Targeting TRP channels – valuable alternatives to combat pain, lower urinary tract disorders and type II diabetes?

Thomas Voets^{1,2, #}, Joris Vriens³, Rudi Vennekens^{1,2}

¹ Laboratory of Ion Channel Research, VIB Center for Brain & Disease Research; Leuven; Belgium

² Department of Cellular and Molecular Medicine; KU Leuven; Leuven; Belgium

³ Laboratory of Endometrium, Endometriosis & Reproductive Medicine, Department of Development and Regeneration, KU Leuven; Leuven; Belgium

Corresponding author

Abstract

Transient Receptor Potential (TRP) channels are a family of functionally diverse and widely expressed cation channels involved in a variety of cell signaling and sensory pathways. Research in the last two decades has not only shed light on the physiological roles of the 28 mammalian TRP channels, but also revealed the involvement of specific TRP channels in a plethora of inherited and acquired human diseases. Considering the historical successes of other types of ion channels as therapeutic drug targets, small molecules that target specific TRP channels hold great promise as treatments for a variety of human conditions. In recent research, important new findings have highlighted the central role of TRP channels in chronic pain, lower urinary tract disorders and type II diabetes, conditions with an unmet medical need. Here, we discuss how these advances support the development of TRP channel-based pharmacotherapies as valuable alternatives to the current mainstays of treatment.

Glossary

Afferent: signaling from peripheral tissue towards the central nervous system.

Allodynia: painful perception of a normally non-painful stimulation.

β cell: insulin-secreting cell in the pancreatic islet of Langerhans.

Efferent: signaling towards the peripheral tissue from the central nervous system.

Gabapentinoids: Derivatives of the inhibitory neurotransmitter γ -aminobutyric acid that bind to the $\alpha_2\delta$ subunit of voltage-gated calcium channels, resulting in analgesia.

Hyperalgesia: abnormally increased sensitivity to pain.

Hyperglycemia: high blood sugar. Levels above 200mg/dl are considered harmful if prolonged.

Hypoglycemia: low blood sugar. Definition is variable, but below 70mg/dl in a diabetic person is a clear diagnosis. Symptoms and risks include trouble talking, confusion, loss of consciousness and even death.

Insulinotropic drug: a compound that increases insulin secretion. This action can be either glucose-dependent or glucose-independent.

Nociceptors: sensory neurons that relay pain signals.

Nocturia: the complaint that an individual has to wake at night one or more times to urinate.

NSAID: non-steroidal anti-inflammatory drug, a widely used class of analgesic drugs; see box 2.

Opioid: a class of drugs acting as agonists of opioid receptor, including naturally occurring compounds such as morphine (from the opium poppy) as well as synthetic opioids such as fentanyl. They are potent analgesic drugs, but can be highly addictive and provoke fatal overdosing due to respiratory depression; see **Box 2**.

T2DM: Type II diabetes mellitus, the most common type of diabetes mellitus, characterized by insulin resistance.

TRP channel: Transient Receptor Potential channel - refers to a class of ion channels. The human genome encompasses 27 different TRP channel genes, classified into six related subfamilies based on sequence homology: the TRP channel subfamily C (canonical, TRPC1-7), the TRP subfamily V (vanilloid, TRPV1-6), the TRP channel subfamily M (Melastatin, TRPM1-8), the TRP subfamily A (Ankyrin, TRPA1), the TRP channel polycystin subfamily (TRPP1-3) and the TRP channel mucolipin subfamily (TRPML1-3)[1]. Four TRP channel subunits coassemble to form functional homo- or heterotetrameric channels, mediating influx of mono- and/or divalent cations, most commonly Ca^{2+} and Na^{+} [2].

Urgency: the need to urinate more often than usual?

The superfamily of TRP channels

The Transient Receptor Potential (TRP) superfamily comprises a group of non-selective cation channels that sense and respond to changes in their local environment [3]. TRP channels are described in many eukaryotes, ranging from single-cell green algae to mammals [4]. Many TRP channels are polymodal, enabling them to act as molecular sensors, coincidence detectors and cellular signal integrators [3, 4]. TRP channels have been implicated in biological processes related to photo-, chemo-, thermo-, and mechanosensation, as well as ion homeostasis.

Considering their diverse physiological roles, it is not surprising that TRP channel dysfunction lies at the origin of diverse acquired and inherited diseases [5, 6]. This has raised high hopes that specific small-molecule modulators of TRP channel activity may be developed as novel drugs for the prevention or treatment of a broad variety of human pathologies across disease areas (for review see [7, 8]). Of particular interest are chronic diseases where current pharmacological therapy options are unsatisfactory for a large number of patients. In this respect, a number of recent studies have greatly advanced our understanding of the role of TRP channels in pain, bladder dysfunction and type II diabetes mellitus (T2DM). Here, we provide a perspective on how TRP channel-targeting drugs may become valuable alternatives for the current pharmacological treatment of these pathologies.

Targeting TRP channels to treat pain

It has been estimated that 1 in 5 adults worldwide suffers from some form of chronic pain that impacts their quality of life [9, 10]. Pain management in patients with chronic pain is challenging, due to a lack of effective and safe pharmacologic therapies [11-13]. The market of analgesic drugs is dominated by compound classes that have been around for many decades (**Box 1**). However, about half of the chronic pain sufferers report inadequate pain control, indicating a high demand for the development of alternative analgesic strategies to treat chronic pain [9, 12].

One attractive strategy is to intercept pain at the source, by targeting the sensory neurons that generate nociceptive signals [14]. Primary sensory neurons of the peripheral somatosensory system are responsible for conveying a wide range of environmental physical and chemical stimuli to the central nervous system. At their nerve endings in the skin and exposed mucosae, distinct molecular sensors translate physical or chemical cues into local depolarization, which, when sufficient to reach the threshold, provokes action potentials that travel to the central nervous system [14]. In particular, several TRP channels are involved in the detection and transduction of environmental stimuli at nerve endings (**Figure 1A**) [4, 14-16].

Such molecular transducers in the peripheral terminals of nociceptive neurons represent particularly valuable targets for drug therapy, since they are pivotal for the initiation of the most common modalities of pain sensation [14-16]. The expression and function in sensory nerve endings has been extensively characterized for TRPV1, TRPA1, TRPM8 and TRPM3, which show distinct expression patterns in subsets of C and A δ fibers originating from both the dorsal root and trigeminal ganglia [17-21]. It is now well established that these four TRP channels play essential roles in the pain responses to cold and hot temperatures and noxious chemicals, both in healthy tissue and in the context of inflammation or nerve injury (**Figure 1A**).

In addition to the initiation of pain signals under the form of action potentials, stimulation of TRPV1, TRPA1 or TRPM3 in peripheral endings of nociceptors can induce the local release of neuropeptides such as substance P and calcitonin gene-related peptide (CGRP) [22-24]. These neuropeptides trigger a biochemical cascade leading to neurogenic inflammation, which encompasses vasodilation, plasma extravasation and attraction and activation of immune cells, and which is implicated in the pathogenesis of various disease states, like migraine and arthritis [25, 26]. All together, these findings indicate that lowering the activity of these nociception-related TRP channels may be a viable strategy to alleviate pain and inflammatory responses associated with a variety of pathologies.

TRPV1

The capsaicin receptor, TRPV1, represents the prototypical nociception-related TRP channel [17]. The channel is activated by heat, low pH, pungent vanilloids, painful venoms from spiders and snakes, and downstream of the activation of phospholipase C, linking TRPV1 to multiple pain pathways and processes [17, 27-29]. TRPV1-deficient mice are less responsive to acute noxious heat [30], though not absolute. Moreover, additional studies showed that TRPV1 is involved in mediating hyperalgesia under inflammatory and neuropathic pain conditions [30, 31]. Thus, TRPV1 was put forward as a novel peripheral target to treat pain, and several approaches have been used to specifically target TRPV1-expressing nociceptor neurons (**Figure 1B**).

The most straightforward approach consisted of the development of specific TRPV1 antagonists compounds of the target. Unfortunately, the development of the first generation of TRPV1 antagonists was generally halted in the preclinical or early clinical phase, as it was observed that inhibition of TRPV1 leads to a significant increase in body temperature in various animals and in humans [32-34]. These results demonstrated the initially underappreciated role of TRPV1 in regulating body core temperature. Based on these results, a second generation of TRPV1 channel antagonists has been developed, which do not inhibit TRPV1 activity when stimulated with low pH and do not induce hyperthermia [35, 36]. Several of these antagonists have been tested in humans in Phase I studies, reporting reduced adverse side effects and inhibiting responses to TRPV1-specific stimuli such as capsaicin [37]. Significant but overall limited analgesic efficacy was reported with TRPV1 antagonists in studies related to post-operative pain [38] and osteoarthritis [39], whereas no effect was observed on esophageal pain in patients with nonerosive reflux disease [40]. TRPV1 antagonists also did not have clinically relevant efficacy in human for non-pain related diseases associated with sensory neuron hyperactivity, including non-allergic rhinitis [41] and chronic cough [37]. Thus, despite substantial preclinical evidence, inhibiting TRPV1 function is not consistently leading to clinically relevant efficacy in various disease states in humans, and further research is needed to determine the patient groups that may benefit from TRPV1 antagonist-based therapy. Finally, TRPV1 antagonism generally leads to an increase in the threshold for heat-induced pain [34, 39]. Therefore, adequate patient counseling may be required to avoid accidental burns in subjects taking TRPV1 antagonist.

Two alternative TRPV1-based approaches have been used, which aim at silencing TRPV1-positive nociceptors altogether. First, excessive activation of TRPV1, for instance by prolonged application of potent vanilloids, is used to induce prolonged desensitization of nociceptors resulting in long-lasting analgesia [42]. This forms the basis of capsaicin transdermal patches, which are now in clinical use for neuropathic pain [43]. Whereas these patches have proven clinical benefit in patients, they can evoke significant pain and irritation upon application, may require multiple applications before efficacy is

achieved, and can result in complete unresponsiveness of the treated area to noxious stimuli [42]. A second approach uses TRPV1 as a route for the selective entry of charged voltage-gated sodium channel blockers into nociceptors, taking advantage of the finding that the pore of activated TRPV1 is wide enough to allow permeation of large cations [44]. QX-314, a membrane-impermeable derivative of the local anesthetic lidocaine, is ineffective in inhibiting voltage-gated sodium channels when applied from the extracellular side of the channel. Interestingly, upon co-application with capsaicin, QX-314 can readily enter TRPV1-positive nociceptors, silencing these neurons by blocking their voltage-gated sodium channels from the cytosolic side [44]. The strategy of using ion channels to deliver voltage-gated sodium channels blockers selectively into nociceptors is, at least in theory, superior to the use of local anesthetics. Indeed, whereas local anesthetics have amply proven their usefulness to prevent or alleviate acute pain during labor or in the context of surgery, they suppress the excitability of all neurons in the treated area, not just sensory nociceptive neurons, leading to block of motor and autonomic function, when only analgesia is desired [44]. In contrast, animal models show that the selective targeting of nociceptors using TRPV1 as delivery pathway results in prolonged local anesthesia while preserving motor and autonomic responses [44]. Since application of capsaicin may cause excruciating pain in patients, more recent studies experiment with a combination of QX-314 and lidocaine to achieve selective entry of QX-314 into nociceptors, based on the observation that high doses lidocaine, cause strong activation of TRPV1 [45]. Overall, the targeted delivery approach seems promising, with significant efficacy in preclinical models [44-46], but confirmation in humans is still outstanding.

TRPA1

TRPA1 stands out by being responsive to an unusually broad variety of chemical stimuli. These include a variety of exogenous chemicals, such as mustard oil (the pungent compound in mustard and wasabi) and cinnamaldehyde (from cinnamon) [18, 47], a variety of other environmental irritants such as acrolein, tear gasses, hypochlorite and nicotine, as well as bacterial endotoxins (lipopolysaccharides). A variety of endogenous chemicals that are released in the context of tissue damage and inflammation also drive TRPA1 channel activation, including prostaglandins, products of lipid peroxidation and methylglyoxal [48-50]. In addition to chemical ligands, TRPA1 has also been implicated in detecting noxious temperatures, both cold and hot [51]. It is, however, still debated whether TRPA1 acts as an intrinsic thermosensor, or rather responds to chemical substances that are released during thermal insults.

Human and animal data implicate TRPA1 in a variety of pain conditions. In rodents, genetic deletion or pharmacological inhibition of TRPA1 was shown to reduce nociceptive behavior in a variety of models of inflammatory and neuropathic pain. In particular, readouts of cold and mechanical pain were most

often impacted [52-55]. On the negative side, one preclinical study reports no or very limited efficacy of a potent TRPA1 antagonist in inflammatory and neuropathic pain, despite full target coverage [56]. In humans, gain-of-function mutations in human TRPA1 were identified as the cause of the rare heritable pain disorder familial episodic pain syndrome, supporting the notion that TRPA1 is a valid target for pain treatment in human [57]. Notably, TRPA1 antagonists do not affect body core temperature or affect acute temperature sensing [53, 58]. At this point, although several clinical studies have been initiated and a patent application from Glenmark documents beneficial effects of a TRPA1 antagonist in a Phase II study with diabetic neuropathy patients, we are not aware of any peer reviewed publication on the effects of systemic TRPA1 antagonism in humans, [59].

TRPM3

TRPM3, a heat-activated channel and ionotropic receptor for the neurosteroid pregnenolone sulfate, has more recently been introduced as a potential target for pain relief. TRPM3-deficient mice have a partial deficit in acute heat sensing, and fail to develop inflammatory thermal hyperalgesia [21]. Several preclinical studies have shown that systemic inhibition of TRPM3 with experimental antagonists, such as the flavonoid isosakuranetin or the anticonvulsant primidone, attenuates acute heat pain as well as thermal and mechanical hypersensitivity in the context of inflammation or nerve injury, without affecting body core temperature [60, 61]. Intriguingly, TRPM3 is also rapidly and efficiently blocked upon μ -opioid receptor activation [62-64]. This raises the important question whether the known peripheral analgesic actions of opioids are, at least in part, related to inhibition of TRPM3 [65]. Further drug development is required to obtain TRPM3 antagonists suitable for testing in humans.

TRPV1, TRPA1 and TRPM3: a heat-sensing triad

Considering that chronic pain is frequently associated with a burning feeling, and that inhibition of TRPV1 in humans increases the risk of accidental burns, the molecular basis of acute heat sensing has been a long-standing question in the field. In recent work, heat-evoked pain was shown to rely on TRPM3, TRPV1, and TRPA1, acting as three functionally redundant heat sensors showing an overlapping expression pattern in heat-sensitive sensory neurons [66]. Combined genetic ablation or pharmacological inhibition of this trio of sensory TRP channels in mice virtually eliminated heat responses in nociceptor neurons. Strikingly, triple knockout mice (*Trpv1^{-/-}Trpm3^{-/-}Trpa1^{-/-}*) lacked the acute withdrawal response to noxious heat, and would sustain burn injuries if not withdrawn from a heat source [66]. Notably, vigorous withdrawal responses were preserved as long as one of the three channels remained functional, indicating that each of the three channels is able to individually signal noxious heat. Yet, comparison of the heat withdrawal latencies of double knockout mice lacking two of the three channels suggests a hierarchy, where TRPV1 is the first sensor to be activated upon

heating, followed by TRPM3 and finally TRPA1 [66]. This provides an explanation for the observation that TRPV1 antagonists cause a substantial increase in heat pain threshold in humans, and suggest that TRPA1 and TRPM3 antagonists are probably less likely to increase the risk of accidental burns.

TRPM8

TRPM8, a channel activated upon lowering temperature and by cooling agents such as menthol, eucalyptol and icilin, is the prototype cold receptor, and is essential in the detection moderate cool [67]. In the context of pain, TRPM8 plays a dual role: on the one hand, activation of TRPM8 can have a soothing effect, as evidenced by the well-known analgesic effect of cooling. Effectively, over-the-counter products containing naturally-occurring TRPM8 agonists such as menthol and eucalyptol are widely used for the topical treatment of pain and itch, and recent animal studies indicate that these products act in a TRPM8-dependent manner [68, 69]. On the other hand, TRPM8 activation contributes to cold-induced pain and cold hypersensitivity [67], and this has fueled significant investments into the development of selective TRPM8 antagonists.

Several TRPM8 antagonists have been assessed in preclinical models of persistent pain. TRPM8 antagonisms was shown to be effective in attenuating cold hypersensitivity in animal models of inflammatory pain, nerve injury or chemotherapy-induced neuropathy, but had little or no effect on mechanical hypersensitivity [67, 70, 71]. As such, the use of TRPM8 antagonists may be restricted to conditions where cold hypersensitivity is a major issue [70, 72]. Pharmacological inhibition of TRPM8 was found to cause hypothermia in mice and rats, which is indicative of its involvement in thermoregulation. However, a selective TRPM8 antagonist, PF-05105679, was assessed in a phase I trial in healthy volunteers, showing efficacy in the cold pressor test without affecting the core body temperature [73]. Unfortunately, a significant number of volunteers reported hot feeling as an adverse event, predominantly in around the mouth, which was nontolerable in some subjects. Assuming that this adverse event represents an on-target side-effect, developing TRPM8 antagonists for treating pain may become challenging [73]. Interestingly, several genome-wide association studies have shown a highly significant association between variations in the TRPM8 gene and migraine [74-76], which has led to the initiation of Phase I clinical studies evaluating TRPM8 antagonists in this context [77].

Targeting TRP channels for lower urinary tract disorders

Roughly 1 billion people worldwide suffer a form of lower urinary tract dysfunction (LUTd). Urinary incontinence, permanent urgency, the inability to efficiently empty the bladder and bladder-associated pain can transform the apparently simple process of urine storage and voiding into a daily burden. Available treatments often lack long-term efficacy, and the societal cost of LUTds is high [78, 79]. Distinct TRP channels are functionally expressed in the different cell types constituting the bladder, in

particular in the urothelial cells lining the bladder lumen and in the endings of sensory neurons innervating the bladder wall [80, 81]. Based on their expression pattern and functional properties, these TRP channels have been put forward as promising novel therapeutic targets to alleviate bladder pain or normalize bladder activity.

Bladder pain

Interstitial cystitis/bladder pain syndrome (IC/BPS) is characterized by chronic symptoms of abdominal pain perceived to be related to the bladder region, mostly associated with frequent urination, but in the absence of an identifiable cause such as bladder infections and with unclear etiology [82]. Current therapy for IC/BPS consists of various treatments aimed at improving the integrity the urothelial barrier [83], as well as symptomatic treatment with classical analgesics drugs (**Box 2**). Pain arising in the bladder and urethra is conveyed via nociceptor sensory neurons from the lumbar and sacral DRGs and express the same palette of sensory TRP channels as found in neurons innervating the skin, including TRPV1, TRPA1, TRPM3 and TRPM8 (**Figure 1; Figure 2**). Therefore, as outlined above, drugs targeting these TRP channels may be promising as alternative analgesics for treating chronic abdominal pain, including IC/BPS.

Bladder storage disorders

Bladder symptoms at the level of urine storage and voiding form the basis of a broad array of other disorders of the lower urinary tract, encompassing symptoms such as incontinence, nocturia, overactive bladder and underactive bladder [84]. Currently available pharmacological treatments to treat dysfunctional bladder storage and voiding are primarily targeting the contractility of the bladder detrusor muscle (**Figure 2; Box 2**). In the last two decades, it has become increasingly clear that normal bladder function critically depends on sensory information that arises in the bladder wall, and that patient symptoms such as urgency can arise without any detectable dysfunction of the detrusor muscle [85]. Therefore, pharmacological treatments aimed at normalizing afferent signaling represent a new avenue to treat various LUTds [80, 81, 85]. TRPV4 represents a particularly attractive target in this respect (**Figure 2**).

TRPV4 is a non-selective cation channel activated by a variety of stimuli including moderate heat, mechanical stimuli, arachidonic acid-derived epoxyeicosatrienoic acids and synthetic ligands such as GSK1016790A [86], and is expressed at high levels in bladder urothelial cells [87] (**Figure 2**). Studies using TRPV4-deficient animals have provided compelling evidence that urothelial TRPV4 is involved in sensing the fullness of the bladder. Urothelial cell stretch leads to activation of TRPV4 in epithelial cells, triggering the release of extracellular messengers such as ATP to stimulate afferent nerves that signal information regarding the filling state of the bladder [88, 89]. Intriguingly, expression of TRPV4 in the

bladder is under the control of the *Clock* (Circadian Locomotor Output Cycles Kaput) gene, a master regulator of circadian rhythm. In particular, TRPV4 expression and ATP release are reduced during the sleep phase in wild type mice, whereas this circadian pattern is disrupted in the *Clock*^{Δ19/Δ19} null mutant mice [90-92]. Notably, these circadian rhythm-deficient mice also show increased frequency of micturition during the sleep phase [91, 93], providing a model for nocturia and suggesting a potential role for TRPV4 in this process.

A growing number of studies has demonstrated that small molecules modulating TRPV4 activity show efficacy in rodent models of bladder dysfunction. Systemic and intravesical administration of the specific TRPV4 antagonists HC06067 and RN1734 improved bladder function in mouse and rat models of cystitis and stress-induced overactivity, reducing the voiding frequency and increasing the bladder capacity [94, 95]. These studies provide a proof-of-principle that pharmacological inhibition of TRPV4 may form the basis for alternative pharmacotherapy for overactive bladder, nocturia, urgency and incontinence. In this respect, it is interesting to note that the potent and selective TRPV4 antagonist GSK2798745 has passed a first-in-human clinical trial, reporting no serious adverse effects in healthy subjects [96].

Recently, intravesical application of the TRPV4 agonist GSK1016790A was reported to increase the voiding frequency and improved the voiding efficiency in a rat model of bladder underactivity provoked by pelvic nerve injury [97, 98]. Whereas these are encouraging results, further development of TRPV4 agonists for treatment of underactive bladder in humans is hampered by the serious consequences of systemic application of TRPV4 agonists. It was shown that i.v. application of GSK1016790A provokes depression of blood pressure followed by circulatory collapse in mice, rats, and dogs, which was attributed to an on-target effect on TRPV4 channels in vascular endothelial cells [99]. However, recent technological advances in intravesical drug delivery, including the use of improved intravesical formulations for prolonged drug release (nanoparticles, liposomes, hydrogels) and indwelling devices that constantly deliver therapeutic drug doses, may be employed to provide a sustained TRPV4 stimulation in the bladder to treat underactivity [100, 101].

TRP channels as target for type II diabetes

Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [102]. This is either due to a reduced amount of insulin secretion by the pancreatic β-cell or an increased insulin resistance (decreased insulin action) in the peripheral tissues like liver and muscle. Chronic hyperglycemia is associated with damage to different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. It is estimated that up to 400 million adults suffer from this condition to date, and that DM is the direct cause of 1.5 million

deaths yearly. In case of Type I DM (T1DM), insulin secreting β cells are absent or degraded, and patients need to control their blood glucose by insulin injections. In case of T2DM, treatment is focused on life-style modifications, increasing insulin secretion from β cells and improving insulin resistance from peripheral tissue (**Box 3**). Diabetes care currently lacks a drug that can prevent or slow down the downward spiral of β cell function [103]. The majority of T2DM patients will become insulin-dependent during the course of the disease. Furthermore, especially in developing countries, affordable and user-friendly medication is often not available. For these reasons, research is still ongoing to understand in detail the processes determining insulin sensitivity and insulin secretion, and to identify novel targets for treatment.

A vast amount of data underscores a role for TRP ion channels in pancreatic β cells and the regulation of insulin secretion [104] (**Figure 3**). Recent data highlight the potential of TRPM5 to modulate insulin secretion and to prevent T2DM progression [105-107]. TRPM5, like its closest homologue TRPM4, is a Ca^{2+} -activated nonselective monovalent cation channel that is impermeable to divalent cations. TRPM5 plays a crucial role in taste receptor cells, where it is essential for the transduction of sweet, bitter and umami tastes [107]. In addition, TRPM5 is expressed in pancreatic β cells, where its activity increases the frequency of glucose-induced calcium oscillations [105]. Loss of TRPM5 expression, and consequently of high frequency bursting, leads to a reduction of glucose-induced insulin release from pancreatic islets and impaired glucose tolerance. Furthermore, it has been suggested that GLP-1 directly stimulates insulin release from the pancreatic β cells through activation of TRPM5 and TRPM4 [108]. The GLP-1 effect on insulin release is absent in *Trpm5*^{-/-} islets [108].

Several lines of evidence link TRPM5 expression and function to T2DM [104, 107]. The blood glucose level in T2DM patients is negatively correlated with TRPM5 expression in the small intestine, and TRPM5 expression is also significantly decreased in islets of morbidly obese, diabetic *Ob/Ob* and *Db/Db* mice [109]. The reduced expression of TRPM5 is related to an impairment of pulsatile insulin release, which is an early marker for β -cell dysfunction in T2DM. Ketterer et al. investigated the impact of nine common SNPs in TRPM5, three of which were found to be related to increased glucose levels during oral glucose tolerance tests [110]. A case-control study in a population suffering from metabolic syndrome (MetS) revealed a polymorphism in the *Trpm5* gene was positively associated with the disease, and showed that TRPM5 expression was significantly reduced in MetS patients [111]. Finally, Wolfram syndrome is a rare autosomal-recessive neurodegenerative disease caused by a mutation in the wolframin gene (WFS1). One of the symptoms of the disease is juvenile onset T2DM. Recently, RNA-seq analysis of pancreatic islets from wild type and *Wfs1*^{-/-} mice revealed a downregulation of TRPM5 expression in the islets of diabetes-prone knockout mice [112]. Taken together, these findings

indicate a negative correlation between TRPM5 expression and the development of T2DM, suggesting that TRPM5 enhancers may have potential as antidiabetic drugs.

So far, synthetic TRPM5 agonists suitable for in human testing have not been reported. However, recent work has identified steviol glycosides as strong potentiators of TRPM5 channel activity [106]. Steviol glycosides are natural, non-caloric sweet-tasting organic molecules in extracts of the scrub plant *Stevia rebaudiana*, which are increasingly used as low calorie high intensity sweeteners in consumer food and beverages. Stevioside and rebaudioside A are the main steviol glycoside compounds in the *Stevia* extract, whereas steviol is the unglycosylated aglycon of these molecules [107]. The frequency of glucose induced Ca^{2+} oscillations in freshly isolated pancreatic islets from WT mice is increased by stevioside, rebaudioside A and steviol. Accordingly, glucose-induced insulin release is augmented by these compounds. Neither of these effects is present in islets from *Trpm5*^{-/-} mice [106]. *In vivo*, acute stevioside administration decreased plasma glucose levels in obese WT but not in *Trpm5*^{-/-} mice. Even more promising, chronic stevioside treatment prevented the development of hyperglycemia in WT mice fed with a high-fat diet [106]. Importantly, stevioside did not affect fasting glucose levels, indicating that it potentiates insulin release in a glucose-dependent manner. This suggests that TRPM5 agonism is not associated with a risk of hypoglycemic episodes, in contrast to currently used insulinotropic agents (**Box 3**). These data underscore that TRPM5 is a promising molecular target of a completely novel strategy to increase insulin secretion from the pancreatic β cell.

Before identification of TRPM5 as their molecular target, stevioside, rebaudioside A and steviol were already known to enhance insulin release from mouse islets in a glucose-dependent manner [113, 114]. In Goto-Kakizaki (a non-obese Wistar rat substrain which develops type II diabetes early in life) and Zucker diabetic fatty rats (an obese, hereditary type II diabetic animal model), acute and chronic stevioside treatment decreased the increase of plasma glucose, increased the insulin response and suppressed the glucagon level in glucose tolerance tests [115, 116]. Taken together, these data indicate that steviol glucosides represent a novel type of insulinotropic medication, which might be helpful for the prevention and treatment of T2DM. To date, studies in humans have been inconclusive because of limited statistical power and incoherent inclusion criteria [107, 117, 118]. Nevertheless, the recent mechanistic insight highlighting TRPM5 as a central molecular target of the actions of steviol glycosides in taste and insulin secretion forms a strong scientific basis for more directed experimental paradigms to test their potential health benefits in humans.

Conclusions and perspectives

Chronic pain, bladder dysfunction and type II diabetes are major health problems, affecting the lives of more than a billion persons worldwide and having an important economic impact. Due to the aging

population and changes in diet and life style, the prevalence of these conditions can be expected to further rise in the coming decades. Considering that only a fraction of patients experience long-term alleviation of their symptoms with the currently available treatments and that several widely-used drugs have significant safety issues, there is a high medical need for alternative therapeutic strategies. As outlined in this brief review, TRP channels have emerged as promising novel pharmacological targets for treating these conditions.

It may sound discouraging to note that, 30 years after the cloning of the first TRP channel, not a single new molecular entity targeting TRP channels has reached the market, despite multi-billion investments, particularly in the development of TRPV1 antagonists. On hindsight, an important reason for the failure of many TRPV1-based drug development endeavors was the initial underestimation of the potential consequences of plain TRPV1 inhibition on thermal homeostasis and acute heat sensing. Nevertheless, these initial drawbacks have significantly expanded our fundamental knowledge of the role of TRPV1 in pain and thermoregulation, and pushed the development of more tailored TRPV1 antagonists that combine *in vivo* efficacy with a better safety profile, and have good chances to progress in clinical development.

At the same time, promising preclinical data have highlighted potential of the small molecules targeting TRPA1, TRPM3 and TRPM8 (for pain), TRPV4 (for bladder dysfunction) and TRPM5 (for T2DM). We expect that increasingly detailed analyses of the structure and function of TRP channels, technological advances in high-throughput automated electrophysiology, and the steadily growing data from genomics and transcriptomics approaches will further accelerate the rational development of TRP channel-targeting drugs (see **Outstanding Questions**), which may reach the clinic in the coming decade. With highly active compounds such as capsaicin, menthol and stevioside, the plant kingdom has shown us the way.

Box 1. Drug therapy for chronic pain

In 1986, the WHO proposed a three-step analgesic ladder to treat cancer pain, which has subsequently also been widely used as a guideline in the treatment of other forms of persistent pain. The first step consists of the use of a non-opioid such as paracetamol (acetaminophen) and non-steroidal anti-inflammatory drugs (NSAIDs). If this does not cause sufficient pain relief, a weak opioid is added to the treatment in step 2. If this is still insufficient, the weak opioid is replaced by a strong one in step 3, until sufficient analgesia is achieved. More recently, new drug classes including the gabapentinoids and antidepressants have been used to treat various forms of chronic pain [12] (**Figure 1**).

NSAIDs (e.g. ibuprofen, aspirin and diclofenac) block the conversion of arachidonic acid to prostaglandins by inhibiting COX-1 and/or COX-2 enzymes. Prostaglandins are the primary noxious mediators released from damaged or inflamed tissue and are responsible for nociceptor activation and sensitization at the spinal cord and periphery [119]. Chronic use of NSAIDs can cause severe gastrointestinal bleeding and ulcers, and its use has been linked to a higher risk of cardiovascular events [120, 121]. Despite its wide use, the precise mechanisms underlying the analgesic and antipyretic actions of **paracetamol** are not fully understood, although an inhibition of central prostaglandin production has been widely discussed [122]. Interestingly, a number of recent studies indicate a potential contribution of TRPA1 and TRPV1, via the formation of metabolites that act as TRP channel agonists in the brain or spinal cord [123-125]. However, it remains largely unclear how the channel agonistic effects of these metabolites promote analgesia instead of enhancing nociceptive signals. Note that, while generally considered safe, paracetamol overdosing can lead to fatal liver damage [122]. **Opioids** (e.g. codeine, morphine and fentanyl) act by binding to opioid receptors, which can lead to strong analgesia via multiple pathways, including inhibition of the release of substance P and other excitatory neurotransmitters from DRG neurons in the dorsal horn as well as supraspinal mechanisms. However, due to opioid tolerance, there are only limited positive effects of opioids in treating chronic pain beyond 6 weeks. Moreover, opioids are highly addictive and overdoses can be fatal, primarily due to respiratory depression. The misuse of and addiction to opioids is a serious national crisis in the US that affects public health as well as social and economic welfare [11]. **Gabapentinoids** (e.g. gabapentin and pregabalin) were originally developed to be antiseizure drugs. By binding to the $\alpha\delta$ subunit of voltage-gated calcium channels, they reduce excitatory neurotransmitter release in the spinal cord and the brain. Whereas they have shown efficacy for treating chronic pain in diabetic neuropathy and postherpetic neuralgia patients, there is little evidence of efficacy on other types of chronic pain [13]. Dizziness, somnolence and cognitive impairments are notable side effects of gabapentinoids. **Antidepressants** including tricyclic antidepressants (e.g. amitriptyline), as well as serotonin and norepinephrine reuptake inhibitors (e.g. duloxetine) are thought to reduce pain

primarily by modulating descending noradrenergic and serotonergic inhibitory pathways. They may also have anti-inflammatory properties and showed efficacy in a subset of patients treating a variety of neuropathic conditions [126].

In comparison to these currently used analgesic drugs, small molecules that specifically target TRP channels have the potential to dampen nociceptive signals at their origin in the sensory endings of nociceptor neurons, without central effects.

Box 2. Drug therapy for bladder disorders

In addition to specific treatments aimed at improving the integrity the urothelial barrier, including oral pentosan polysulfate or intravesical instillations of hyaluronic acid, chondroitin sulfate or even DMSO (dimethyl sulfoxide), pain in IC/BPC is generally treated with analgesics drugs including NSAIDs, tricyclic antidepressants and opioids (see **Box 1**).

Antimuscarinics are the most widely prescribed drugs for the treatment of overactive bladder and urinary incontinence. Targeting the muscarinic acetylcholine receptors prevents contraction of the detrusor muscle triggered by acetylcholine, which is released from motor neurons that innervate the bladder (**Figure 2**). However, despite their frequent use and associated high costs, only a fraction of patients experience prolonged benefits of antimuscarinic drugs, and many cease treatment due to lack of efficacy and side-effects including dry mouth, blurred vision and constipation [84]. As an alternative, **$\beta 3$ adrenergic receptor agonists** have recently been introduced for the treatment of overactive bladder. Activation of these receptors in the detrusor muscle leads to cAMP-dependent relaxation (**Figure 2**). However, only a subset of patient experiences significant improvement of their symptoms with $\beta 3$ receptor agonists, and their use is also associated with adverse effects, in particular elevated blood pressure [84]. Finally, direct injection of **botulinum toxin A** into the bladder wall, thereby inhibiting the exocytosis of acetylcholine-containing vesicles from motor neurons, has been used in patients that were non-responsive to antimuscarinics (**Figure 2**). While clear improvement was observed, an increase in bladder infections and urine retention was observed [127].

On the other hand, bladder underactivity has been treated with drugs that enhance stimulation of the muscarinic acetylcholine receptors in the detrusor muscle, including **muscarinic receptor agonists** and **acetyl cholinesterase inhibitors**, but there is little evidence of the long-term effectivity of such treatments [128].

Targeting TRP channels in the bladder wall represents a promising, and mechanistically distinct, approach to treat bladder storage dysfunction, as it aims at normalizing afferent signaling from the bladder to the central nervous system, rather than dampening efferent signaling towards the detrusor muscle (**Figure 2**).

Box 3. Drug therapies to improve insulin secretion

The primary aim of any anti-diabetic therapy is reducing hyperglycemia [129]. In case of T2DM, this can be achieved by improving endogenous insulin secretion by the pancreatic β cell. Alternatively, several strategies improve glycemic control relatively independent of the β cell. These include life-style adjustments (weight loss and exercise), metformin therapy (which reduces hepatic glucose production), thiazolidinedione therapy (which improve the insulin response of adipose tissue, skeletal muscle and liver), alpha-glucosidase inhibitors (that prevent digestion of polysaccharides in the gut), SGLT-2 inhibition (which reduces glucose uptake in the kidney) and, finally, insulin injections. A healthier diet and more active lifestyle can be very successful in helping T2DM patients achieve weight loss in the short term. Unfortunately, this approach alone fails to achieve adequate glycemic control within 1 year for the majority of patients (i.e. failure to make lifelong changes in their diet and habits), and most will ultimately require pharmacotherapy to restore normoglycemia [103].

Several strategies are available to enhance insulin secretion from the β cell (**Figure 3**). In short, insulin secretion is the result of glucose uptake by the β cell, closure of ATP-sensitive K^+ channels, depolarization of the β cell and opening of voltage-dependent Ca^{2+} channels. The increase in intracellular Ca^{2+} concentration triggers exocytosis of insulin containing vesicles. **Sulfonylureas** (glybenclamide, glimiperide, glyburide) and **meglitinides** (nateglinide, repaglinide) inhibit ATP-sensitive K^+ channels, independently of glucose uptake and thereby initiate glucose-independent insulin release. An important drawback of these drugs is their potential to trigger hypoglycemic episodes, especially in elderly and in patients with renal impairment [130]. Incretin-based therapies are more successful in this regard. **Incretins**, mainly GLP-1 and GIP, are hormones that are released after eating, which increase insulin secretion from the pancreatic β cell, and slow down gastric emptying. Several synthetic agonists of the GLP-1 receptor are available (exenatide, albiglutide). An important advantage of this pathway is that it potentiates glucose-dependent insulin secretion, absence of hypoglycemia and weight loss. However, on the downside is the need for injections, a high incidence of nausea, vomiting, and diarrhea, especially during initial treatment, and their cost [129]. Alternative to GLP-1, GIP and synthetic incretin receptor agonists, **DPP4-inhibitors** (anagliptin, linagliptin) are increasingly used. These compounds inhibit rapid breakdown of endogenous GLP-1 and GIP by dipeptidyl peptidase-4 [131].

Whereas these therapies all show important improvements in the maintenance of normoglycemia, none of them have proven successful to prevent the progression of T2DM. And even more important, none of them are useful for prevention of diabetes. In this respect, the preclinical data with steviol glycoside TRPM5 agonists are highly promising, as they indicate a beneficial effect both on glucose-dependent insulin release and on the development of hyperglycemia.

Highlights

- TRP channel dysfunction is associated with a growing number of acquired and inherited pathologies, fueling research into specific TRP channel modulating drugs to treat a variety of human disease conditions. Specific indications include chronic pain, lower urinary tract disorders and type II diabetes, global health problems for which the current mainstays of treatment are unsatisfactory.
- Small-molecular inhibitors of TRPV1 showed efficacy in various preclinical models of inflammatory and neuropathic pain, but the clinical development of first generation TRPV1 antagonists was halted due to dangerous on-target side effects. More tailored approaches to silence TRPV1-positive nociceptors or block specific modalities of TRPV1 activation still have potential as novel analgesic treatments.
- Drug development efforts are also ongoing for other TRP channels expressed in sensory neurons, in particular TRPA1, TRPM8 and TRPM3, for treatment of conditions including inflammatory and neuropathic pain as well as migraine.
- TRPV4, highly expressed in urothelial cells, is involved in sensing the fullness of the bladder; its pharmacological modulation improves bladder function in models of overactive and underactive bladder.
- TRPM5 plays a dual role in glucose sensing: as a transduction channel for sweet taste in the tongue, and as positive regulator of glucose-dependent electrical activity and insulin release in the pancreas. Pharmacological enhancement of TRPM5 activity represents a promising route to improve glucose homeostasis in type II diabetes.

Outstanding questions

- What elements determine the translatability of results obtained with TRP channel-targeting drugs from animal models to humans?
- Is it feasible to develop TRP channel-based therapeutic strategies for chronic pain relief without affecting the physiological functions of TRP channels in acute nociception?
- Considering that TRPV1, TRPA1 and TRPM3 are co-expressed in nociceptors and have overlapping roles in pain sensation, which is the best target for analgesic drug development?
- Are there adverse effects of long-term usage of TRPV4 antagonists, and how do they compare to the adverse effects of antimuscarinics for treatment bladder storage diseases?
- Is it possible to develop orally available TRPV4 antagonists that specifically target the bladder urothelium as treatment for bladder underactivity?
- Are TRPM5 potentiating compounds, such as steviol glycosides, useful for prevention or halting progression of type II Diabetes in humans?
- Considering the expression of TRPM5 in chemosensory cells in the tongue and the gut, and its crucial role in the signal transduction cascade downstream of chemoreceptors, which are the systemic effects of TRPM5 modulating compounds apart from its effect on insulin release?

References

1. Clapham, D.E. et al. (2005) International Union of Pharmacology. XLIX. Nomenclature and structure-function relationships of transient receptor potential channels. *Pharmacological Reviews* 57 (4), 427-450.
2. Mulier, M. et al. (2017) TRP channel pores and local calcium signals. *Cell Calcium* 66, 19-24.
3. Clapham, D.E. (2003) TRP channels as cellular sensors. *Nature* 426 (6966), 517-24.
4. Vangeel, L. and Voets, T. (2019) Transient Receptor Potential Channels and Calcium Signaling. *Cold Spring Harb Perspect Biol*.
5. Smani, T. et al. (2015) Functional and physiopathological implications of TRP channels. *Biochim Biophys Acta* 1853 (8), 1772-82.
6. Nilius, B. (2007) TRP channels in disease. *Biochim Biophys Acta* 1772 (8), 805-12.
7. Nilius, B. and Szallasi, A. (2014) Transient Receptor Potential Channels as Drug Targets: From the Science of Basic Research to the Art of Medicine. *Pharmacological Reviews* 66 (3), 676-814.
8. Moran, M.M. (2018) TRP Channels as Potential Drug Targets. *Annu Rev Pharmacol Toxicol* 58, 309-330.
9. Reid, K.J. et al. (2011) Epidemiology of chronic non-cancer pain in Europe: narrative review of prevalence, pain treatments and pain impact. *Curr Med Res Opin* 27 (2), 449-62.
10. Grayson, M. (2016) Pain. *Nature* 535 (7611), S1.
11. Florence, C.S. et al. (2016) The Economic Burden of Prescription Opioid Overdose, Abuse, and Dependence in the United States, 2013. *Med Care* 54 (10), 901-6.
12. Finnerup, N.B. et al. (2015) Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol* 14 (2), 162-73.
13. Goodman, C.W. and Brett, A.S. (2017) Gabapentin and Pregabalin for Pain - Is Increased Prescribing a Cause for Concern? *N Engl J Med* 377 (5), 411-414.
14. Dubin, A.E. and Patapoutian, A. (2010) Nociceptors: the sensors of the pain pathway. *J Clin Invest* 120 (11), 3760-72.
15. Julius, D. (2013) TRP channels and pain. *Annu Rev Cell Dev Biol* 29, 355-84.
16. Vriens, J. et al. (2014) Peripheral thermosensation in mammals. *Nat Rev Neurosci* 15 (9), 573-89.
17. Caterina, M.J. et al. (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 389 (6653), 816-24.
18. Jordt, S.E. et al. (2004) Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* 427 (6971), 260-5.
19. McKemy, D.D. et al. (2002) Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 416 (6876), 52-8.
20. Peier, A.M. et al. (2002) A TRP channel that senses cold stimuli and menthol. *Cell* 108 (5), 705-15.
21. Vriens, J. et al. TRPM3 is a nociceptor channel involved in the detection of noxious heat. *Neuron* 70 (3), 482-94.
22. Kilo, S. et al. (1997) Peripheral CGRP release as a marker for neurogenic inflammation: a model system for the study of neuropeptide secretion in rat paw skin. *Pain* 73 (2), 201-7.
23. Kunkler, P.E. et al. (2011) TRPA1 receptors mediate environmental irritant-induced meningeal vasodilatation. *Pain* 152 (1), 38-44.
24. Held, K. et al. (2015) Activation of TRPM3 by a potent synthetic ligand reveals a role in peptide release. *Proc Natl Acad Sci U S A* 112 (11), E1363-72.
25. Russell, F.A. et al. (2014) Calcitonin gene-related peptide: physiology and pathophysiology. *Physiol Rev* 94 (4), 1099-142.
26. Edvinsson, L. et al. (2018) CGRP as the target of new migraine therapies - successful translation from bench to clinic. *Nat Rev Neurol* 14 (6), 338-350.
27. Prescott, E.D. and Julius, D. (2003) A modular PIP2 binding site as a determinant of capsaicin receptor sensitivity. *Science* 300 (5623), 1284-8.
28. Geron, M. et al. (2017) Animal Toxins Providing Insights into TRPV1 Activation Mechanism. *Toxins (Basel)* 9 (10).

29. Siemens, J. et al. (2006) Spider toxins activate the capsaicin receptor to produce inflammatory pain. *Nature* 444 (7116), 208-12.
30. Caterina, M.J. et al. (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 288 (5464), 306-13.
31. Davis, J.B. et al. (2000) Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature* 405 (6783), 183-7.
32. Gavva, N.R. et al. (2007) The vanilloid receptor TRPV1 is tonically activated in vivo and involved in body temperature regulation. *J Neurosci* 27 (13), 3366-74.
33. Gavva, N.R. et al. (2008) Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. *Pain* 136 (1-2), 202-10.
34. Szallasi, A. and Sheta, M. (2012) Targeting TRPV1 for pain relief: limits, losers and laurels. *Expert Opin Investig Drugs* 21 (9), 1351-69.
35. Garami, A. et al. (2018) TRPV1 antagonists that cause hypothermia, instead of hyperthermia, in rodents: Compounds' pharmacological profiles, in vivo targets, thermoeffectors recruited and implications for drug development. *Acta Physiol (Oxf)* 223 (3), e13038.
36. Garami, A. et al. (2010) Contributions of different modes of TRPV1 activation to TRPV1 antagonist-induced hyperthermia. *J Neurosci* 30 (4), 1435-40.
37. Belvisi, M.G. et al. (2017) XEN-DO501, a Novel Transient Receptor Potential Vanilloid 1 Antagonist, Does Not Reduce Cough in Patients with Refractory Cough. *Am J Respir Crit Care Med* 196 (10), 1255-1263.
38. Quiding, H. et al. (2013) TRPV1 antagonistic analgesic effect: a randomized study of AZD1386 in pain after third molar extraction. *Pain* 154 (6), 808-12.
39. Manitsipitkul, P. et al. (2018) A multiple-dose double-blind randomized study to evaluate the safety, pharmacokinetics, pharmacodynamics and analgesic efficacy of the TRPV1 antagonist JNJ-39439335 (mavarep). *Scand J Pain* 18 (2), 151-164.
40. Krarup, A.L. et al. (2013) Randomized clinical trial: inhibition of the TRPV1 system in patients with nonerosive gastroesophageal reflux disease and a partial response to PPI treatment is not associated with analgesia to esophageal experimental pain. *Scand J Gastroenterol* 48 (3), 274-84.
41. Murdoch, R.D. et al. (2014) TRPV1 inhibition does not prevent cold dry air-elicited symptoms in non-allergic rhinitis. *Int J Clin Pharmacol Ther* 52 (4), 267-76.
42. Derry, S. et al. (2017) Topical capsaicin (high concentration) for chronic neuropathic pain in adults. *Cochrane Database Syst Rev* 1, CD007393.
43. Backonja, M. et al. (2008) NGX-4010, a high-concentration capsaicin patch, for the treatment of postherpetic neuralgia: a randomised, double-blind study. *Lancet Neurol* 7 (12), 1106-12.
44. Binshtok, A.M. et al. (2007) Inhibition of nociceptors by TRPV1-mediated entry of impermeant sodium channel blockers. *Nature* 449 (7162), 607-10.
45. Roberson, D.P. et al. (2011) Targeting of sodium channel blockers into nociceptors to produce long-duration analgesia: a systematic study and review. *Br J Pharmacol* 164 (1), 48-58.
46. Talbot, S. et al. (2015) Silencing Nociceptor Neurons Reduces Allergic Airway Inflammation. *Neuron* 87 (2), 341-54.
47. Bandell, M. et al. (2004) Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron* 41 (6), 849-57.
48. Andersson, D.A. et al. (2008) Transient receptor potential A1 is a sensory receptor for multiple products of oxidative stress. *J Neurosci* 28 (10), 2485-94.
49. Eberhardt, M.J. et al. (2012) Methylglyoxal activates nociceptors through transient receptor potential channel A1 (TRPA1): a possible mechanism of metabolic neuropathies. *J Biol Chem* 287 (34), 28291-306.
50. Trevisani, M. et al. (2007) 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. *Proc Natl Acad Sci U S A* 104 (33), 13519-24.
51. Moparthy, L. et al. (2016) Human TRPA1 is a heat sensor displaying intrinsic U-shaped thermosensitivity. *Sci Rep* 6, 28763.

52. Bautista, D.M. et al. (2006) TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell* 124 (6), 1269-82.
53. Chen, J. et al. (2011) Selective blockade of TRPA1 channel attenuates pathological pain without altering noxious cold sensation or body temperature regulation. *Pain* 152 (5), 1165-72.
54. del Camino, D. et al. (2010) TRPA1 contributes to cold hypersensitivity. *J Neurosci* 30 (45), 15165-74.
55. Horvath, A. et al. (2016) Transient receptor potential ankyrin 1 (TRPA1) receptor is involved in chronic arthritis: in vivo study using TRPA1-deficient mice. *Arthritis Res Ther* 18, 6.
56. Lehto, S.G. et al. (2016) Selective antagonism of TRPA1 produces limited efficacy in models of inflammatory- and neuropathic-induced mechanical hypersensitivity in rats. *Mol Pain* 12.
57. Kremeyer, B. et al. (2010) A gain-of-function mutation in TRPA1 causes familial episodic pain syndrome. *Neuron* 66 (5), 671-80.
58. de Oliveira, C. et al. (2014) Transient receptor potential channel ankyrin-1 is not a cold sensor for autonomic thermoregulation in rodents. *J Neurosci* 34 (13), 4445-52.
59. Koivisto, A. et al. (2018) TRPA1 Antagonists for Pain Relief. *Pharmaceuticals (Basel)* 11 (4).
60. Jia, S. et al. (2017) Antinociceptive Effects of Isosakuranetin in a Rat Model of Peripheral Neuropathy. *Pharmacology* 100 (3-4), 201-207.
61. Krugel, U. et al. (2017) Primidone inhibits TRPM3 and attenuates thermal nociception in vivo. *Pain* 158 (5), 856-867.
62. Badheka, D. et al. (2017) Inhibition of Transient Receptor Potential Melastatin 3 ion channels by G-protein betagamma subunits. *Elife* 6.
63. Dembla, S. et al. (2017) Anti-nociceptive action of peripheral mu-opioid receptors by G-beta-gamma protein-mediated inhibition of TRPM3 channels. *Elife* 6.
64. Quallo, T. et al. (2017) G protein betagamma subunits inhibit TRPM3 ion channels in sensory neurons. *Elife* 6.
65. Stein, C. (2016) Opioid Receptors. *Annu Rev Med* 67, 433-51.
66. Vandewauw, I. et al. (2018) A TRP channel trio mediates acute noxious heat sensing. *Nature* 555 (7698), 662-666.
67. McKemy, D.D. (2013) The molecular and cellular basis of cold sensation. *ACS Chem Neurosci* 4 (2), 238-47.
68. Liu, B. et al. (2013) TRPM8 is the principal mediator of menthol-induced analgesia of acute and inflammatory pain. *Pain* 154 (10), 2169-77.
69. Palkar, R. et al. (2018) Cooling Relief of Acute and Chronic Itch Requires TRPM8 Channels and Neurons. *J Invest Dermatol* 138 (6), 1391-1399.
70. Lehto, S.G. et al. (2015) AMG2850, a potent and selective TRPM8 antagonist, is not effective in rat models of inflammatory mechanical hypersensitivity and neuropathic tactile allodynia. *Naunyn Schmiedebergs Arch Pharmacol* 388 (4), 465-76.
71. Bertamino, A. et al. (2018) Identification of a Potent Tryptophan-Based TRPM8 Antagonist With in Vivo Analgesic Activity. *J Med Chem*.
72. Uvin, P. et al. (2015) Essential role of transient receptor potential M8 (TRPM8) in a model of acute cold-induced urinary urgency. *Eur Urol* 68 (4), 655-61.
73. Winchester, W.J. et al. (2014) Inhibition of TRPM8 channels reduces pain in the cold pressor test in humans. *J Pharmacol Exp Ther* 351 (2), 259-69.
74. Chasman, D.I. et al. (2011) Genome-wide association study reveals three susceptibility loci for common migraine in the general population. *Nat Genet* 43 (7), 695-8.
75. Freilinger, T. et al. (2012) Genome-wide association analysis identifies susceptibility loci for migraine without aura. *Nat Genet* 44 (7), 777-82.
76. Dussor, G. and Cao, Y.Q. (2016) TRPM8 and Migraine. *Headache* 56 (9), 1406-1417.
77. Horne, D.B. et al. (2018) Discovery of TRPM8 Antagonist (S)-6-(((3-Fluoro-4-(trifluoromethoxy)phenyl)(3-fluoropyridin-2-yl)methyl)carbamoyl)nicotinic Acid (AMG 333), a Clinical Candidate for the Treatment of Migraine. *J Med Chem* 61 (18), 8186-8201.
78. Aoki, Y. et al. (2017) Urinary incontinence in women. *Nat Rev Dis Primers* 3, 17042.

79. Kinsey, D. et al. (2016) The psychological impact of overactive bladder: A systematic review. *J Health Psychol* 21 (1), 69-81.
80. Franken, J. et al. (2014) TRP channels in lower urinary tract dysfunction. *Br J Pharmacol* 171 (10), 2537-51.
81. Merrill, L. et al. (2016) Receptors, channels, and signalling in the urothelial sensory system in the bladder. *Nat Rev Urol* 13 (4), 193-204.
82. Malde, S. et al. (2018) Guideline of guidelines: bladder pain syndrome. *BJU Int* 122 (5), 729-743.
83. Neuhaus, J. and Schwalenberg, T. (2012) Intravesical treatments of bladder pain syndrome/interstitial cystitis. *Nat Rev Urol* 9 (12), 707-20.
84. McDonnell, B. and Birder, L.A. (2017) Recent advances in pharmacological management of urinary incontinence. *F1000Res* 6, 2148.
85. Griffiths, D. (2015) Neural control of micturition in humans: a working model. *Nat Rev Urol* 12 (12), 695-705.
86. Nilius, B. and Voets, T. (2013) The puzzle of TRPV4 channelopathies. *EMBO Rep* 14 (2), 152-63.
87. Everaerts, W. et al. (2010) Functional characterization of transient receptor potential channels in mouse urothelial cells. *Am J Physiol Renal Physiol* 298 (3), F692-701.
88. Gevaert, T. et al. (2007) Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding. *J Clin Invest* 117 (11), 3453-62.
89. Mochizuki, T. et al. (2009) The TRPV4 cation channel mediates stretch-evoked Ca²⁺ influx and ATP release in primary urothelial cell cultures. *J Biol Chem* 284 (32), 21257-64.
90. Ihara, T. et al. (2018) The time-dependent variation of ATP release in mouse primary-cultured urothelial cells is regulated by the clock gene. *Neurourol Urodyn* 37 (8), 2535-2543.
91. Ihara, T. et al. (2017) The Clock mutant mouse is a novel experimental model for nocturia and nocturnal polyuria. *Neurourol Urodyn* 36 (4), 1034-1038.
92. Ihara, T. et al. (2017) Clock Genes Regulate the Circadian Expression of Piezo1, TRPV4, Connexin26, and VNUT in an Ex Vivo Mouse Bladder Mucosa. *PLoS One* 12 (1), e0168234.
93. Negoro, H. et al. (2012) Involvement of urinary bladder Connexin43 and the circadian clock in coordination of diurnal micturition rhythm. *Nat Commun* 3, 809.
94. Everaerts, W. et al. (2010) Inhibition of the cation channel TRPV4 improves bladder function in mice and rats with cyclophosphamide-induced cystitis. *Proc Natl Acad Sci U S A* 107 (44), 19084-9.
95. Merrill, L. and Vizzard, M.A. (2014) Intravesical TRPV4 blockade reduces repeated variate stress-induced bladder dysfunction by increasing bladder capacity and decreasing voiding frequency in male rats. *Am J Physiol Regul Integr Comp Physiol* 307 (4), R471-80.
96. Goyal, N. et al. (2019) Clinical Pharmacokinetics, Safety, and Tolerability of a Novel, First-in-Class TRPV4 Ion Channel Inhibitor, GSK2798745, in Healthy and Heart Failure Subjects. *Am J Cardiovasc Drugs*.
97. Deruyver, Y. et al. (2018) Intravesical Activation of the Cation Channel TRPV4 Improves Bladder Function in a Rat Model for Detrusor Underactivity. *Eur Urol* 74 (3), 336-345.
98. Takaoka, E.I. et al. (2018) Effect of TRPV4 activation in a rat model of detrusor underactivity induced by bilateral pelvic nerve crush injury. *Neurourol Urodyn* 37 (8), 2527-2534.
99. Willette, R.N. et al. (2008) Systemic activation of the transient receptor potential vanilloid subtype 4 channel causes endothelial failure and circulatory collapse: Part 2. *J Pharmacol Exp Ther* 326 (2), 443-52.
100. Cima, M.J. et al. (2014) Single compartment drug delivery. *J Control Release* 190, 157-71.
101. Kolawole, O.M. et al. (2017) Advances in intravesical drug delivery systems to treat bladder cancer. *Int J Pharm* 532 (1), 105-117.
102. American Diabetes, A. (2014) Diagnosis and classification of diabetes mellitus. *Diabetes Care* 37 Suppl 1, S81-90.
103. van Raalte, D.H. and Verchere, C.B. (2017) Improving glycaemic control in type 2 diabetes: Stimulate insulin secretion or provide beta-cell rest? *Diabetes Obes Metab* 19 (9), 1205-1213.
104. Philippaert, K. and Vennekens, R. (2017) The Role of TRP Channels in the Pancreatic Beta-Cell. In *Neurobiology of TRP Channels* (nd and Emir, T.L.R. eds), pp. 229-250.

105. Colsoul, B. et al. (2010) Loss of high-frequency glucose-induced Ca²⁺ oscillations in pancreatic islets correlates with impaired glucose tolerance in *Trpm5*^{-/-} mice. *Proc Natl Acad Sci U S A* 107 (11), 5208-13.
106. Philippaert, K. et al. (2017) Steviol glycosides enhance pancreatic beta-cell function and taste sensation by potentiation of TRPM5 channel activity. *Nat Commun* 8, 14733.
107. Vennekens, R. et al. (2018) TRPM5 in the battle against diabetes and obesity. *Acta Physiol (Oxf)* 222 (2).
108. Shigeto, M. et al. (2015) GLP-1 stimulates insulin secretion by PKC-dependent TRPM4 and TRPM5 activation. *J Clin Invest* 125 (12), 4714-28.
109. Colsoul, B. et al. (2013) Insulin downregulates the expression of the Ca²⁺-activated nonselective cation channel TRPM5 in pancreatic islets from leptin-deficient mouse models. *Pflugers Arch* 466 (3), 611-21.
110. Ketterer, C. et al. (2011) Genetic variation within the TRPM5 locus associates with prediabetic phenotypes in subjects at increased risk for type 2 diabetes. *Metabolism* 60 (9), 1325-33.
111. Tabur, S. et al. (2015) Role of the transient receptor potential (TRP) channel gene expressions and TRP melastatin (TRPM) channel gene polymorphisms in obesity-related metabolic syndrome. *Eur Rev Med Pharmacol Sci* 19 (8), 1388-97.
112. Ivask, M. et al. (2016) RNA-sequencing of WFS1-deficient pancreatic islets. *Physiol Rep* 4 (7).
113. Jeppesen, P.B. et al. (2000) Stevioside acts directly on pancreatic beta cells to secrete insulin: actions independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K⁺-channel activity. *Metabolism* 49 (2), 208-14.
114. Abudula, R. et al. (2004) Rebaudioside A potently stimulates insulin secretion from isolated mouse islets: studies on the dose-, glucose-, and calcium-dependency. *Metabolism* 53 (10), 1378-81.
115. Dyrskog, S.E. et al. (2005) Preventive effects of a soy-based diet supplemented with stevioside on the development of the metabolic syndrome and type 2 diabetes in Zucker diabetic fatty rats. *Metabolism* 54 (9), 1181-8.
116. Jeppesen, P.B. et al. (2006) Can stevioside in combination with a soy-based dietary supplement be a new useful treatment of type 2 diabetes? An in vivo study in the diabetic goto-kakizaki rat. *Rev Diabet Stud* 3 (4), 189-99.
117. Geuns, J.M. et al. (2007) Metabolism of stevioside by healthy subjects. *Exp Biol Med (Maywood)* 232 (1), 164-73.
118. Gregersen, S. et al. (2004) Antihyperglycemic effects of stevioside in type 2 diabetic subjects. *Metabolism* 53 (1), 73-6.
119. FitzGerald, G.A. (2003) COX-2 and beyond: Approaches to prostaglandin inhibition in human disease. *Nat Rev Drug Discov* 2 (11), 879-90.
120. Bally, M. et al. (2017) Risk of acute myocardial infarction with NSAIDs in real world use: bayesian meta-analysis of individual patient data. *BMJ* 357, j1909.
121. Bjarnason, I. et al. (2018) Mechanisms of Damage to the Gastrointestinal Tract From Nonsteroidal Anti-Inflammatory Drugs. *Gastroenterology* 154 (3), 500-514.
122. Ghanem, C.I. et al. (2016) Acetaminophen from liver to brain: New insights into drug pharmacological action and toxicity. *Pharmacol Res* 109, 119-31.
123. Andersson, D.A. et al. (2011) TRPA1 mediates spinal antinociception induced by acetaminophen and the cannabinoid Delta(9)-tetrahydrocannabinol. *Nat Commun* 2, 551.
124. Mallet, C. et al. (2010) TRPV1 in brain is involved in acetaminophen-induced antinociception. *PLoS One* 5 (9).
125. Ohashi, N. et al. (2017) Acetaminophen Metabolite N-Acylphenolamine Induces Analgesia via Transient Receptor Potential Vanilloid 1 Receptors Expressed on the Primary Afferent Terminals of C-fibers in the Spinal Dorsal Horn. *Anesthesiology* 127 (2), 355-371.
126. Kremer, M. et al. (2016) Antidepressants and gabapentinoids in neuropathic pain: Mechanistic insights. *Neuroscience* 338, 183-206.

127. Mangera, A. et al. (2014) An updated systematic review and statistical comparison of standardised mean outcomes for the use of botulinum toxin in the management of lower urinary tract disorders. *Eur Urol* 65 (5), 981-90.
128. Chai, T.C. and Kudze, T. (2017) New therapeutic directions to treat underactive bladder. *Investig Clin Urol* 58 (Suppl 2), S99-S106.
129. Nathan, D.M. (2015) Diabetes: Advances in Diagnosis and Treatment. *JAMA* 314 (10), 1052-62.
130. van Dalem, J. et al. (2016) Risk of hypoglycaemia in users of sulphonylureas compared with metformin in relation to renal function and sulphonylurea metabolite group: population based cohort study. *BMJ* 354, i3625.
131. Gomez-Peralta, F. et al. (2018) Safety and Efficacy of DPP4 Inhibitor and Basal Insulin in Type 2 Diabetes: An Updated Review and Challenging Clinical Scenarios. *Diabetes Ther* 9 (5), 1775-1789.

Figure legends

Figure 1: Targeting TRP channels in the pain pathway. Non-myelinated C fibers and thinly myelinated A δ fibers have their cell bodies in the dorsal root ganglia, and their pseudo-unipolar axons convey signals from peripheral tissue to the dorsal horn. Via spinal interneurons, these signals can lead to activation of motoneurons that mediate a rapid avoidance reaction, and via ascending second-order sensory neurons, provoke pain sensation and emotional reactions in higher brain centers. As indicated, currently used analgesic drugs act both peripherally and centrally to reduce pain. At the nerve endings of sensory neurons, various TRP channels act as primary sensors of thermal and chemical stimuli. Their opening causes local membrane depolarization, which can result in activation of voltage-gated Na⁺ and Ca²⁺ channels and action potential firing. TRPA1, TRPV1 and TRPM3 show an overlapping expression profile in a large subset of nociceptor neurons, and play redundant roles in the detection of acute noxious heat. TRPM8 expressing cold-sensitive neurons form a separate neuronal subset. Drugs that specifically target these channels have the potential to inhibit pain without central effects.

Three TRP channel-based approaches have been developed to reduce nociceptor activity. Selective TRP channel antagonism inhibits TRP channel-mediated pain responses but leaves TRP channel-independent responses intact. Treatment with high doses of agonist can lead to overstimulation of sensory neurons, resulting in functional desensitization as well as degeneration of nerve endings. An example is the use of patches with high concentrations of the TRPV1 agonist capsaicin, which leads to prolonged analgesia in neuropathic pain patients due to the defunctionalization of nociceptors. The pore of activated nociceptor TRP channels such as TRPV1 and TRPM3 is wide enough to allow passage of large molecules, including membrane-impermeant inhibitors of voltage-gated sodium channels. An example is the combined application of capsaicin and QX-314, a membrane-impermeable derivative of the local anesthetic lidocaine, leading to selective silencing of nociceptive responses in animal models.

Figure 2: TRP channels and the neuronal control of bladder function. Schematic view of the afferent (left) and efferent (right) innervation of the bladder. During the filling stage, post-ganglionic sympathetic neurons release noradrenalin, leading to contraction of the internal sphincter (via α 1 receptors) and relaxation of the detrusor muscle (via β 3 receptors). β 3 receptor agonists mimic and enhance the latter. During voiding, post-ganglionic parasympathetic neurons release acetylcholine, leading to detrusor muscle contraction, mainly via M3 muscarinic acetylcholine receptors. Antimuscarinics inhibit the contractile effect of acetylcholine on smooth muscle cells, whereas Botulinum toxin A blocks the vesicular release of acetylcholine from the parasympathic neurons.

Several TRP channels lie at the basis of afferent signaling. TRPV4, highly expressed in urothelial cells, responds to membrane stretch and signals the filling state of the bladder towards somatosensory neurons. The free endings of these neurons also contain TRP channels involved in pain signaling (see **Figure 1**). Targeting TRP channels represents a promising alternative to treat bladder pain and storage disorders.

Figure 3: Signal transduction cascades and TRP channels in pancreatic β cells. Glucose enters the β cell via GLUT2 transporters. Upon its metabolization, the intracellular ATP level increases, leading to closure of ATP-sensitive K^+ channels (K_{ATP} channels) and membrane depolarization. The ensuing pattern of bursts of action potentials and influx of Ca^{2+} via voltage-dependent Ca^{2+} channels (VDCC) triggers Ca^{2+} -dependent exocytosis of insulin-containing vesicles. Sulfonylureas induce glucose-independent insulin secretion by blocking K_{ATP} channels, whereas drugs targeting GLP1-receptor signaling enhance glucose-induced insulin secretion via PKA- and PKC-dependent signal transduction cascades. Various Ca^{2+} -permeable TRP channels expressed in the β cell may modulate insulin secretion as alternative entryways for Ca^{2+} , but their actual contribution to the secretory process remains largely obscure. TRPM5, a Ca^{2+} -impermeable cation channel activated by an increase in intracellular Ca^{2+} , increases the frequency of glucose-induced membrane potential oscillations. Consequently, potentiation of TRPM5 activity by steviol glycosides amplifies glucose-induced insulin secretion. TRPM5 and the closely related TRPM4 are also targets of the GLP1 receptor, which potentiates their activity via a PKC-dependent pathway.