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DIFFERENCES IN ION CHANNEL PHENOTYPE AND FUNCTION BETWEEN HUMANS AND ANIMAL MODELS

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Abstract

Ion channels play crucial roles in regulating a broad range of physiological processes. They form a very large family of transmembrane proteins. Their diversity results from not only a large number of different genes encoding for ion channel subunits but also the ability of subunits to assemble into homo- or heteromultimers, the existence of splice variants, and the expression of different regulatory subunits. These characteristics and the existence of very selective modulators make ion channels very attractive targets for therapy in a wide variety of pathologies. Some ion channels are already being targeted in the clinic while many more are being evaluated as novel drug targets in both clinical and preclinical studies. Advancing ion channel modulators from the bench to the clinic requires their assessment for safety and efficacy in animal models. While extrapolating results from one species to another is tempting, doing such without careful evaluation of the ion channels in different species presents a risk as the translation is not always straightforward. Here, we discuss differences between species in terms of ion channels expressed in selected tissues, differing roles of ion channels in some cell types, variable response to pharmacological agents, and human channelopathies that cannot fully be replicated in animal models.

Keywords

Ion channels; disease; drug development; animal model; target validation; pharmacology

2. Introduction

The use of animal models is integral to the understanding of physiological and pathophysiological processes, to the validation of new drug targets, and to the development of novel therapeutics. However, the choice of incorrect animal models too often leads to the generation of information that cannot be translated to humans or to the failure of clinical trials. Many factors should influence the choice of animal model, for example the age and gender of the animals compared to the target patient population (1). It is also important to take in account the relevance of the animal model in terms of molecular drug targets. Here,

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we will focus on ion channels as targets for therapy and diagnosis of a wide range of diseases.

More than 300 different ion channels, belonging to 13 superfamilies, have been identified in the human genome (2). Ion channels are proteins located in the cell membranes; they form pores in the membrane and tightly control the influx or efflux of ions in cells or organelles (3). Most channels require the assembly of multiple subunits to form functional pores. A channel subunit can be part of a homomultimer in a cell type and also be part of a heteromultimer in another cell type, leading to an even higher diversity in channels, but also to a restricted tissue distribution of most channels. In addition, diversity in ion channels is increased by the expression, in some tissues, of regulatory subunits that interact with pore-forming subunits to regulate their expression, localization, and function.

Historically, ion channels have been called different names by different groups, generating confusion in the literature. For example, the large conductance voltage- and calcium-dependent potassium channel is known as KCa1.1, BK, Maxi-K, or Slo1, while the gene encoding for its pore-forming α subunit is called either *slo1* or *KNCMA1*. In an attempt to reduce confusion, we will use the nomenclature established by the International Union of Basic and Clinical Pharmacology (IUPHAR) (4) to name all ion channels throughout this review. We will also provide other commonly used names, as appropriate.

Ion channels regulate a wide range of physiological and pathophysiological processes. Because of their diversity, their restricted tissue distribution, and their important roles in regulating critical cell functions, they are very attractive therapeutic or diagnostic targets in a wide range of diseases (5, 6). For example, Ziconotide (SNX-111), the synthetic version of ω-conotoxin MVIIA from the venom of the marine snail *Conus magus*, is a powerful analgesic drug that selectively blocks a voltage-gated calcium channel and is approved for the treatment of severe chronic pain (5, 7). Chlorotoxin, a chloride channel blocker from the venom of the scorpion *Leiurus quinquestriatus*, is undergoing clinical trials as a potential tool for the diagnosis of glioma (8). Also undergoing clinical trials is ShK-186 (Dalazatide), a synthetic analog of ShK, a $K_V1.3$ potassium channel blocking peptide from the venom of the sea anemone *Stichodactyla helianthus*, for its selective immunomodulatory properties on effector memory T lymphocytes in autoimmune diseases (9–11).

It can be tempting to extrapolate data obtained with an ion channel in one species to another species. While some ion channel phenotypes and functions are highly reproducible between humans and common laboratory animal models, in some tissues dramatic difference in ion channels expression, function, and sensitivity to pharmacological agents have been identified over the years. Here, we will review differences in ion channel expression, function, and response to pharmacological agents between human cells and tissues and mammalian animal models using selected examples in the literature to highlight the complexity of choosing the appropriate animal model for the question to be answered.

3. DIFFERENTIAL EXPRESSION OF ION CHANNELS IN HUMANS AND OTHER MAMMALS

Investigators have often assumed that the identification of one or more ion channels in a specific tissue or cell type could be directly translated to another species. While this can be the case, as for example when comparing the potassium channel phenotype of human and rat fibroblast-like synoviocytes (12, 13), it is not always true in other cell types or when focusing on other ion channels. This has led to testing potential novel therapeutics in the incorrect animal model. In this section, we highlight a few examples of differences in ion channel expression between different species.

3.1. Expression differences of potassium, sodium, and calcium channels in the cardiovascular system

The cardiovascular system consists of the heart, the blood vessels, and the blood that circulates through the body. This system is responsible for transporting oxygen, nutrients, cellular waste, and hormones to all organs. The heart has four chambers; the upper chambers are the atria and the lower chambers are the ventricles. The septum, a wall of muscle, separates the left and right atria and the left and right ventricles. Valves regulate blood flow through the different chambers of the heart.

The electrocardiogram measures electrical activity in the heart and is an important tool in the diagnosis of patients suffering from cardiac disease. Mice are often used as models of cardiovascular diseases, however the heart rate of mice is approximately 10-fold higher than that of humans, which requires shorter action potentials and different repolarizing potassium currents. Indeed, the electrocardiogram of mice and humans is very different (14, 15). The most prominent difference is the presence of a J wave and the absence of an isoelectric ST segment in the mouse electrocardiogram. These differences can be explained by differences in ion channels expressed in human and mouse hearts.

A transient outward current (I_{to}) plays a role in the prominent early phase of action potential repolarization. I_{to} was originally attributed to chloride ions but additional work showed it is carried by potassium ions. It has been identified in atrial and ventricular myocytes in most mammals, including humans, rats, mice, ferrets, cats, rabbits, and dogs (16). It was also identified in sinoatrial and atrioventricular nodal cells (16). I_{to} has two components, the fast ($I_{to,f}$) and the slow ($I_{to,s}$) components. Their density varies between myocytes within a species and also between species (16). In human and ferret, $I_{to,f}$ densities are higher in epicardial cells while $I_{to,s}$ is predominant in endocardial myocytes. In rat ventricles, $I_{to,f}$ densities are higher in epicardial cells than endocardial and septal cells; $I_{to,s}$ densities are similar. In mouse, $I_{to,f}$ densities are higher in right than left ventricle myocytes; they are low in the interventricular septum. $I_{to,s}$ is only expressed in the mouse septum. In human, dog, and mouse atrial myocytes, only $I_{to,f}$ is expressed whereas $I_{to,s}$ is predominant in rabbit atrial myocytes. In all species studied (rat, ferret, dog, human, and mouse), $I_{to,s}$ is carried by $K_V1.4$ channels (16). $I_{to,f}$ modulates calcium handling, the action potential profile, and contractility and hypertrophy in cardiomyocytes. Alpha subunits of the voltage-gated K_V4 channel underlies $I_{to,f}$. In human and canine ventricle cardiomyocytes, the $K_V4.3$ potassium

channels is responsible for $I_{to,f}$ (17). In contrast, rodent and ferret ventricle myocytes express both $K_V4.2$ and $K_V4.3$ (18–20). Importantly, Kv4.3 was detected in only a few cardiac cells in the Göttigen minipig (21). As a result of these differences, the study of $K_V4.3$ modulators may have drastically different effects on the cardiac function of humans and rodents, Göttigen minipigs, or ferrets.

Voltage-gated sodium (Na_V) channels mediate the rapid increase in sodium permeability during the initial phase of the action potential. Blechschmitt *et al.* compared expression levels of Na_V1 isoforms in the hearts of humans, pigs, rats, and mice (22). While the pig heart resembles most the human heart in terms of transcript levels of Na_V1 channels, rat and mouse heart Na_V1 expression patterns are very different from one another and from the human heart. In both the human and the pig heart, $Na_V1.5$ is the predominant Na_V channel with low expression of $Na_V1.1$, $Na_V1.3$, and $Na_V1.4$. Interestingly, while $Na_V1.5$ is the dominant isoform in both male and female human hearts, levels of the three other isoforms vary with gender. Whereas $Na_V1.1$ accounts for 5% of all sodium channel transcripts in the rat heart, much lower levels were detected in human, mouse, and pig hearts. $Na_V1.2$ transcripts are the highest in mouse hearts in which they account for ~7% of all sodium channel transcripts but are much lower in the rat heart and negligible in human and pig hearts. Approximately 15% of all sodium channel transcripts in the mouse heart are $Na_V1.3$, but are only ~2% in the other three species studied. $Na_V1.4$ transcripts represent ~7% of all sodium channel transcripts in the mouse heart but are expressed at much lower levels in the human and pig heart and its level is negligible in rat hearts.

The primary function of arterial smooth muscle cells is contraction and relaxation. During contraction, vascular smooth muscle cells shorten and therefore decrease the diameter of the blood vessel and enhance blood pressure. Voltage-gated calcium (Ca_V) channels play important roles in the transduction of vascular smooth muscle contractility. Human cerebral artery smooth muscle cells express $Ca_V1.2$ (a.k.a. L-type calcium channel), $Ca_V3.2$ (a.k.a. T-type calcium channel), and $Ca_V3.3$ (a.k.a. T-type calcium channel) (23). Mouse and rat arterial smooth muscle cells also express $Ca_V1.2$ and $Ca_V3.2$, but $Ca_V3.3$ is replaced by $Ca_V3.2$ in these two species (24, 25). Human, mouse, and rat smooth muscle cell inward calcium currents have the same voltage dependence. However, in going with the switch between $Ca_V3.3$ and $Ca_V3.2$, inward current densities were lower in human smooth muscle cells than their rat or mouse counterparts (23). In addition, the reversal potential of rat currents is depolarized compared to human or mouse whereas whole-cell currents inactivate faster in mouse smooth muscle cells than their human or rat counterparts (23). The switch in a single Ca_V isoform between species can therefore drastically affect cell function.

3.2. Human and mouse T lymphocytes express different potassium channels

The immune system is a network of organs, tissues, cells, and secreted molecules that interact to protect the body against infectious agents and tumor cells and to balance each other to prevent over-reaction to threats and damage to the host itself. Cells of the immune system have been divided into innate and adaptive immune cells. T lymphocytes are cells of the adaptive immune system. They are activated by antigens presented by antigen-presenting cells, such as dendritic cells, macrophages, or B lymphocytes and this activation leads to

their proliferation, migration to sites of inflammation, and production of mediators of inflammation. Loss of T lymphocyte function can leads to immunodeficiency and a heightened risk of infections, and can be lethal. Dysregulation of T lymphocytes can lead to autoimmunity or cancer.

Because of their crucial roles in the immune system, T lymphocytes have been extensively studied, including the phenotype and function of their ion channels. Two potassium channels were described in human T lymphocytes in the mid-1980s, the voltage-gated $K_V1.3$ channel (a.k.a. the n channel or *KCNA3*) and the calcium-activated $K_{Ca}3.1$ channel (a.k.a. IKCa1, SK4, or *KCNN4*) (26, 27). In humans, these channels regulate the membrane potential of T cells and promote Ca^{2+} influx through Ca^{2+} release-mediated Ca^{2+} channels; they therefore regulate T cell activation and subsequent proliferation and secretion of cytokines and chemokines (28). The relative contribution of $K_V1.3$ and $K_{Ca}3.1$ to these functions depends on the channel expressed at higher levels in a given human T cell. Human T lymphocytes can be separated into three distinct populations based on the surface expression of the chemokine receptor CCR7 and the phosphatase CD45RA (29). Naïve T cells express both CCR7 and CD45RA whereas central memory T cells are $CCR7^+$ and $CD45RA^-$ and effector memory T cells are $CCR7^-$ and $CD45RA^-$. Quiescent T cells from all three populations express 200–300 $K_V1.3$ channels/cell and 8–20 $K_{Ca}3.1$ channels/cell (30–32). After activation, $CCR7^+$ T cells up-regulate the $K_{Ca}3.1$ channel to express 400–500 $K_V1.3$ channels/cell and 200–500 $K_{Ca}3.1$ channels/cell. In contrast, $CCR7^-$ T cells up-regulate $K_V1.3$ channels upon activation to express ~ 1500 $K_V1.3$ channels/cell and only ~ 10 $K_{Ca}3.1$ channels/cell. This phenotype is observed in both $CD4^+$ and $CD8^+$ human T cells.

Rat naïve, central memory, and effector memory T lymphocytes display similar $K_V1.3$ and $K_{Ca}3.1$ channel phenotypes to their human counterparts (32–34), as do rhesus macaque T lymphocytes (35). Mouse T lymphocytes, however, exhibit significant differences in their potassium channel patterns (32). Quiescent mouse T cells express only 10–30 potassium channels/cell. Besides $K_V1.3$, $CD4^+$ mouse T cells also express $K_V1.1$, $K_V1.2$, and $K_V1.6$ channels whereas $CD8^+$ mouse T cells express $K_V3.1$ instead of $K_V1.3$ (36–39). Activation of mouse T cells induces an increase in potassium channels to 200–300/cell. Repeated activation of mouse T cells induces a progressive up-regulation of both K_V and $K_{Ca}3.1$ channels whereas the same protocol induces an up-regulation of $K_V1.3$ and a down-regulation of $K_{Ca}3.1$ by human and rat T lymphocytes (31–33, 40). In addition, whereas the membrane potential of human and rat T lymphocytes is maintained by potassium channels, in mouse T cells it is maintained by the Na-K-ATPase (41). Finally, $K_V1.3$ transcripts are abundant in the mouse thymus whereas in humans they are predominantly expressed in mature T cells (32).

The differences in potassium channel expression by various T lymphocyte populations in different species have drastic consequences on the outcomes of inhibiting the expression or function of a given channel (10, 32, 42). Human and rat $CCR7^-$ effector memory T lymphocytes are highly sensitive to $K_V1.3$ channel blockers that inhibit their activation, proliferation and ability to secrete cytokines (31, 33, 43–45). On the other hand, human and rat $CCR7^+$ naïve and central memory T lymphocytes, initially sensitive to $K_V1.3$ blockers, up-regulate $K_{Ca}3.1$ channels, rendering them resistant to $K_V1.3$ blockers and sensitive to

$K_{Ca}3.1$ blockers (30). As a result, $K_V1.3$ channel blockers show efficacy in reducing inflammation in rat models of $CCR7^-$ effector memory T cell-mediated inflammatory diseases, such as experimental autoimmune encephalomyelitis, pristane-induced arthritis, asthma, or autoimmune periodontitis without preventing the clearance of acute infections (34, 43, 44, 46–49). Similar results were obtained in a mouse model of psoriasis induced by a xenograft of human lesioned skin (50) and a selective $K_V1.3$ channel blocker showed efficacy in patients with active plaque psoriasis (11). $K_V1.3$ knockout mice are resistant to a model of multiple sclerosis (51), demonstrating similar effects as blocking $K_V1.3$ in rat models of this disease (34, 46, 47). However, blocking $K_{Ca}3.1$ is also effective in reducing severity in a mouse model of multiple sclerosis (52) whereas it provides little benefits in a rat model of this disease (34).

3.3. Potassium channels in human, mouse, and rat spermatocytes and spermatozoa

Sperm is the male reproductive cell, named after sperma, the Greek word for “seed”. Spermatozoa are equipped with a strong flagellum used to propel them for fertilization of the egg.

Potassium channels regulate membrane potential and cell motility; they therefore are indispensable for normal sperm physiology. In 1998, Schreiber et al. demonstrated the presence of the calcium-activated potassium channel $K_{Ca}5.1$ (a.k.a. Slo3, *KCNU1*), a pH sensitive potassium channel, in mouse spermatocytes, the precursors of spermatozoa (53). This work was extended approximately a decade later by the demonstration that $mK_{Ca}5.1$ underlies the potassium current (mKSper) recorded in mouse spermatozoa (54, 55). At the time, the assumption was that $K_{Ca}5.1$ carries the potassium current in spermatocytes and spermatozoa of all mammalian species although mouse spermatozoa are twice larger than human spermatozoa but have only about half the capacitance (56, 57). However, whole-cell patch-clamp studies of rat spermatocytes demonstrated the expression of the large-conductance $K_{Ca}1.1$ channel (a.k.a. Slo1, BK, MaxiK, *KCNMA1*) and of a 4-aminopyridine-sensitive K_V channel (58). Subsequent work with human spermatozoa demonstrated that their potassium current (hKSper) is sensitive to intracellular calcium but not to intracellular alkalinization (59). A combination of RT-PCR, immunoblotting, immunocytochemistry, and electrophysiology showed that $K_{Ca}1.1$ underlies the hKSper current (59). More recent work comparing the pharmacological profile of human and mouse $K_{Ca}5.1$ (detailed in section 5.3) further complicates the findings, even suggesting that hKSper could be composed of a combination of $K_{Ca}1.1$ and $K_{Ca}5.1$. The identification of $K_{Ca}1.1$ in human spermatozoa also concluded to the expression of β3 regulatory subunits of $K_{Ca}1.1$ in these cells (59). The finding that different splice variants of the β3 regulatory subunit of $K_{Ca}1.1$ are expressed in primates and non-primates (detailed in section 4.2) adds to the complication of the precise identification of the potassium channels that underlie mKSper and hKSper.

3.4. Potassium channel differences in fibroblast-like synoviocytes

Fibroblast-like synoviocytes (FLS) are one of the primary cell types that compose the synovial lining of diarthrodial, freely moveable joints. Within healthy joints, FLS have a role in maintaining joint homeostasis through the secretion of components of synovial fluid, such

as lubricin and hyaluranon (60, 61). During rheumatoid arthritis, an autoimmune disease involving inflammation of the synovial joints, FLS develop a pathogenic phenotype characterized by a high degree of invasiveness and protease secretion, leading to extracellular matrix and bone degradation, as well as the secretion of pro-inflammatory cytokines and chemokines (60, 61).

FLS isolated from patients with rheumatoid arthritis express the pore-forming α subunit of $K_{Ca}1.1$ (a.k.a. Slo1, BK, MaxiK, *KCNMA1*) at their plasma membranes, as well as $K_{Ca}3.1$ channels (*a.k.a.* IKCa1, SK4, or *KCNN4*) (12, 62–64). $K_{Ca}1.1$ is upregulated on highly invasive FLS from patients with rheumatoid arthritis compared to those from patients with osteoarthritis, whose FLS are less invasive (63). Furthermore, the regulatory β3 subunit of $K_{Ca}1.1$ is associated with $CD44^{high}$, highly invasive FLS from patients with rheumatoid arthritis, whereas the β1 subunit of $K_{Ca}1.1$ is found in $CD44^{low}$, less invasive FLS (see section 4.2 for more details about $K_{Ca}1.1$ regulatory subunits) (63). A similar expression and upregulation of $K_{Ca}1.1$ α is observed in FLS isolated from rats with the pristane-induced arthritis model of rheumatoid arthritis (13). However, FLS cultured from rabbit synovium express mRNA for $K_V1.1$, 1.4, 1.5, and 1.6 and have outward currents mainly from $K_V1.1$ channels (65) and not from $K_{Ca}1.1$, as described in humans and rats.

Selectively blocking $K_{Ca}1.1$ reduces the *in vitro* invasion and proliferation of both human and rat FLS, along with reducing their secretion of cytokines and proteases. Systemic treatment of rats with pristane-induced arthritis or collagen-induced arthritis with the small molecule $K_{Ca}1.1$ blocker paxilline significantly reduces disease severity, indicating the potential of $K_{Ca}1.1$ to be used as a therapeutic target for rheumatoid arthritis (13). The similarities between potassium channels in rat and human FLS highlight the importance of using the proper animal model to study drug efficacies that have the potential to be translated to humans.

$K_{Ca}3.1$ has also been identified on FLS from rheumatoid arthritis patients and is found at similar expression levels at the mRNA level as $K_{Ca}1.1$ (62). $K_{Ca}3.1$ block with the small molecule inhibitor TRAM-34 reduces FLS production of several cytokines, as well as causes a reduction in cell proliferation (62). However, in this study TRAM-34 was used in concentrations between 20 and 40 μM, doses at which it is no longer selective for $K_{Ca}3.1$ over $K_{Ca}1.1$ and other channels (66) and the observed effects of TRAM-34 on FLS phenotypes could be due in part to off-target effects. Furthermore, siRNA-mediated knockdown of $K_{Ca}3.1$ did not recapitulate all of the effects of TRAM-34 on FLS. This is in contrast to $K_{Ca}1.1$ knockdown, which mimics the effects of $K_{Ca}1.1$ blockers (13, 62). This further indicates the possibility of off-target effects of TRAM-34 used at high doses. However, $K_{Ca}3.1$ knockout mice are resistant to the development of collagen-induced arthritis, a model of rheumatoid arthritis, indicating the potential value of $K_{Ca}3.1$ as a target for rheumatoid arthritis (67). However, the potassium channel repertoire of mouse FLS is currently unknown and the observed resistance to inflammatory arthritis in $K_{Ca}3.1$ knockout mice may therefore be due to changes in other cell types involved with disease progression, such as T cells that express $K_{Ca}3.1$ (see section 3.2).

4. DIFFERENT ROLES OF ION CHANNELS IN SPECIFIC CELL TYPES IN HUMANS AND OTHER MAMMALS

Ion channels play crucial, yet diverse, roles in the functions of many different cell types. They regulate ion homeostasis, cell volume and morphology, cell activation and migration, ability of cells to secrete growth factors, hormones and other soluble peptides and proteins, contraction of skeletal muscles, propagation of nerve conduction, and many other functions. When a specific channel is expressed in a cell type in multiple species, it often performs the same function. For example, the calcium release-activated calcium channel expressed by T lymphocytes mediates the calcium influx necessary for T cell activation in all species in which it has been studied (28). In some cases, however, the same ion channel in expressed in different species but, in the same cell type, doesn't play the same role, as discussed in this section.

4.1. Regulation of F-actin organization in astrocytes

Astrocytes are the most numerous and diverse cells in the central nervous system. They display remarkable heterogeneity in both morphology and function. They are important in the development and maintenance of the brain architecture and homeostasis, they store and distribute energy substrates, they regulate the development of neural cells, and they play a role in brain defense.

Water transport is highly regulated in tissues. In the central nervous system, it is involved in the regulation of cell volume, in the production of cerebrospinal fluid, and in the pathogenesis of edema. Aquaporin-4 (AQP4; a.k.a. the mercury-insensitive water channel) is the main water channel in the brain. It was identified in the perivascular membranes of human, mouse, and rat astrocytes. While it is expressed by astrocytes in all three species, some of its functions differ between species (68). The knock down of AQP4 in rat astrocyte primary cultures leads to dramatic changes in cell morphology and reduction in membrane water permeability (69). Similar changes in astrocyte morphology can be observed when using human cells (68). In contrast, the knock down of AQP4 in primary mouse astrocytes induces only moderate changes in cell morphology (68). Interestingly, while the knock down of AQP4 in mouse astrocytes induces only moderate alterations in cell morphology, it induces profound structural cytoskeleton changes in which the cortical layer of F-actin is completely replaced by fibers with a star-like organization. In human and rat astrocytes, AQP4 knock down results in actin depolymerization. These findings demonstrate a role for AQP4 in regulating the actin cytoskeleton in astrocytes but the mechanism of action is different in mouse and rat or human astrocytes.

4.2. Species-specific expression and effects of splice variants of K_{Ca}1.1 regulatory β subunits

The pore of the large-conductance K_{Ca}1.1 potassium channel (a.k.a. Slo1, BK, MaxiK, *KCNMA1*) is formed by the tetrameric assembly of four α subunits (70). In some cells, K_{Ca}1.1 channels are also composed of regulatory subunits. Four regulatory subunits were first identified and named β1, β2, β3, and β4; they are encoded by the *KCNMB1*, *KCNMB2*, *KCNMB3*, and *KCNMB4* genes (71–74). More recently, four more regulatory

subunits of $K_{Ca}1.1$ were identified in a different structural family. These were named γ1 - γ4; they are encoded by the *LRRC26*, *LRRC52*, *LRRC55*, and *LRRC38* genes (75, 76). These auxiliary subunits play important roles in regulating the voltage- and calcium-dependence of the $K_{Ca}1.1$ channel, its gating kinetics, inactivation, and ion current rectification, and its sensitivity to modulators (77–79). The ability of β and γ subunits of $K_{Ca}1.1$ to modify both the biophysical and pharmacological fingerprint of the channel make them highly relevant to the identification of the expression and role of $K_{Ca}1.1$ in different cell types and to the generation of selective modulators of this channel for the development of both research tools and of potential novel therapeutics.

The β1, β2, and β4 regulatory subunits of $K_{Ca}1.1$ from mice and humans share 83.2%, 95.3%, and 93.8% homology, respectively (80). In contrast, the mouse $K_{Ca}1.1$ β3 subunit shares only 62.8% identity with its human counterpart when N-terminal splice variants are not included in the study. Different splice variants of the human β3 subunit of $K_{Ca}1.1$ have been identified and named β3a-d (81, 82). In contrast, screening of mouse cDNA libraries and searches in the mouse genome revealed the expression of only two N-terminal splice variants of $K_{Ca}1.1$ β3: β3a and β3b (80). A further analysis of the genome of 28 species sets of vertebrates and 18 species sets of placental mammals showed that the β3c and β3d splice variants are primate-specific are not expressed in rodents and other non-primate species (80).

Co-expression of the α subunit of $K_{Ca}1.1$ with different human splice variants of β3 subunits leads to different effects on the resulting potassium current, such as inactivation and voltage- and calcium-dependence (80, 81). Human β3a and β3c induce inactivation of the $K_{Ca}1.1$ current whereas β3d does not affect it (81, 82). Interestingly, human β3b induces a very fast inactivation, but only in a small number of the cells tested (N = 3/7 and 4/10 of the cells tested) (81, 82). Although β3a and β3b are expressed by both humans and mice, they have different effects on $K_{Ca}1.1$ currents (80). Mouse β3a induces a faster and more complete $K_{Ca}1.1$ current inactivation than human β3a. Whereas some of the cells expressing human β3b display a very fast but incomplete inactivation of the $K_{Ca}1.1$ current, such inactivation is not observed with mouse β3b (80). Interestingly, the N-terminus of human and mouse β3b vary. Mouse β3b contains a deletion of 6 amino acids and the phenylalanine in position 4 of human β3b is replaced by a leucine in mouse β3b.

The β3 subunit of KCa1.1 has been identified in a number of cell types. Since its splice variants have very different effects on $K_{Ca}1.1$ currents in humans and other species, a careful investigation of the models used for future studies is necessary before any conclusion can be drawn for potential translation to human health. For example, minimally invasive FLS (see section 3.4) from patients with osteoarthritis or rheumatoid arthritis express the β1 subunit of $K_{Ca}1.1$ whereas highly invasive FLS from patients with rheumatoid arthritis express β3b (63). Translation of these findings to animal models of rheumatoid arthritis or osteoarthritis will require verification of the phenotype in the chosen species.

4.3. Regulation of ion fluxes in pancreatic beta cells

The pancreas is an organ in the abdomen. The bulk of this organ consists of the exocrine pancreas that plays an important role in digestion by releasing enzymes into the gastrointestinal tract. The pancreas also has an endocrine function with islets of Langerhans

that release the hormones insulin and glucagon into the circulation to regulate glucose levels. Within the islets of Langerhans, the beta cells are the cells that produce insulin.

In human pancreatic beta cells, the $K_{Ca}1.1$ channel (a.k.a. Slo1, BK, MaxiK, *KCNMA1*) carries a rapidly activated potassium transient that can be blocked by selective and non-selective blockers of $K_{Ca}1.1$ (83). While mouse pancreatic beta cells also express the $K_{Ca}1.1$ channel, it doesn't play a major role in glucose-induced electrical activity or insulin secretion (84). The second, delayed, component of the potassium current is carried by voltage-gated K_V2 channels. The $K_V2.1$ isoform is predominant in mouse islets (85) whereas transcripts of $K_V2.2$ are more abundant in human islets. However, staining for $K_V2.1$, but not $K_V2.2$, overlaps with insulin staining showing that in human beta cells, as in mouse beta cells, $K_V2.1$ is the predominant isoform (86). Blocking $K_V2.1/2.2$ channels with stromatoxin stimulates insulin secretion in mouse but not human pancreatic beta cells (85, 87). In rats, pancreatic beta islets also express $K_V1.4$, which is not found in human or mouse beta cells (85, 86, 88).

Human and dog pancreatic beta cells express voltage-dependent sodium channels that can be activated at physiologically relevant membrane potentials (-70 mV and higher) (89, 90), whereas voltage-dependent sodium current in mouse pancreatic beta cells are only detected at hyperpolarized holding potentials (91).

5. CHANNELS WITH DIFFERENT RESPONSES TO PHARMACOLOGICAL AGENTS IN DIFFERENT SPECIES

When ion channels are conserved between species and expressed in the same cell types, it is assumed that they will exhibit the same response to pharmacological agents. This is often correct, for example, ShK, a peptide from the venom of the sea anemone *Stichodactyla helianthus*, blocks $K_V1.3$ channels of human, mouse, and rat origin with similar IC_{50}s in the low picomolar range (31, 34, 92). This assumption can however also be incorrect and lead to errors in data interpretation when comparing cells from different species. Indeed, we highlight here a few examples of ion channels with different sensitivities to pharmacological agents in different species.

5.1. Mouse and rat, but not human, TRPV2 channels are sensitive to 2APB

Transient receptor potential (TRP) channels are non-selective cation channels classified into seven families: TRPC, TRPV, TRPM, TRPN, TRPA, TRPP, and TRPML. All share the membrane topology of voltage-gated potassium channels. TRPV2 (a.k.a. Osm-9-like TRP channel 2, OTRPC2) is the only member of the TRPV family that does not play a role in temperature sensing but rather acts as a mechanosensor. The sequence identity between human, rat, and mouse TRPV2 orthologs ranges from 75 to 90% (93). Mouse and rat TRPV2 channels are activated by 2-aminoethoxydiphenyl borate (2APB) with EC50s of 187 μM and 59 μM, respectively. In contrast, the human TRPV2 channel is resistant to this compound when tested at concentrations up to 300 μM (94). Human TRPV2 exerts a dominant-negative effect on 2APB activation on native mouse TRPV2 channels.

5.2. Different pharmacological profile and response to cold of TRPA1 channels in different species

The transient receptor potential ankyrin-1 (TRPA1) channel is expressed at high levels in a subpopulation of C-fiber primary afferent neurons. Mustard oil and wasabi cause a rapid and intense burning sensation; this is mediated by the binding of the active ingredient in mustard oil, allyl isothiocyanate, to TRPA1 channels and its action as an agonist (95). These channels have therefore emerged as potential targets for the development of analgesic and anti-inflammatory drugs.

Four different trichloro(sulfanyl)ethyl benzamides (AMG2504, AMG5445, AMG7160 and AMG9090) are potent antagonists of human TRPA1 (hTRPA1) (96). Whereas two of these (AMG2504 and AMG7160) act as weak antagonists of rat TRPA1 (rTRPA1), the other two (AMG5445 and AMG9090) act as partial agonists.

Nagatomo and Kubo reported in 2008 agonistic effects of caffeine on mouse TRPA1 (mTRPA1) and its antagonist effects on hTRPA1 (97) (Table 1). A few years later, further work was done to compare the pharmacological profile of TRPA1 channels from different species (98). The orthologs were transiently expressed in HEK293-F cells using patch-clamp electrophysiology and calcium influx assays. TRPA1 from four different species were compared: hTRPA1 was compared to rhesus macaque TRPA1 (rhTRPA1), rTRPA1, and mTRPA1. The pharmacological profile of hTRPA1 is most similar, but not identical, to that of rhTRPA1. The profiles of mTRPA1 and rTRPA1 are similar to each other but different from that of hTRPA1 and rhTRPA1. The agonist mustard oil opens hTRPA1, mTRPA1, and rTRPA1 with similar EC_{50}s in the low micromolar range, respectively. It is less active on rhTRPA1 with an EC_{50} of 75 μM. Another agonist, 4-hydroxynonenal, opens hTRPA1, mTRPA1, and rTRPA1 with similar EC_{50}s in the low micromolar range, but has no effects on rhTRPA1 at doses up to 100 μM. Farnesyl thiosalicylic acid is a potent agonist of hTRPA1 (EC_{50} 5 μM) with a lower activity on rhTRPA1 (EC_{50} 167 μM), rTRPA1 (EC_{50} 102 μM), and mTRPA1 (EC_{50} 85 μM). Similarly, 3′-carbamoylbiphenyl-3-yl cyclohexylcarbamate is an agonist of hTRPA1 (EC_{50} 7.5 μM), mTRPA1 (EC_{50} 74 μM), and rTRPA1 (EC_{50} 129 μM) with no effect on rhTRPA1 at 100 μM. Trinitrophenol is an agonist of mTRPA1 (EC_{50} 30 μM), rTRPA1 (EC_{50} 71 μM), and hTRPA1 (107 μM) with no effect on rhTRPA1 at 300 μM. Menthol is a much more potent agonist of mTRPA1 and rTRPA1 (EC_{50}s 5 and 7 μM, respectively) than of hTRPA1 (EC_{50} 278 μM) and has no effects on rhTRPA1 at 300 μM. (E)-1-(4-fluoro-phenyl)-2-methyl-pent-1-en-3-one oxime is a potent antagonist of hTRPA1 and slightly less potent antagonist of rTRPA1, rhTRPA1, and mTRPA1. 2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-purin-7yl)-*N*-(4-isopropyl-phenyl)-acetamide is an antagonist of TRPA1 of all species studied with IC_{50}s in the low micromolar range. (E)-4-(4-chloro-phenyl)-3-methyl-but-3-en-2-one oxime is an antagonist of TRPA1 channels in all four species studied. Ruthenium red is a stronger antagonist of hTRPA1 and rhTRPA1 (IC_{50}s 63 and 78 μM, respectively) than of rTRPA1 and mTRPA1 (IC_{50}s 98 and 146 μM, respectively). Interestingly, 4-methyl-*N*-[2,2,2-trichloro-1-(4-nitro-phenylsulfanyl)-ethyl]-benzamide is a potent agonist of mTRPA1 and rTRPA1 (EC_{50}s of 900 nM for both) but acts as an antagonist of hTRPA1 and rhTRPA1 with IC_{50}s of 1 and 3 μM, respectively. As previously shown (97), caffeine too is an agonist of mTRPA1 and rTRPA1 (EC_{50}s of 62

and 96 μM, respectively) but a weak antagonist of hTRPA1 (EC_{50} 988 μM) and has no effects on rhTRPA1 at 1 mM.

Several TRP channels can sense heat or cold with various threshold for activation. Detection of heat by snakes occurs through a specialized organ, the pit organ that detects infrared radiation. The infrared receptors on the sensory neurons of pit-bearing snakes (vipers, pythons, and boas) were identified as TRPA1 channels (99). TRPA1 channels from western clawed frogs and from green anoles are also sensitive to heat (100), as are Drosophila TRPA1 channels that participate in avoidance of elevated temperatures (101, 102). In contrast, mTRPA1 and rTRPA1 are activated by cold, displaying an opposite response from reptile and fruit fly TRPA1 channels (102, 103), whereas hTRPA1 and rhTRPA1 do not respond to cold stimuli (103).

Some, but not all, of these differences in sensitivity to pharmacological agents and to heat and cold stimuli can directly be related to the evolutionary divergence of TRPA1 channels in the different species assessed (Table 2).

5.3. Different pharmacological profile of human and mouse $K_{Ca}5.1$ channels

In section 3.3 we discussed the different channels that carry potassium currents in human and mouse sperm. While $mK_{Ca}5.1$ (a.k.a. Slo3, *KCNMC1*, or *KCNU1*) underlies the potassium current (mKSper) recorded in mouse spermatozoa (54, 55), $K_{Ca}1.1$ underlies the human KSper current (59). In addition to the differences in potassium channels expressed in human and mouse sperm, comparisons of $mK_{Ca}5.1$ and $hK_{Ca}5.1$ in an expression system demonstrates a species-specific pharmacological profile for $K_{Ca}5.1$ (104).

While all reports agree that clofilium (4-Chloro-N,N-diethyl-N-heptylbenzenebutanaminium) blocks $mK_{Ca}5.1$, the literature provides conflicting results as to its ability to block human $K_{Ca}5.1$ with one study showing it is resistant while another shows the same sensitivity of $mK_{Ca}5.1$ and $hK_{Ca}5.1$ for clofilium (104, 105). Charybdotoxin, a peptide from the venom of the scorpion *Leiurus quinquestriatus* known to block human, mouse, and rat $K_{Ca}1.1$, $K_{Ca}3.1$, $K_V1.2$ and $K_V1.3$ channels (106), also blocks human $K_{Ca}5.1$ with an IC_{50} of approximately 100 nM (104). Mouse $K_{Ca}5.1$ channels are however insensitive to charybdotoxin at that concentration (107). Such a difference in sensitivity to a pharmacological agent could be the result of sequence differences between the human and mouse $K_{Ca}5.1$ channels as they share only 68% identity.

5.4. Human, rat, and mouse $K_V7.1$ channels are sensitive to PKC whereas guinea pig $K_V7.1$ channels are not

The voltage-gated $K_V7.1$ potassium channels (a.k.a. minK or *KCNQ1*) are required for the repolarization phase of the human cardiac action potential. While human $K_V7.1$ ($hK_V7.1$) more closely aligns with guinea pig $K_V7.1$ than mouse or rat $K_V7.1$ (Table 3), their response to PKC diverge most.

Human, mouse, and rat $K_V7.1$ channels contain a serine phosphorylation site on serine 102. This site is absent from guinea pig $K_V7.1$ channels in which serine 102 is replaced by an asparagine in the equivalent position (108, 109). As a result from this single amino acid

substitution between species, the PKC-induced inhibition of human, mouse, and rat $K_V7.1$ currents is not observed with guinea pig $K_V7.1$ channels. This species-specific PKC-mediated inhibition of $K_V7.1$ is mediated by clathrin-mediated endocytosis of the channel upon S102 phosphorylation (110).

6. SOME HUMAN CHANNELOPATHIES CANNOT BE FULLY REPLICATED IN ANIMAL MODELS

Human channelopathies resulting from a well-defined mutation can often be replicated in animal models, leading to a phenotype identical or very similar to the human disease. For example, *de novo* gain of function mutations in the *SCN8A* gene, encoding the $Na_V1.6$ channel, induces early infantile epileptic encephalopathy with an increased risk of sudden unexplained death in epilepsy. A gain of function mutation of *SCN8A* in mice induced a phenotype similar to that described in humans (111). Similarly, loss of $Ca_V1.3$ function induces bradycardia and congenital deafness in both humans and mice (112). In some cases, however, channelopathies in animal models do not fully recapitulate the human symptoms. Below are a few examples of such channelopathies.

6.1. Manipulating CFTR does not recapitulate all aspects of cystic fibrosis in different species

Cystic fibrosis affects all ethnicities but is the most common inherited disease in Caucasians with an incidence of 1 for every 2,500 newborns. It affects 70,000 individuals worldwide (113, 114). Cystic fibrosis is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulatory gene (*CFTR*). CFTR is a small conductance ATP- and cAMP-dependent chloride channel found at the apical border of epithelial cells in most exocrine glands. Its primary function is to regulate liquid volume on epithelial surfaces. Almost 2,000 mutations of the *CFTR* gene have been described although the most common in cystic fibrosis is the deletion of phenylalanine at codon 508 (p.Phe508del or F508del), occurring in ~70% of patients. While lung disease is the most likely to lead to premature death in cystic fibrosis, the disease manifests in many organs.

Models of cystic fibrosis have been created in five different species: mouse, rat, ferret, pig, and zebrafish (114, 115). However, none of these models recapitulate all the aspects of the human disease. While all display aberrant chloride transport, other aspects of cystic fibrosis are either enhanced or absent from the animal models (114). Besides humans, only the pig and ferret models show obstructive lung disease and diabetes mellitus. While humans develop intestinal obstructions, these are more severe in pigs, ferrets, mice, and rats andare not observed in zebrafish. Growth disturbance, observed in humans and rats, was more prominent in pigs, ferrets, and mice but absent in zebrafish. An anomalous vas deferens was observed in all species but mice and zebrafish while maldevelopment of the trachea was observed in humans and all models, except the zebrafish. Finally, dysfunction of the liver and the exocrine pancreas was seen to a similar extent in humans and ferrets, was enhanced in pigs, and absent in the other species studied.

Some of the differences in phenotype might be explained by differences between the mutation in humans and the changes made to *CFTR* during generation of the animal model. Of the almost 2,000 variants of *CFTR* identified in cystic fibrosis, 40% cause substitution of an amino acid, 36% affect RNA processing via nonsense or frameshift mutations and alternative splicing, 14% are neutral variants, 3% involve large rearrangements, and 1% affect promoter regions. The effects of the remainning 6% of mutations remains unclear (114). The most common mutation of *CFTR* in cystic fibrosis, F508del, causes aberrant protein folding, resulting in its degradation. The minority of CFTR that reaches the plasma membrane has aberrant chloride channel function. In rats, the model was created by introducing a 16 base pair deletion in exon 3, leading to the loss of CFTR expression (116). In zebrafish, the model was induced by loss of CFTR function (117). In the ferret, the model was a whole knock-out of *CFTR* (118). In pigs, both a whole knock-out of *CFTR* and an F508del mutation of *CFTR* were used as models with similar outcomes (119, 120).

The inability of the animal models to completely mimic the human disease suggests that CFTR may not have the same tissue distribution in the different species studied. Alternatively, the expression of another chloride channel may provide redundancy in some tissues in some species but not others, or CFTR may regulate different functions in different species.

6.2. Loss of Kv11.1 or K_V7.1 function induces long QT syndrome in humans but not in mice

Human ventricular cardiomyocytes express K_V11.1 (a.k.a. HERG or *KCNH2*) and Kv7.1 (a.k.a. minK or *KCNQ1*) (121), as do Göttigen minipig ventricular myocytes (21). In humans, loss of function mutations in either K_V7.1 or K_V11.1 leads to long QT syndrome, an inherited cardiac disorder that causes syncopes, seizures, and sudden death in otherwise healthy individuals (121, 122). Long QT syndrome can also be a result of pharmacological agents that inhibit the function of K_V11.1 or K_V7.1. Off-target effects of therapeutics on these channels is therefore a major safety concern and has led to the withdrawal of high-profile drugs from the market, including cisapride, designed to enhance motility in the upper gastrointestinal tract, and astemizole, an antihistamine (123).

The G628S mutation in K_V11.1 is associated with a particularly severe form of long QT syndrome in humans. It was used for the generation of transgenic mice in which the K_V11.1 G628S mutation exerts a dominant negative effect. These mice, however, exhibit electrocardiograms indistinguishable from those of their wild type counterparts with normal ventricular action potentials (124, 125).

Two *KCNQ1*$^{-/-}$ mouse models have been generated by deletion of either exon 1 (126) or exon 2 (125, 127). Both exhibit Shaker-waltzer behavior that consists of hyperactivity, head shaking and circling (127) as well as gastric hyperplasia and hypochloric gastric fluid (126). The latter anomalies have not been described in patients with long QT syndrome. Interestingly, the two mouse models display different cardiac phenotypes. The exon 2 *KCNQ1*$^{-/-}$ mice display prolonged QT intervals, abnormal P and T waves, and delayed atrioventricular conduction. In contrast, exon 1 *KCNQ1*$^{-/-}$ mice have normal heart rates, heart rate variability, ventricular repolarization, and atrioventricular conduction.

These differences in phenotypes between human and mice highlight the fact that the presence of the same channel in the same cells in two different species does not always lead to this channel playing the same role. It can be a crucial component in one species and less critical in another.

6.3. Perinatal lethality in ORAI1- and STIM1-deficient mice

Influx of calcium via store-operated calcium entry is important in many non-electrically excitable cells and in some electrically excitable cells. Of these channels, the calcium release-activated calcium channel is the best characterized. It is formed by the interaction of ORAI1, the pore-forming subunit present in the plasma membrane, and stromal interaction molecule 1 (STIM1), located in the endoplasmic reticulum membrane.

Humans lacking ORAI1 or STIM1 are severely immunocompromised and succumb during their first years of life. In contrast, ORAI1- and STIM-1 deficient mice die perinatally of unknown causes (128). It should however be noted that the penetrance of this lethality depends on the mouse strain in which the deficiency was introduced (129, 130).

This difference in phenotype between humans and mice suggest that that ORAI1 and STIM1 are either expressed and necessary at different stages of human and mouse development, that their tissue distribution varies between the two species, or that they have different functions in the two species, regardless of tissue distribution.

7. PERSPECTIVES FOR EVALUATING ANIMAL MODELS

While crucial for the development of novel therapeutics, animal models, if not chosen correctly to answer the question, may mislead investigators and generate results that do not translate to human care. In an ideal situation, the same cells and tissues can be studied in humans and animals to choose the most appropriate animal model. This is relatively easy to accomplish with cells that can be obtained through non-invasive or minimally-invasive procedures, such as the collection of induced-sputum or blood to study circulating immune cells, platelets, or erythrocytes (31, 44, 131). Some cells can also be extracted from tissues removed following surgical procedures performed for therapeutic purposes, as in severe rheumatoid arthritis, osteoarthritis, or solid tumors (12, 132). However, many cells, such as neurons or cardiomyocytes, are difficult to access from live volunteers for obvious ethical reasons. Similarly, obtaining healthy tissues can present difficulties as surgeons will aim at removing only diseased tissues with limited impact on the remainder of the surrounding tissues. This precludes or limits the direct comparison of human and animal model tissues in some situations.

The study of ion channels in primary cells maintained in culture should also take in account potential changes in channel phenotype over time. For example, repeated activation of naïve T lymphocytes will led to their differentiation into memory T lymphocytes and thus to a change in potassium channel phenotype (31).

Even when the same channel is identified in cells from different species, care should be taken to determine whether other channels are present in one of the samples that could play

a redundant role, such as $K_V1.1$, $K_V1.2$, and $K_V1.6$ channels in $CD4^+$ mouse T lymphocytes that can compensate for the loss of $K_V1.3$ channels or preclude the binding of $K_V1.3$ selective blockers through the formation of heterotetramers (36–39). Care should also be taken to verify that minute amino acid differences do not dramatically alter the response to a signaling molecule or a ligand, as was shown for $K_V7.1$ and PKC (123).

The difference in results between blocking $K_V11.1$ channels in humans and in mice has been especially problematic when developing novel ion channel modulators, as well as other drugs. Safety studies in mice are unlikely to detect unexpected off-target effects of a compound on this channel. Other assays must therefore be used, such as high throughput binding assays on cells expressing $K_V11.1$ or even electrophysiology on cultured cardiomyocytes (123).

While some animal models do not fully replicate all aspect of a human disease, such as animal models of cystic fibrosis, they can still be useful to answer specific questions provided that animal models are chosen wisely for each specific question. For example, the study of growth disturbance induced by cystic fibrosis should be conducted in rats whereas this model is not appropriate to study obstructive lung disease or diabetes mellitus in cystic fibrosis. Overall, the studies highlighted here demonstrate the necessity for diligence in choosing the proper animal model for answering a particular question in order for high quality, translatable research to be completed.

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Abbreviations

2APB	2-aminoethoxydiphenyl borate
AQP4	aquaporin-4
Ca_V	voltage-gated calcium (channel)
CFTR	cystic fibrosis transmembrane conductance regulatory
FLS	fibroblast-like synoviocyte
IUPHAR	International Union of Basic and Clinical Pharmacology
K_{Ca}	calcium-activated potassium (channel)
K_V	voltage-gated potassium (channel)
KSper	sperm potassium (current)
Na_V	voltage-gated sodium (channel)
STIM1	stromal interaction molecule 1

TRP transient receptor potential (channel)

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Table 1

Effects of TRPA1 channel modulators in different species (97, 98, 103, 133)

Agonists are highlighted in blue and antagonists in red. Compounds with no effects are highlighted in grey. ND, not determined.

TRPA1 modulators	Human	Rhesus macaque	Rat	Mouse	Rattle snake	Green anole	Western clawed frog	Fruit fly
2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-purin-7yl)-*N*-(4-isopropyl-phenyl)-acetamide	<O>IC_{50} 7.4 μM	<O>IC_{50} 7.3 μM	IC_{50} 7.6 μM	IC_{50} 6.8 μM	ND	ND	ND	ND
3′-carbamoylbiphenyl-3-yl cyclohexylcarbamate	EC_{50} 7.5 μM	No effect at 100 μM	EC_{50} 129 μM	EC_{50} 74 μM	ND	ND	ND	ND
4-methyl-*N*-[2,2,2-trichloro-1-(4-nitro-phenylsulfanyl)-ethyl]-benzamide	IC_{50} 1 μM	IC_{50} 3 μM	EC_{50} 900 nM	EC_{50} 900 nM	ND	ND	ND	ND
4-hydroxynonenal	EC_{50} 10 μM	No effect at 100 uM	EC_{50} 6 μM	EC_{50} 5 μM	ND	ND	ND	ND
Caffeine	EC_{50} 988 μM	No effect at 1 mM	IC_{50} 96 μM	IC_{50} 62 μM	ND	ND	ND	No effect
(E)-1-(4-fluoro-phenyl)-2-methyl-pent-1-en-3-one oxime	IC_{50} 89 μM	IC_{50} 600 nM	IC_{50} 330 nM	IC_{50} 750 nM	ND	ND	ND	ND
(E)-4-(4-chloro-phenyl)-3-methyl-but-3-en-2-one oxime	IC_{50} 9 μM	IC_{50} 56 μM	IC_{50} 40 μM	IC_{50} 33 μM	ND	ND	ND	ND
Farnesyl thiosalicylic acid	EC_{50} 5 μM	EC_{50} 167 μM	EC_{50} 102 μM	EC_{50} 85 μM	ND	ND	ND	ND
Menthol	EC_{50} 278 μM	No effect at 300 μM	EC_{50} 7 μM	EC_{50} 5 μM	ND	ND	ND	ND
Mustard oil	EC_{50} 8 μM	EC_{50} 75 μM	EC_{50} 9 μM	EC_{50} 5 μM	ND	ND	ND	ND
Trichloro(sulfanyl)ethyl benzamide, AMG2504	IC_{50} 105 nM	ND	IC_{50} > 30 μM	ND	ND	ND	ND	ND
Trichloro(sulfanyl)ethyl benzamide, AMG7160	IC_{50} 28 nM	ND	IC_{50} > 30 μM	ND	ND	ND	ND	ND
Trichloro(sulfanyl)ethyl benzamide, AMG5445	IC_{50} 29 nM	ND	EC_{50} 115 nM	ND	ND	ND	ND	ND
Trichloro(sulfanyl)ethyl benzamide, AMG9090	IC_{50} 7 nM	ND	EC_{50} 66 nM	ND	ND	ND	ND	ND
Trinitrophenol	EC_{50} 107 μM	No effect at 300 μM	EC_{50} 71 μM	EC_{50} 30 μM	ND	ND	ND	ND
Ruthenium red	IC_{50} 63 μM	IC_{50} 78 μM	IC_{50} 98 μM	IC_{50} 146 μM	ND	ND	ND	ND

TRPA1 modulators		Human	Rhesus macaque	Rat	Mouse	Rattle snake	Green anole	Western clawed frog	Fruit fly
Heat		No effect	No effect	No effect	No effect				
Cold		No effect	No effect			ND	ND	ND	ND

Table 2

Evolutionary divergence of TRPA1 channels (103).

Species	Identity to hTRPA1 (%)
Rhesus macaque	97
Rat	79
Mouse	79
Anole lizard	63
Rattle snake	63
Western clawed frog	56
Drosophila	36

Table 3

Evolutionary divergence of $K_V7.1$ channels.

Species	Identity to $hK_V7.1$ (%)
Rat	89
Mouse	88
Guinea pig	91