro-differentiating TRPC1/4/5/6/7 were reported in the epidermis and isolated keratinocytes of psoriatic patients. In addition, cultured psoriatic keratinocytes exhibited substantial defects in Ca^{2+} influx in response to high extracellular Ca^{2+} levels (Leuner et al. 2011), which may be explained by the suppressed TRPC channel expressions.

Non-ion Selective Channels

Elevated levels of AQP9 were described in lesional skin of psoriatic patients (Suárez-Fariñas et al. 2011). Likewise, highly upregulated levels of Cx26, which was shown to inhibit epidermal keratinocyte differentiation and hence barrier

formation, were identified in human psoriatic plaques and in hyperplastic warts (Lucke et al. 1999). Of clinical importance, the highly elevated Cx26 levels in psoriatic lesions were significantly suppressed after treatment of psoriasis with methotrexate and PUVA (Shaker and Abdel-Halim 2012) which suggests the role of Cx26 in the pathogenesis of the disease.

3.6.3 Non-melanoma Skin Cancers

Voltage-Gated Channels

Expression of mRNA of Kv3.4 K^+ channel was found to be increased in SCC. In addition, inhibition of Kv3.4 suppressed growth of oral SCC cells (Chang et al. 2003) which argues for that K^+ channel activities support malignant cell growth.

Ligand-Gated Channels

Expression of NMDAR1 in cutaneous SCC was found to inversely correlate with the degree of malignancy. Namely, very low (if any) expression was identified in un-differentiated SCC samples (Kang et al. 2009) whereas the reactive (non-neoplastic) epithelium surrounding the SCC showed strong NMDAR1 levels (Nahm et al. 2004). These data, on the one hand, further support the pro-differentiating role of NMDAR1-coupled signaling in keratinocytes; on the other hand, they also argue for that NMDAR1 may be a prognostic indicator for cutaneous SCC.

Human papillomaviruses are recognized as important human tumor promoters in the development of non-melanoma skin cancers (Biliris et al. 2000; Greig et al. 2006). Interestingly, in human skin warts as well as in raft cultures of CIN 612 cells, a model of keratinocytes infected with human papillomavirus type 31 (Ozbun 2002), up-regulation of the expression of P2X5 receptors was detected. In addition, P2X5 and P2X7 receptors were found in the nuclei of koilocytes, the abnormal keratinocytes characteristic of human papillomavirus infection (Greig et al. 2006). Based on these findings, as well as on the expression pattern of P2X receptor in the epidermis, it is therefore proposed that P2X5 receptors are likely to be involved in keratinocyte differentiation and P2X7 receptors are likely to be part of the machinery of end stage terminal differentiation/apoptosis of keratinocytes (Burnstock 2006; Gorodeski 2009; Burnstock et al. 2012). As an additional factor, the promoting role of these receptors in the anti-viral immune response may also be involved (see under Sect. 3.3.2).

Indeed, the pro-apoptotic role of P2X7 was also demonstrated in a two-stage (DMBA/TPA) mouse model of skin papilloma and SCC. In this model, the P2X7 specific agonist BzATP inhibited formation of tumors. Moreover, in cultured mouse keratinocytes BzATP induced prolonged Ca^{2+} influx and caspase-9 coupled apoptosis. Importantly, apoptosis was much less efficient in SCC keratinocytes

which exhibited four- to fivefold lower levels of P2X7 in cancer tissues. Therefore, activation of P2X7-dependent apoptosis (and possibly of the pro-differentiating P2X5 receptors) in skin papillomas and cancers as well as in melanomas may represent novel therapeutic tools.

TRP Channels

In BCC samples, the lack of epidermal expression of the pro-differentiating TRPC1/TRPC4 was observed (Beck et al. 2008) which was correlated with the impaired differentiation and enhanced proliferation of tumor cells. In addition, topical treatment with triterpenes of actinic keratosis, an in situ form of SCC, promoted cellular differentiation, most probably via the stimulation of TRPC6-mediated Ca^{2+}-influx to the cells (Woelfle et al. 2010).

In addition, TRPV1 knockout mice were shown to exhibit a highly increased susceptibility to induction of skin carcinogenesis (Bode et al. 2009). Since TRPV1 was described to inhibit proliferation and induce apoptosis in keratinocytes (see above under Sect. 3.1.3), it is proposed that TRPV1 (just as the above TRPCs) may be protective against skin tumor formation.

Non-ion Selective Channels

Further supporting the promoting role of AQP3 in epidermal proliferation, highly increased levels of AQP3 were identified in human SCC when compared to control skin (Hara-Chikuma and Verkman 2008c). In addition, in a multistage murine carcinogenesis model, AQP3 knockouts were found to be resistant to induction of tumorigenesis, also arguing for the pro-mitogenic role of AQP3 (Hara-Chikuma and Verkman 2008c). As tumor cells generally exhibit an aggressive energy metabolic profile (Gatenby and Gillies 2007), the glycerol transport mediated by AQP3 and the concomitant accumulation of cellular ATP may act as an important determinant of skin tumorigenesis. Hence, inhibition of AQP3 activity may provide a rational basis for the therapy of skin (and possibly other) cancers associated with overexpression of aquaglyceroporins.

3.6.4 Malignant Melanoma

Voltage-Gated Channels

The tumor-promoting roles of various K^+ channels are suggested in malignant melanoma. Indeed, on cell cultures of the human melanoma cell line SK MEL 28, inhibition of the expressed inwardly rectifying (K_{ir}) K^+ channels or K_{Ca} channels inhibited cell-cycle progression (Lepple-Wienhues et al. 1996). Likewise, in metastatic human melanoma cell lines, activation of $K_{Ca}3.1$ channels was shown to

promote the secretion of melanoma inhibitory activity, a soluble melanoma-derived factor which, by interacting with cell adhesion molecules and hence facilitating cell detachment, stimulates the formation of metastases (Schmidt et al. 2010).

Based on these results, it is proposed that membrane depolarization following the inhibition of these voltage-gated K^+ channels most probably reduces the driving force for the influx of Ca^{2+}, a key messenger in the mitogenic signal cascade of human malignant cells, which eventually results in cell cycle arrest. Therefore, voltage-gated K^+ channel inhibitors may represent novel therapeutic tools in the treatment of malignant melanoma.

Finally, it should be mentioned that silencing of the two-pore K^+ channel TASK-3, which is predominantly localized in the mitochondria in malignant melanoma cells (Rusznák et al. 2008), impaired cellular integrity and viability as well as proliferation of these cells (Kosztka et al. 2011).

Ligand-Gated Channels

Cultured melanocytes were shown to express the AMPARs GluA2 and 4 and the NMDAR2A and 2C whose activation by AMPA and NMDA resulted in elevation of intracellular Ca^{2+} concentration (Hoogduijn et al. 2006). Melanocytes also express specific glutamate transporters and decarboxylases; yet, glutamate production or release was not found. Glutamate treatment of human melanocytes did not affect melanin production and cell survival. However, application of AMPARs and NMDARs inhibitors induced disorganization of actin and tubulin microfilaments. In addition, the AMPA receptor inhibitor CFM-2 markedly suppressed the expression of microphthalmia-associated transcription factor, a key regulator of melanocyte differentiation and proliferation. Therefore, further studies are invited to define the potential role of ionotropic glutamatergic signaling in malignant melanoma.

In addition, increased expression of P2X7 receptors were identified in malignant melanomas (Slater et al. 2003) whose stimulation resulted in a Ca^{2+} influx-dependent induction of apoptosis (Deli et al. 2007). Therefore, just as described under Sect. 3.6.3, P2X7-targeted approaches may be beneficial not only in non-melanoma skin cancers, but also in malignant melanomas.

TRP Channels

Human epidermal melanocytes also express TRPM1 whose level was shown to correlate with melanin content of the cells indicating that functional TRPM1 channels are critical for normal melanocyte pigmentation (Devi et al. 2009; Oancea et al. 2009). Indeed, decreased expression of the *trpm1* gene was found to be associated with the coat spotting patterns of the Appaloosa horse (*Equus caballus*) (Bellone et al. 2008). In part similar to these findings, two mutations in the gene encoding TRPML3 were found to be correlated with the diluted coat color of the varitint-waddler mouse (Di Palma et al. 2002; Cuajungco and Samie 2008).

Certain TRPM channels also seem to be involved in the development of cutaneous melanoma. Namely, expression of the *trpm1* gene, which encodes the pro-apoptotic TRPM1, was found to exhibit an inverse correlation with the in vivo metastatic potential of skin melanoma (Deeds et al. 2000; Duncan et al. 2001; Miller et al. 2004; Zhiqi et al. 2004; Lu et al. 2010). Therefore, down-regulation of TRPM1 in the tumor was proposed as a prognostic marker for metastasis (Deeds et al. 2000; Duncan et al. 2001; Miller et al. 2004; Zhiqi et al. 2004). Likewise, upregulation of antisense, tumor-enriched (TE) transcripts of TRPM2 (another growth-inhibitory TRPM channel) was identified in human cutaneous melanoma (Orfanelli et al. 2008). Accordingly, functional knockout of TRPM2-TE or overexpression of wild-type TRPM2 in melanoma-derived cells augmented susceptibility to apoptosis (Orfanelli et al. 2008). Interestingly, an increased (and not decreased) level of TRPM8-specific transcripts, were also demonstrated in malignant melanoma (Tsavaler et al. 2001). Since activation of TRPM8 in human cultured melanoma cells induced Ca^{2+}-dependent cell death (Slominski 2008; Yamamura et al. 2008a), the functional significance of these findings are not currently understood.

Non-ion Selective Channels

Panx1 expression, which was found to be low in normal mouse melanocytes, increased in tandem with tumor cell aggressiveness in mouse malignant melanoma cell lines (Penuela et al. 2012b). In addition, gene silencing of Panx1 in BL6 mouse melanoma cell lines resulted in a marked suppression of in vitro cellular growth and migration and the down-regulation of the malignant melanoma markers vimentin and β-catenin. Likewise, the growth rate and metastasis-forming capacity of Panx1 knock-down cells also significantly decreased in a xenograft model (Penuela et al. 2012b). Although we lack human data, these findings collectively argue for the putative pathogenetic role of Panx1 (at least in murine) melanoma. In addition, these results also raise the possibility of a future management of the malignancy by inhibiting and/or down-regulating Panx1.

AQP1 channels are also expressed on human cultured melanocytes; however, their role in melanogenesis and melanocyte/melanoma growth is not known (Boury-Jamot et al. 2006). In addition, the elevated expressions of pro-proliferating Cx26 and Cx30 (but not of the pro-differentiating Cx43) were identified in the epidermal tumor microenvironment of malignant melanoma which correlated to the degree of malignancy (Haass et al. 2010).

3.6.5 Other Skin Diseases

Olmsted Syndrome

As we have shown above (under Sects. 3.2.3 and 3.3.3), a gain-of-function (Gly573Ser) mutation of the *trpv3* gene resulted in a spontaneous hairless phenotype and the development of AD-like itchy dermatitis in mice. Of greatest

importance, most recently, similar gain-of-function mutations of *trpv3* were identified in Olmsted syndrome, a rare congenital disorder characterized by palmoplantar and periorificial keratoderma, alopecia, and severe itching (Lin et al. 2012). In heterologous systems, mutant TRPV3 channels conveyed increased membrane currents and mediated augmented apoptosis which was also detected in the epidermis of the diseased patients. Therefore, Olmsted syndrome can be regarded as the first "truly cutaneous TRPpathy".

Smith–Lemli–Opitz Syndrome

Smith–Lemli–Opitz syndrome (SLOS) is an inherited disorder of cholesterol synthesis caused by mutations of the *dhcr7* gene which encodes the final enzyme in the cholesterol synthesis pathway (Tint et al. 1994). In this disease, 7-dehydrocholesterol accumulates in various cells and impairs key cell functions including those of skin fibroblasts (Honda et al. 1997; Wassif et al. 2002). In membrane caveolae of dermal fibroblasts of SLOS patients, impaired activity and markedly suppressed protein levels of BK K_{Ca} channel were observed (Ren et al. 2011). Since BK K_{Ca} channels were shown to co-migrate with caveolin-1, a key component of lipid rafts and hence regulator of a multitude of cell membrane-localized proteins (channels, receptors, transporters, etc.) and their signaling (Rothberg et al. 1992; Simons and Ikonen 1997; Ren et al. 2011), alterations in BK K_{Ca} channel functions may contribute to the pathophysiology of SLOS.

Pemphigus Vulgaris

Pemphigus vulgaris (PV) is a severe autoimmune blistering disease. In the pathogenesis of the dermatosis, the role of autoantibodies targeting (and then destroying) desmoglein-3, a key cell adhesion molecule of the epidermis, are suggested (Amagai and Stanley 2012). Intriguingly, α9 nAChRs were also found to be targeted by PV. Of further importance, inhibition of α9 nAChRs activity in keratinocyte cultures resulted in the development of PV-like morphology (acantholysis) which findings, besides further supporting the role of the ion channel in keratinocytes adhesion processes (see above under Sect. 3.1.2), argue for a potential pathogenetic role of α9 nAChRs in PV (Nguyen et al. 2000).

Mal de Meleda

Mal de Meleda is an autosomal recessive inflammatory and keratotic palmoplantar skin disorder due to mutations in the gene encoding SLURP-1 (secreted mammalian Ly-6/uPAR-related protein 1) (Fischer et al. 2001). Interestingly, SLURP-1 was shown to potentiate the effect of ACh on α7 nAChR channels (Chimienti et al. 2003; Chernyavsky et al. 2012). Since, as was detailed above, α7 nAChR receptors play

multiple roles in skin function, the authors hypothesized that the lack of this potentiation by SLURP-1 downregulation may contribute to the development of the characteristic skin symptoms of the disease.

Darier's Disease

TRPC1 is overexpressed in keratinocytes of patients with Darier's disease (DD) (or keratosis follicularis), a genetic disorder with loss-of-function mutations in the *SERCA2b* gene encoding endoplasmic reticulum Ca^{2+}-pumps, which is characterized by abnormal keratinization of the epidermis (Barfield et al. 2002; Pani et al. 2006). Importantly, cultured DD keratinocytes exhibited a greatly enhanced TRPC1-mediated (store-operated) Ca^{2+} influx, proliferation, and apoptosis resistance suggesting that TRPC1 may be involved in the pathological differentiation program (Pani et al. 2006).

Prurigo Nodularis

Markedly elevated TRPV1 levels were detected in the highly hyperkeratotic lesions of skin samples of prurigo nodularis patients (Stander et al. 2004). Furthermore, chronic (for several months) topical capsaicin treatment of the prurigo lesions (and hence the prolonged activation of the apoptosis-promoting TRPV1 expressed on keratinocytes) not only mitigated the intense pruritus, but also markedly reduced the epidermal hyperplasia and the hyper-orthokeratosis of the skin (Stander et al. 2001).

Diseases of the Adnexal Structures

K_{Ca} channels on normal sweat gland-derived cells exhibited similar functional properties to those expressed by cells from patients with cystic fibrosis (Henderson and Cuthbert 1991a), a common genetic disease characterized by, among others, defective sweat gland functions (Quinton 2007). However, eccrine sweat gland cells from these patients additionally expressed Ca^{2+}-independent, small-conductance, outwardly rectifying K^+ channels which were practically absent on cells from healthy donors (Henderson and Cuthbert 1991b). The impact of these findings is not yet known.

Although expressions of a huge variety of nAChR subunits were described in various compartments of the HF and sebaceous glands (summarized in Kurzen 2004; Kurzen et al. 2004, 2007; Kurzen and Schallreuter 2004; Grando et al. 2006), the functional role of these channels in pilosebaceous unit biology is not revealed. Likewise, it is also unknown whether ACh and the cutaneous cholinergic system is involved in mediating the effect of smoking to significantly increase the prevalence and disease severity of acne vulgaris (Schäfer et al. 2001).

However, with respect to the pathology of the pilosebaceous unit and the relationship with smoking, intriguing observations were made during the assessment of skin samples of hidradenitis suppurativa (HS) patients. Clinically, HS (a.k.a. acne inversa) is a chronic inflammatory skin disease emerging from the pilosebaceous units of the intertriginous areas (e.g. underarms, thighs, groin). Importantly, HS is considered as a nicotine-dependent dermatosis as more than 80 % of patients are active smokers (Jansen et al. 2001). In organotypic epidermal culture system, chronic nicotine exposure (12 days) resulted in epidermal thickening which was very similar to the hyperplastic epidermis seen in HS. In addition, highly elevated α7 nAChR levels were identified in HS epidermis, especially in the follicular infundibulum. These results propose that the cutaneous cholinergic system, most probably by promoting infundibular epithelial hyperplasia and thus follicular occlusion, may have a pathogenetic role in HS (Hana et al. 2007).

Another smoking-associated inflammatory skin disease, which is related to adnexal skin structures, is palmoplantar pustulosis (PPP) characterized by pustules, erythema and scaling on the soles and palms. Apparently, sweat glands are involved in the pathogenesis of PPP (impaired structure of the acrosyringium, outward migration of granulocytes from the acrosyringium to the str. corneum to form pustules). Importantly, in involved PPP skin, significant expressions of ChAT and α3 nAChRs were observed in the infiltrating granulocytes indicating a role for ACh in inflammation. Moreover, irregular expression patterns of α3 and α7 nAChRs were found throughout the epidermis. Currently, the exact mechanisms for the effect of nicotine/smoking in PPP is still unknown; yet, it is noteworthy that cessation of smoking improved all skin symptoms characteristics for PPP (Hagforsen 2007).

Finally, it might be of clinical importance that decreased AQP5 levels were detected in the secretory part of eccrine sweat glands of patients with Sjögren's syndrome, but not in skin affected by idiopathic segmental anhidrosis or idiopathic pure sudomotor failure (Iizuka et al. 2012).

Chronic Venous Insufficiency

The expression patterns of P2X5 and P2X7 receptors were found to be altered in the epidermis of patients with chronic venous insufficiency (CVI). In CVI, elevated P2X5 receptor levels were found mainly in the spinosal layer and extending further into the str. granulosum whereas expressions of P2X7 receptors were reduced in the str. corneum. It is proposed that the above alterations may contribute to the appearance of the thinner epidermis seen in CVI (Metcalfe et al. 2006).

"Connexin Diseases"

Further supporting its potential role in epidermal biology, Cx26 mutations were found to be associated with numerous hyperkeratotic skin disorders (palmoplantar keratoderma, keratitis-ichthyosis deafness syndrome, Vohwinkel

syndrome, hystrix-ichthyosis deafness syndrome, and Bart-Pumphrey syndrome) which are characterized by pathologically altered epidermal growth and differentiation (reviewed in Lee and White 2009).

3.6.6 Skin Ageing

As we have presented above (Sect. 3.3.3), TRPV1 was shown to mediate the effect of UV exposure and heat to induce skin inflammation and to upregulate MMP-1. Since chronic UV exposure is suggested to promote skin ageing, the role of TRPV1 in these processes is suggested. Indeed, applications of both heat and UV elevated expression of TRPV1 proteins in human skin in vivo. Moreover, as further support for its pro-ageing role, increased TRPV1 levels were found both in photoaged and intrinsically aged skin samples when compared to the expressions of the channel found in skins obtained from sunprotected areas and from young individuals, respectively (Lee et al. 2009a, 2012).

Interestingly, AQP3 expression was lower in aged than in young skin (Li et al. 2010), which is one of the key factors resulting in lower epidermal/skin water content and dry skin conditions seen in the elderly. Furthermore, UVB irradiation of NHEKs upregulated the expression of the pro-apoptotic P2X7 receptors (Inoue et al. 2005), which may lead to premature cell death.

Of further importance, comparison of dermal fibroblasts obtained from young, elderly and centenarian donors revealed age-dependent changes in K^+ channel expression and function. K^+ current amplitude was significantly smaller in fibroblasts from elderly than from young donors. In addition, expression of voltage-gated (K_v) shaker $K_v1.1$ channels was found to be higher in fibroblasts of elderly and centenarians whereas the BK K_{Ca} channel β1 subunit showed lower expression levels in fibroblasts of centenarians. It is possible, therefore, that the age-related remodeling of dermal fibroblast K^+ channel subtypes in centenarians might be associated with "successful" aging and hence provide a "predictive marker of longevity" (Zironi et al. 2010).

Finally, it should be noted that chronic nicotine exposure of cultured human dermal fibroblasts markedly altered the expression patterns of α3, α5, α7, β2, and β4 nAChR subunits (Arredondo et al. 2003). Therefore, it can also be postulated that premature ageing of the skin and impaired wound healing seen in chronic smokers may be related to pathological alterations of the fibroblast cholinergic system.

4 Concluding Remarks

In this paper, we have attempted to review the roles of ion and non-ion selective channels on non-neuronal cell types of the skin. Moreover, we have detailed recent evidence suggesting the involvement of certain channels in various skin diseases.

The major messages of this review are the followings:

- A plethora of ion and non-ion selective channels are expressed by various non-neuronal cell populations of the skin.
- On these cells, numerous channels, their endogenous activators/inhibitors, and their related signaling mechanisms were shown to play central roles in such key cutaneous processes as e.g. cellular growth, differentiation, and survival; formation, maintenance, and regeneration of the epidermal physical-chemical barrier; wound healing; inflammatory and immune responses, exocrine functions, etc.
- In addition, certain channels expressed by epidermal keratinocytes also contribute to the sensory functions of the skin (e.g. thermo-, osmo-, and pH sensation), via the release of soluble intercellular mediators which stimulate cutaneous sensory afferents.
- It is important to note, that the involved channels may exert both synergistic as well as antagonistic effects in the regulation of the above processes. This is especially remarkable during the recovery of the epidermal barrier following its disruption.
- Finally, pathological alterations in the homeostatic channel-coupled mechanisms are implicated in various dermatoses (e.g. AD, psoriasis, skin cancers, autoimmune and genetic diseases, etc.) and in skin ageing.

Evidently, more extensive in vitro and in vivo studies are urgently needed to reveal the exact molecular roles of these channels in skin physiology and pathophysiology. Yet, we strongly believe that the presented intriguing findings will encourage future, highly sophisticated pre-clinical and clinical trials to explore the seemingly rich potential of channel-targeted management of various skin diseases.

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