Trends in Molecular Medicine

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REVIEWS

Opinion

TRPV1: A Potential Therapeutic Target in Type 2 Diabetes and Comorbidities?

Dorte X. Gram,[1,*] Jens J. Holst,[2] and Arpad Szallasi[3]

With an estimated 422 million affected patients worldwide in 2016, type 2 diabetes (T2D) has reached pandemic proportions and represents a major unmet medical need. T2D is a polygenic disease with a chronic, low-grade inflammatory component. Second-generation transient receptor potential vanilloid-1 (TRPV1) antagonists are potent anti-inflammatory agents with proven clinical safety. In rodent models of T2D, TRPV1 blockade was shown to halt disease progression and improve glucose metabolism. Thus, we propose that TRPV1 antagonists merit further study as novel therapeutic approaches to potentially treat T2D and its comorbidities.

Trends

T2D is being increasingly recognized as a disease with a chronic, low-grade inflammatory component; in preclinical models, elimination of this inflammatory response improves glucose homeostasis and halts disease progression.

T2D is a heterogeneous disease, so a 'one-size-fits-all' approach in drug development may not be feasible (elicits the need for personalized medicine).

Novel antidiabetic agents that not only return blood glucose to near normal levels but also treat comorbidities such as obesity and hyperlipidemia at the same time are needed.

We posit that TRPV1 antagonists may serve as putative antidiabetic agents to treat T2D; current clinical trials are investigating this possibility.

The Human and Monetary Cost of Type 2 Diabetes: A Need for Novel Therapeutic Interventions

Globally, the estimated number of diabetic patients has increased from 108 million in 1980 to 422 million in 2016 and the disease has thus reached pandemic proportions[i].

In the United States, the prevalence of **Type 2 diabetes** (**T2D**, see Glossary and Box 1) is also on the rise, following the global trend. It is estimated that one in three adults have prediabetes and the number of Americans with T2D in 2015 exceeded 30 million[ii]. Moreover, the number of diabetic children is also rapidly growing due to an increasing number of overweight youths [1]. The cost of this increase and the prevalence of diabetes have the potential to be crippling; in 2012, Americans had spent over US$100 billion on diagnosis and medications, and another US $145 billion on the treatment of diabetic complications [2].

T2D is typified by insulin resistance in the skeletal muscle and liver combined with progressive pancreatic β-cell failure [3]. Among T2D comorbidities, **cardiovascular disease** in particular is a leading cause of disability and premature death [4]. Unfortunately, current therapeutic strategies to manage hyperglycemia [5] do not halt (or reverse) disease progression and may even cause undesirable adverse effects and comorbidities on their own. For example, treatment with insulin, sulfonylureas, and glinides may lead to weight gain [6], whereas thiazolidinediones have been associated with bone fractures [7] and an increased risk for congestive heart failure [8]. In addition, antidiabetic agents currently in existence (such as metformin and sulfonylureas) might lose efficacy over time, usually within 4 years[iii]. Indeed, according to the European Medicines Agency, approximately half of T2D patients might need a new drug to maintain adequate glucose control[iii]. This necessitates the development of new therapeutic antidiabetic agents (Box 1).

There is increasing recognition among both clinicians and researchers that T2D has a low-grade inflammatory component. T2D is also heterogenous, so a 'one-size-fits-all' approach in

[1]Pila Pharma, Malmö, Sweden
[2]Panum Institute, University of Copenhagen, Copenhagen, Denmark
[3]Baptist Medical Center, Jacksonville, FL, USA

*Correspondence:
dxg@pilapharma.com (D.X. Gram).

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Box 1. Type 2 Diabetes (T2D): The Facts

- Globally, more than 422 million people are already affected and the number of T2D patients is still on the rise to reach pandemic proportions[i]. Only half of the patients who have the disease are actually diagnosed with it; it is diagnosed when fasting and postprandial blood glucose levels are found to be elevated.
- T2D is a result of impaired insulin secretion and insulin resistance and is associated with obesity and cardiovascular disease.
- At an early stage, lifestyle changes (healthy diet and more exercise) can revert blood glucose levels to normal. If lifestyle changes do not normalize blood glucose, an oral antidiabetic agent (usually metformin) is prescribed. If metformin alone is not sufficient, two to three other oral antidiabetic agents are added. If oral antidiabetic agents do not return blood glucose to near normal levels, an injectable antidiabetic agent (GLP-1 or insulin) is prescribed.
- Many T2D patients require a new antidiabetic agent every 3–4 years to maintain adequate blood glucose control[iii].
- No ideal antidiabetic agent exists (with convenient delivery, good efficacy, no side effects, and low cost). New antidiabetic agents should reduce blood glucose to near normal levels, reduce blood lipids and body weight, and thereby reduce the risk of cardiovascular disease.
- Potential new antidiabetic agents may be second-generation TRPV1 antagonists (that do not cause a febrile reaction or compromise noxious heat sensation) – they are well tolerated, can be delivered as tablets, may be produced at low cost, and – if efficacious in regulating glycemia – may provide additional efficacy (body weight, inflammation, pain, etc.).

drug development does not seem feasible. Here, we present evidence that transient receptor potential vanilloid-1 (TRPV1)-expressing sensory neurons may play a pivotal role in initiating and maintaining the inflammatory component of T2D in preclinical models. We argue that such TRPV1-mediated inflammation could also be relevant for T2D patients. If this hypothesis holds true, a subset of T2D patients might potentially benefit from treatment using TRPV1 antagonists.

A Brief Introduction to TRPV1-Positive Sensory Neurons

In mammals, including humans, a specific subset of **peptidergic sensory neurons** is distinguished by their unique sensitivity to **capsaicin**, the pungent component in hot peppers [9]. Capsaicin and its ultrapotent analog, **resiniferatoxin**, are collectively termed **vanilloids** [9]. The vanilloid (capsaicin) receptor in these neurons was identified as **TRPV1** [10]. TRPV1 is highly expressed on capsaicin-sensitive sensory neurons [9]. It is also present, albeit at much lower levels, in rodent and human brain nuclei [11], as well as in rodent inflammatory cells (including macrophages and T lymphocytes) [12] and skeletal muscle [13]. Sensory nerves that express TRPV1 innervate all organs including the pancreas [14,15] and adipose tissue [16]. Of note, TRPV1 expression has also been reported in rat pancreatic β cells [17] and rat and human adipocytes [18]. However, the physiological role, if any, of the non-neuronal TRPV1 receptor in adipocytes and pancreatic β cells remains unknown. Nevertheless, the endings of TRPV1-expressing nerves have been identified as sites of release of the neuropeptides **substance P (SP)** and **calcitonin gene-related peptide (CGRP)**, both of which are known to initiate the biochemical cascade referred to as **neurogenic inflammation** in both experimental animals and humans [9].

Probing the role of TRPV1-expressing sensory neurons has largely relied on approaches that render relevant cells quiescent – either transiently or permanently. The initial excitation of TRPV1-positive neurons by capsaicin (or resiniferatoxin) is followed by a long-lasting refractory state, traditionally referred to as **capsaicin desensitization**, in which the neuron is unresponsive not only to repeated capsaicin challenge, but also to various unrelated stimuli, including **noxious heat** [9]. Per definition, capsaicin desensitization is reversible. However, modifying the treatment protocol can make the loss of neuronal function permanent. For example, treating neonatal rodents with high-dose capsaicin [50 mg/kg subcutaneously (s.c.)] can kill neurons [19]. This treatment has the expected functional consequences: adult rats treated with capsaicin as newborns show remarkably reduced sensitivity to noxious heat and are capsaicin insensitive, similar to the TRPV1 knockout (KO) mouse [20,21]. Indeed, sensory ganglia removed from these rats show a significant loss (over 50%) of small peptidergic neurons compared to vehicle controls [22].

Glossary

Adiponectin: a protein secreted by adipocytes. The gene encoding adiponectin *ADIPOQ* localizes to chromosome 3q27, a locus linked to genetic susceptibility to obesity and T2D in humans. In mouse models, adiponectin supplementation can improve blood glucose control. In humans, low adiponectin levels herald the development of insulin-resistant T2D.
B2 cells: subclass of lymphocytes involved in adaptive immunity. They differ from B1 cells in their immunoglobulin (Ig) class switch (B1 cells preferentially produce IgM, whereas B2 cells secrete IgG). Obese adipose tissue can recruit B2 cells, known as ATB2 cells to promote insulin resistance.
Calcitonin gene-related peptide (CGRP): neuropeptide with potent vasodilator and proinflammatory actions. A main source of CGRP is the capsaicin-sensitive neuron. CGRP has been implicated in the pathogenesis of migraine, hypertension, and T2D.
Capsaicin: pungent principle in hot chili peppers. A subdivision of sensory neurons is distinguished by its unique sensitivity to capsaicin. Acutely, capsaicin causes pain and triggers neurogenic inflammation. Chronically, capsaicin 'silences' neurons.
Capsaicin desensitization: capsaicin is unique among naturally occurring irritant compounds in that the initial irritation it causes is followed by a long-lasting (up to several weeks) refractory state (referred to as desensitization) in which the previously activated sensory neuron remains unresponsive to various unrelated chemicals and physical stimuli. Desensitization to capsaicin is in clinical use as a form of pain relief.
Cardiovascular disease (CVD): includes various complications (such as stroke and heart attack), many of which are related to atherosclerosis. Obesity is a major risk factor for CVD. In turn, CVD is a leading cause of premature death in patients with T2D.
C-reactive protein (CRP): biomarker of inflammation; elevated in obesity and CVD.
Diabetic foot ulcer: a late complication of diabetes that affects 15% of patients. It is the leading

It is noteworthy that sensory nerve desensitization can not only abolish the response to repeated vanilloid challenge, but might also 'silence' (defunctionalize) the whole neuron [9]. Thus, the effect of ablating TRPV1-expressing sensory neurons from an animal may be even broader than the phenotype of TRPV1 KO mice, given that the former also lose their ability to respond to stimulation pathways that are independent of TRPV1, including the transient receptor potential cation channel, subfamily A, member 1 (TRPA1) activator formalin [9]. This observation suggests that TRPV1-independent pathways that are expressed in the same neurons as TRPV1 will also be lost if TRPV1-sensory neurons are ablated [9].

TRPV1-Expressing Sensory Neurons in T2D

Chemical ablation of sensory nerves by neonatal capsaicin administration (50 mg/kg s.c.) has been shown to improve insulin sensitivity in the adult rat [23]. Inspired by this observation, investigators explored the role of capsaicin-sensitive nerves in blood glucose control and T2D pathogenesis. Specifically, they discovered that sensory nerve desensitization prevented the development of spontaneous hyperglycemia in adult **Zucker diabetic fatty (ZDF) rats** [14]. Diabetes in these rats is preceded by obesity caused by their excessive hunger (food intake), which is secondary to the absence of the leptin receptor due to a missense mutation in the leptin receptor gene, *Lepr* (*fa/fa* genotype) [24]. Obese ZDF rats also show progressive scarring of the pancreas as fibrous tissue replaces degenerating β-cell islets [25], resulting in frank diabetes. Consequently, the ZDF rat is considered a good model of human T2D.

Subsequent studies in rats showed that vanilloid desensitization improved glucose homeostasis by a combination of increased insulin secretion in response to oral glucose challenge in ZDF rats [14,26] and in Zucker obese rats [27], and enhanced insulin sensitivity in Zucker obese rats [27] and in normal rats [28]. Endogenous **dipeptidyl peptidase-4** levels – a serine peptidase involved in the regulation of hormones such as **glucagon-like peptide 1 (GLP-1)** – were reduced in diabetic ZDF rats desensitized to resiniferatoxin (0.01 mg/kg s.c.) [26]. Furthermore, when given to overtly diabetic ZDF rats, resiniferatoxin prevented further deterioration of glucose homeostasis [26].

Moreover, innervation by sensory nerves expressing CGRP was greatly diminished upon capsaicin administration in pancreatic islets, as determined from immunohistochemistry [14]. This effect was accompanied by increased insulin secretion in response to oral glucose [14]. In addition, increased plasma CGRP levels (16 pmol/L in obese animals compared to 6.9 pmol/L in lean littermates) preceded the development of obesity in Zucker obese rats [29]. Based on these observations, a hypothesis was developed which posited that a low-grade neurogenic inflammatory response in the pancreas (and perhaps also in other tissues with deposition of adipose tissue) maintains sustained CGRP release that inhibits insulin secretion, thus contributing to T2D (Figure 1, Key Figure). The mechanism by which CGRP inhibits insulin secretion and promotes the development of T2D is most likely complex and is only partially understood. For example, CGRP is thought to exert a direct inhibitory effect on insulin secretion, presumably by interacting with its receptor on pancreatic islet β cells [30].

Since defunctionalizing TRPV1-expressing neurons show preclinical benefit in animal models of diabetes, researchers have asked if inhibition of TRPV1 *per se* might be a promising therapeutic approach. First, in one study, TRPV1 KO mice fed a high-fat diet did not develop glucose intolerance and exhibited improved glucose-induced insulin secretion [31]. Second, the first generation of a TRPV1 antagonist, **BCTC [*N*-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2H)-carbox-amide]**, given orally, was found to improve glucose tolerance in Zucker obese rats by stimulating insulin secretion and reducing oxidative stress and inflammation in mesenteric white fat [31]. This was evidenced by the suppressed levels of **inducible nitric oxide synthase** protein, and by a reduced number of fat-infiltrating

cause of nontraumatic foot amputations in the United States.
Diacylglycerol: functions as a second messenger signaling lipid that activates protein kinase C (PKC) and facilitates its transport from the cytosol to the plasma membrane.
Dipeptidyl peptidase-4 (DPP-4): serine exopeptidase that cleaves x-proline dipeptides. DPP-4 has been implicated in the inactivation of GLP-1, rendering it a therapeutic target in T2D. DPP-4 inhibitors (so-called gliptins) are in clinical use, mostly prescribed for diabetic patients not responding well to metformin or sulfonylureas.
Endovanilloid: endogenous activator (vanilloid) of the capsaicin receptor, TRPV1.
Fibrinogen: blood protein produced by the liver; essential for clot formation.
First phase of insulin secretion: starts within 2 min of nutrient digestion and lasts for 10–15 min. It promotes peripheral utilization of glucose and blocks hepatic glucose production. It keeps postprandial blood glucose levels within a physiological range and is disturbed in T2D.
Glucagon-like peptide 1 (GLP-1): produced in the pancreas (α cells) and in the gut (enterochromaffin L cells). It increases insulin secretion and, at the same time, inhibits glucagon production, slowing down gastric emptying.
HbA_{1c}: fraction of hemoglobin that has become glycosylated when blood glucose is elevated; it is used as a biomarker of long-term blood glucose control.
Inducible nitric oxide synthase: key enzyme generating nitric oxide (NO), an important regulator of insulin secretion.
Leukotrienes: lipid signaling molecules generated by white blood cells through the oxidation of arachidonic acid. Leukotrienes have been linked to inflammation and asthma.
M1 macrophages (classical macrophage activation): produce NO to fight pathogenic organisms (innate immunity). They also generate high amounts of proinflammatory cytokines.
M2 macrophages (alternative route of macrophage activation): M2 macrophages, with a distinct cytokine profile, eliminate immune

Key Figure

The 'Gram Hypothesis' of CGRP-Expressing Sensory Nerves 'Overdrive' Leading to Type 2 Diabetes and Comorbidities.

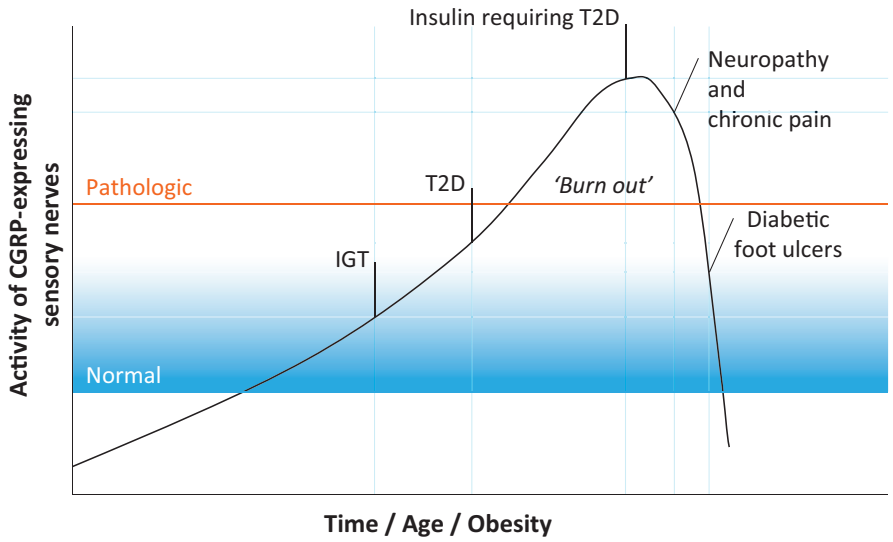

Figure 1. In rats desensitized to vanilloids, CGRP-containing sensory nerves are no longer functional. In these animals, glucose homeostasis is improved by a combination of increased insulin secretion and decreased insulin resistance. Other metabolic improvements have also been uncovered. 'The Gram hypothesis' posits that a low-grade neurogenic inflammatory response maintains sustained CGRP release during T2D that, in turn, might inhibit insulin secretion and potentially contribute to T2D pathogenesis. This model assumes that CGRP-expressing sensory nerves that coexpress transient receptor potential vanilloid-1 (TRPV1) are not dormant but maintain basal activity. This basal activity is maintained by 'endovanilloids' (endogenous TRPV1 activators) generated by obese adipose tissue. If the TRPV1-positive neurons are overstimulated for a prolonged time, they might degenerate due to exhaustion (burn out), where neuropathy and chronic pain may be overt. Eventually, decreased activity of these neurons (below the normal range) might cause late-stage complications such as diabetic foot ulcers because the skin is deprived from the tonic action of substance P. If this hypothesis holds true, TRPV1 antagonists are predicted to be beneficial in T2D patients by preventing the 'overdrive' of CGRP-containing neurons. This prediction is currently being tested in clinical trials using the TRPV1 antagonist XEN-D0501. CGRP, calcitonin gene-related peptide; IGT, impaired glucose tolerance. (Figure adapted from D.X. Gram, PhD thesis defense, University of Copenhagen, Denmark, 2003.)

macrophages [31]. Together, these findings supported the 'Gram hypothesis', stating that low-grade neurogenic inflammation in obese adipose tissue could maintain CGRP release, as well as inhibit insulin secretion and induce insulin resistance, thus potentially contributing to T2D pathogenesis, although this has yet to be directly validated (Figure 1). Accordingly, a patent application on the use of TRPV1 antagonists in 'obesity and obesity-associated diseases and disorders' was filed [31].

Similar to obese Zucker rats, ***ob/ob* obese mice** (a mutant strain that eats excessively due to a leptin mutation [32]) present with elevated plasma CGRP levels [33]. In these animals, oral BCTC administration was reported not only to normalize plasma CGRP levels, but also to stimulate insulin release from β cells, thus improving insulin sensitivity in target tissues such as the liver and skeletal muscle [33]. Lipid metabolism was also improved [33]. Of note, preliminary

complexes and fight fungi and helminths.

***N*-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl) tetrahydropyrazine-1(2H)-carboxamide (BCTC):** a potent, first-generation small-molecule TRPV1 antagonist that inhibits capsaicin activation of rat Trpv1 with an IC_{50} value of 35 nM.

Neurogenic inflammation: caused by the local release of proinflammatory neuropeptides such as SP and CGRP from sensory nerve endings. It has been implicated in the pathomechanisms of migraine, vasomotor rhinitis, and inflammatory bowel disease.

Neurokinin-1 receptor (NK-1R): receptor for SP. NK-1R antagonists have been developed for indications such as pain and asthma. They were discontinued due to cardiovascular side effects.

Noxious heat: thermal stimulus with the potential to cause tissue damage. Noxious heat activates thermoreceptors, including most capsaicin-sensitive neurons. Although the capsaicin receptor TRPV1 is heat responsive, the main heat target that initiates the protective responses remains to be identified.

***Ob/ob* obese mouse:** murine model of human T2D. It eats excessively due to a missense mutation in the gene encoding the appetite-regulating peptide leptin (*lep*).

Painful diabetic neuropathy: a common (to some degree it may affect up to 90% of patients) and potentially debilitating complication of diabetes. It may cause anxiety and sleep disturbances. Once it develops, it is irreversible. Therefore, prevention by strict blood glucose control is a major objective of the medical management of diabetes.

Peptidergic sensory neuron: major subdivision of the peripheral nervous system that releases neuropeptides (e.g., CGRP and SP) upon stimulation. Many peptidergic neurons coexpress the capsaicin receptor TRPV1.

Protein kinase C (PKC): enzyme controlling the function of various proteins via phosphorylation. PKC was shown to sensitize TRPV1 to painful stimuli. Some studies suggest that PKC can also directly activate TRPV1.

Resiniferatoxin: ultrapotent capsaicin analog isolated from the cactuslike perennial, *Euphorbia*

data have suggested that the TRPV1 antagonist, AZV1 (Astra Zeneca), might reduce fructosamine and lactate levels in obese mice, presumably via enhanced insulin sensitivity[iv], but additional studies are required to confirm these findings. Overall, these observations suggest that the therapeutic potential of TRPV1 antagonism in T2D warrants further investigation and may constitute a promising area of research.

Adipose Tissue: Inflammation, Insulin Resistance, and TRPV1

T2D is closely associated with, and often preceded by, obesity and sedentary lifestyle. Biochemical markers of low-grade inflammation [e.g., acute-phase reactants such as **fibrinogen** and **C-reactive protein (CRP)**] are persistently elevated in both obese individuals [34] and T2D patients [35], implying a relationship between obesity and T2D as an (at least in part) inflammatory disorder [36]. CRP has also been found to be a marker of endothelial dysfunction in obesity [34], and identified as a risk marker of cardiovascular death and heart failure [37]. Consequently, low-grade inflammation can be considered a common factor in obesity, T2D, and cardiovascular disease.

Proponents of this theory speculate that obesity is associated with a proinflammatory phenotype in adipocytes which, in turn, plays a causative role in generating insulin resistance and associated comorbidities such as T2D. Indeed, obesity has been associated with the activation of major inflammatory pathways [36,38]. A portion of what has been established about the inflammatory response during obesity has come from studies in adipose tissue and its interaction with the immune system. Mammalian fat contains inflammatory cells [including macrophages and **T-helper (Th) lymphocytes**] that patrol fat tissue and maintain the hormonal sensitivity of adipocytes [39]. Furthermore, rodent adipose tissue is densely innervated by sensory nerve endings that express TRPV1 [17]. In lean animals, most inflammatory T cells are of the **Th2 type**, which help maintain a balance of resident macrophages toward an **M2 macrophage** phenotype, capable of antagonizing prototypic inflammatory responses [40]. By contrast, **Th1 lymphocytes** predominate in obese animals, which produce proinflammatory cytokines such as interferon-γ and interleukin-2 (IL-2) [40]. These cytokines, in turn, recruit proinflammatory **M1 macrophages**, which generate tumor necrosis factor-α (TNF-α), IL-6, and IL-1β. The generation of these cytokines has been positively correlated with the development of insulin resistance and metabolic syndrome [40]. Accordingly, obese T2D patients present high serum TNF-α levels [41]. This is important because TNF-α has also been shown to reduce insulin sensitivity in the periphery by influencing the phosphorylation state of the insulin receptor [42]. These findings establish that TNF-α generation by M1 macrophages can result in insulin receptor phosphorylation, thus providing a link between obese adipose tissue and increased insulin resistance via this pathway.

Macrophages also seem to play a key role in pancreatic islet inflammation as seen in T2D [43]. The best-studied mechanism by which islet-infiltrating macrophages appear to cause pancreatic β-cell dysfunction is through secretion of IL-1β [38,44,45], and blockade of IL-1β activity has been shown to improve glucose control in experimental T2D [46]. In addition, studies carried out with IL-1 receptor antagonists (e.g., anakinra [47]) or IL-1β antagonism (gevokizumab, canakinumab, and LY2189102 [48,49]) in T2D patients have shown beneficial effects on **$HbA1_c$** levels (a marker of long-term blood glucose control) and β-cell function in parallel to a decrease in systemic markers of inflammation such as IL-6 and CRP.

Adipose tissue **B2 cells** (B lymphocytes that regulate local inflammatory responses) are also thought to play a central role in the development of insulin resistance during obesity [50]. Specifically, obese adipose tissue contains increased numbers of B2 cells, and high fat diet-induced insulin resistance is mitigated in B2-deficient (B-null) mice [51]. Moreover, adoptive

resinifera Berg. Intrathecal resiniferatoxin is being tested in the clinic as a 'molecular scalpel' to ablate nociceptive neurons and achieve permanent analgesia in cancer patients with chronic, intractable pain.

Substance P (SP): long-acting (tachykinin) neuropeptide coexpressed with CGRP in capsaicin-sensitive sensory neurons. SP is thought to play a key role in nociception, asthma, and mood disorders. The receptor for SP is known as the neurokinin-1 receptor (NK-1R).

***Tac1* gene:** encodes the four members of the tachykinin peptide hormone family (substance P, neurokinin A, neuropeptide K, and gamma).

T-helper (Th) lymphocytes: T-lymphocyte subset that influences the function of other inflammatory cells by secreting cytokines. **Th1** cells predominate in obese experimental animals. Th1 cells generate proinflammatory cytokines. **Th2** cells are the major Th lymphocyte subset in lean animals.

Transient receptor potential vanilloid-1 (TRPV1): formerly known as the capsaicin receptor or vanilloid receptor, VR1. Highly expressed on a specific subset of sensory neurons (polymodal nociceptors). Thought to be an integrator of painful signals, including noxious heat, acids, pungent plant products (like capsaicin in hot peppers), but also venoms in jelly fish, snakes, and tarantula.

Type 1 diabetes (T1D): diabetes mellitus (juvenile diabetes) is considered an autoimmune disease. Autoreactive T cells destroy pancreatic β cells that produce insulin.

Type 2 diabetes (T2D): disease in which the insulin resistance that develops in targets organs (such as skeletal muscle and liver) leads to increased insulin demand and eventual pancreatic β-cell exhaustion. T2D is intimately related to obesity and CVD.

Vanilloids: capsaicin and its ultrapotent analog, resiniferatoxin, share a homovanillyl moiety essential for bioactivity. In a strict sense, the term 'vanilloid' is used to collectively describe capsaicin and resiniferatoxin structural analogs. More broadly, vanilloids are TRPV1 activators.

transfer of B2 cells from obese, diabetic mice to B-null animals has rendered recipient mice diabetic [51]. B2 cells produce **leukotrienes**, such as leukotriene B4 (LTB4), which can stimulate TRPV1-expressing neurons both directly (by interacting at TRPV1) and indirectly [via its own receptor, leukotriene B4 receptor 1 (LTB4R1)] [52]. In other words, B2 cells may generate **endovanilloids** (endogenous TRPV1 activators) that are capable of activating TRPV1 either directly or indirectly. Consistent with this notion, hypertrophic adipose tissue has been considered to be a source of endovanilloids. For example, lipoxygenase derivatives [53] and polyunsaturated omega-3 fatty acids [54] may function as direct TRPV1 agonists. Furthermore, free fatty acids can stimulate **protein kinase C** via **diacylglycerol** formation [55]. Protein kinase C can, in turn, phosphorylate the TRPV1 channel protein, leading to sensitization [56] or even activation *in vitro*, across several species [57].

In nonobese diabetic mice, TRPV1 regulates pancreatic inflammation and insulin resistance [58]. Nonobese diabetic mice are genetically prone to develop **Type 1 diabetes (T1D)** and carry a hypofunctional TRPV1 mutant localized to the *Idd4.1* diabetes-risk locus. Elimination by capsaicin of SP- and CGRP-containing neurons confers protection against the development of diabetes in these animals, despite the persistence of pathogenic T-cell populations [58].

Further experiments should help clarify the role of all these immune cell populations and their precise relationship to triggering/perpetuating inflammation, obesity, T2D, and other metabolic disorders in the context of TRPV1 activation.

As outlined earlier, there is preliminary evidence that TRPV1 activity may be increased by obesity and diabetes. However, the exact relationship between obesity and TRPV1 remains ill defined and controversial. For example, TRPV1 KO mice have been reported to stay lean [59], or, conversely, become obese [60]. Immunoblotting of adipose tissue obtained from obese individuals has also shown reduced TRPV1-like immunoreactivity [18]. By contrast, a different study reported increased capsaicin responses (including CGRP release) in obese rats, implying a sensitized TRPV1 [61]. Further studies should help elucidate these confounding results.

How Might TRPV1 Antagonists Exert Their Effects in Type 2 Diabetes?

SP and CGRP are coexpressed in the majority of capsaicin-sensitive neurons, and are released together during neurogenic inflammation [9]. In rodents, these two neuropeptides might work in concert to increase blood glucose levels. As discussed earlier, an elevation of plasma CGRP levels heralds the onset of obesity in Zucker obese rats [29]. Similarly, overweight humans also present higher plasma CGRP levels (32.26 pg/ml) than individuals of normal body weight (21.64 pg/ml) [62]. A study reporting that an anti-CGRP antibody could improve the **first phase of insulin secretion** in mice in the oral glucose tolerance test suggested that CGRP might not only serve as a biomarker of T2D, but also potentially act as a pathogenic agent in T2D [63]. In fact, the direct inhibitory effect of CGRP on insulin secretion was first documented 30 years ago in both rodents [64] and pigs [65]. Recently, these observations have been questioned by the finding that the long-acting CGRP analog, α-CGRP, reduced fasting blood glucose in *ob/ob* obese mice [66]. This action was attributed to a positive metabolic effect combined with increased GLP-1 secretion [66]. These conflicting observations are not easy to reconcile. Of note, a similar bimodal effect was reported for SP in a murine model of T1D [58]. Both SP depletion and exogenous SP injection (two conflicting interventions) produced the same outcome: protection against T1D [58].

Human mesenteric preadipocytes express the SP receptor, **neurokinin-1 receptor (NK1R)** [67]. In experimental rat models, SP injection may inhibit insulin-mediated glucose uptake,

XEN-D0501: a potent, small molecule TRPV1 antagonist (previously known as BAY 69-9426) that does not evoke a febrile reaction in humans at doses that block capsaicin-induced responses (a second-generation TRPV1 antagonist). It has demonstrated good oral bioavailability and has been well-tolerated in healthy volunteers, elderly women with overactive bladder disease, and chronic cough patients.

Zucker diabetic fatty (ZDF) rat: model of human T2D. ZDF rats overeat and become fat due to a missense mutation in the leptin receptor gene *Lepr*.

leading to hyperglycemia [68]. Moreover, when kept on a high-fat diet, ***Tac1*** KO mice (the gene that encodes SP) exhibit increased **adiponectin** levels, resulting in enhanced insulin sensitivity and reduced blood glucose [69]. In addition, animals harboring a genetically inactivated NK1R express a similar phenotype, presenting lower weight gain and improved blood glucose control [70]. Whether these observations translate to humans remains to be tested.

Nevertheless, based on all these observations, one can visualize that sustained activation of TRPV1-expressing nerves can increase the release of vasoactive neuropeptides SP and CGRP, which, in turn, can inhibit insulin secretion and impair insulin sensitivity (Figure 2). Second, as impaired insulin sensitivity progresses, TRPV1-expressing nerves may become dysfunctional, as evidenced by the development of **painful diabetic neuropathy**. Indeed, occlusive patches containing a high concentration (8%) of capsaicin (NGX-4010, Qutenza) are in clinical use to relieve this associated neuropathic pain without any significant side effects [71].

According to these hypotheses, a TRPV1 antagonist might be able to improve glucose control in T2D patients by suppressing pathogenic CGRP and SP release. As an added benefit, these drugs might also prevent (or at least delay) the development of late comorbidities such as **diabetic foot ulcers** and painful diabetic neuropathy (see Figure 1), presumably by preserving the trophic function of sensory nerves. It is known that SP can exert a trophic effect on keratinocytes [72], and chemical ablation of SP-expressing nerve endings by capsaicin leads to the development of skin ulcers in rats [73]. Conversely, topical SP promotes wound healing in diabetic rats and mice [74]. In diabetic human skin, the density of TRPV1-positive nerves is decreased, as is the innervation by SP- and CGRP-immunoreactive fibers [75]. To be clear, this remains an untested hypothesis, but we believe that it could be easily addressed in the near future.

Is Impaired TRPV1 Signaling a Faulty Proinflammatory, Neuroimmune Link to T2D?

Based on the aforementioned findings, we propose the following one can visualiz 'Vicious Circle Hypothesis' (Figure 2) in which (i) sensitization/activation of capsaicin-sensitive nerves by proinflammatory substances generated during obesity might lead to sustained SP and CGRP release; (ii) SP and CGRP might block insulin-mediated glucose uptake, leading to compensatory hyperinsulinemia; (iii) insulin might promote TRPV1 transport to the plasma membrane [76]; and (iv) increased TRPV1 might amplify an abnormal SP and CGRP response. Of note, TRPV1-positive neurons not only release SP but also possess NK-1 (SP) receptors [77], suggesting that another harmful autocrine loop may exist in which SP that is released from sensory neurons could further stimulate its own release. However, this remains to be tested.

Nonetheless, if this model holds true, breaking this vicious circle via TRPV1 blockade might improve blood glucose control via improvement of both insulin secretion and insulin sensitivity. Furthermore, TRPV1 antagonists could potentially protect the pancreas from ongoing inflammatory damage, as TRPV1 antagonists have already been shown to ameliorate pain and tissue damage during experimental acute pancreatitis in rodents [78,79]. Indeed, TRPV1 antagonists have been patented to treat obesity, insulin resistance, and diabetes [80–82].

TRPV1 Antagonists in Clinical Trials: Current Challenges and Future Outlook

The molecular cloning of TRPV1 in 1997 [10] triggered a flurry of research into small molecule antagonists of the channel. Astonishingly, it took less than a decade for the first potent TRPV1 antagonists to enter Phase 1 clinical trials [83]. Yet, despite the large investment of the pharmaceutical industry in TRPV1 research, there are still no clinical development candidates in Phase 3 [84]. The two major adverse effects preventing TRPV1 antagonists from entering

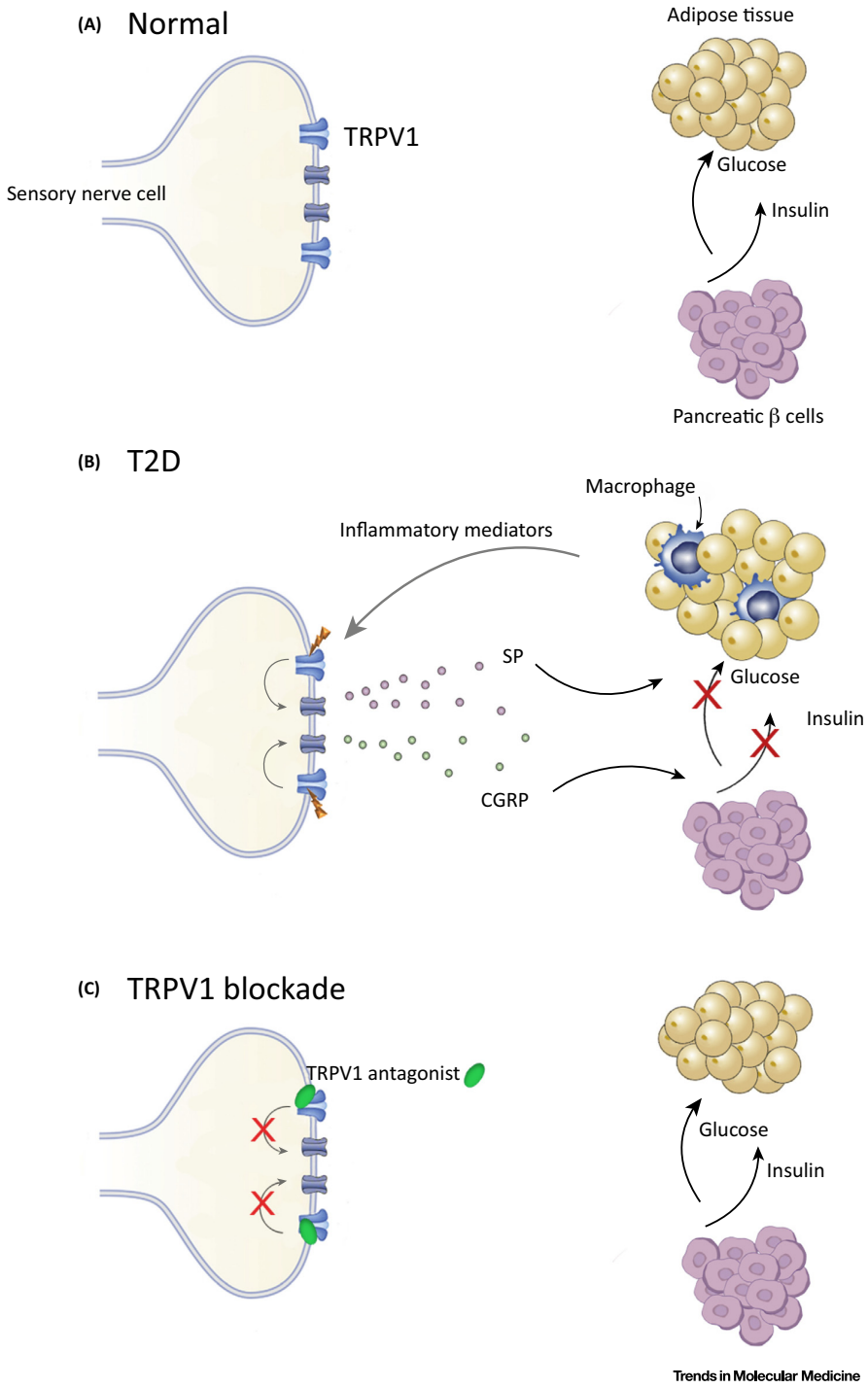

Figure 2. The 'Vicious-Circle Hypothesis': The Role of CGRP-Expressing Sensory Nerves in Obesity and Type 2 Diabetes (T2D). Transient receptor potential vanilloid-1 (TRPV1)-positive CGRP-expressing sensory neurons that contain CGRP innervate both the pancreatic islets and adipose tissue. (A) In healthy subjects, insulin maintains blood glucose in the normal range by regulating glucose uptake in the insulin-sensitive tissues such as liver,

Phase 3 trials have been the transient increase in body temperature (febrile reaction), and long-lasting compromise of noxious heat sensation leading to burn injuries [84]. Neither side effect was completely unexpected (see below), though the underlying molecular mechanisms remain poorly understood.

By contrast, TRPV1 agonists such as capsaicin are well known to induce a transient drop in body temperature in experimental animals including rats and dogs [9]. It is speculated that capsaicin may cause a similar cooling effect in humans (capsaicin as an air conditioner), which might explain the popularity of red chili pepper (capsaicin)-flavored foods in tropical climates [85]. If TRPV1 activation leads to hypothermia, TRPV1 antagonism may be expected to cause hyperthermia. As expected, most TRPV1 antagonists were reported to elevate body temperature in clinical studies, though the duration and magnitude of the febrile reaction varied significantly based on the compounds tested [83,84]. With regard to the noxious heat response, TRPV1 KO mice are deficient in acute heat pain sensation [20,21]. Furthermore, resiniferatoxin desensitization has been reported to markedly increase the temperature required to evoke a noxious heat response in treated rats [86]. These findings are likely due to the fact that noxious heat is a major stimulus of TRPV1 activation [10].

There is, however, increasing evidence that these side effects can be avoided, or at least minimized. Second-generation TRPV1 antagonists have been reported to be well tolerated with either no or only minimal effects on body temperature in healthy volunteers [87,88]. At present, the clinically safe TRPV1 antagonist, **XEN-D0501** [88,89], is being developed as an oral therapy for T2D. XEN-D0501 has effectively blocked capsaicin responses in volunteers, verifying receptor occupancy [89]. Of note, XEN-D0501 or the TRPV1 antagonist SB705498 has not shown any antitussive activity in chronic cough patients [89,90], suggesting that TRPV1 signaling might not be implicated in the pathogenesis of chronic cough. Results from an ongoing clinical trial in patients with T2D (EudraCT-number 2016-003843-12) will be an important step in determining the therapeutic utility of targeting TRPV1 in diabetes.

Concluding Remarks

In rodent models of T2D, pharmacological blockade of TRPV1 by small molecule antagonists can improve insulin secretion, minimize insulin resistance, and halt disease progression. We speculate that in T2D and obesity, inflammatory mediators produced by adipose tissue might continuously activate TRPV1 to evoke sustained CGRP release. Elevated circulating CGRP levels, in turn, might contribute to impaired insulin secretion and, presumably, to the development of insulin resistance, leading to T2D. This vicious circle might be potentially disrupted by TRPV1 antagonism. Consequently, we posit that TRPV1 antagonists warrant further investigation into their potential as novel candidate antidiabetic agents. Evidently, numerous questions and limitations exist (see Outstanding Questions and Box 2); therefore, extensive research will be required to prove that second-generation TRPV1 antagonists can be clinically safe and effective antidiabetic agents. It will be exciting to see whether TRPV1 antagonists are able to interfere in any way with the progression of T2D and/or any of its comorbidities.

Outstanding Questions

Can the beneficial effect of TRPV1 antagonism from preclinical T2D models be effectively translated to T2D patients?

If so, is there a well-defined patient subpopulation that could benefit from TRPV1 antagonist therapy?

How do we identify these patient populations?

Can TRPV1 antagonists be used as a single drug in T2D patients or do they need to be part of a multidrug protocol?

Are TRPV1 antagonists safe when given in a long-term treatment regimen?

What impact would these antagonists have on T2D comorbidities, if any?

Can TRPV1 antagonists effectively reduce the risk for cardiovascular disease?

Is there any additional health benefit from TRPV1 antagonist therapy?

muscle, and adipose tissue. (B) Obese adipose tissue recruits inflammatory cells (e.g., macrophages) that in turn can secrete inflammatory mediators (endovanilloids) that activate TRPV1. When activated, TRPV1 on sensory nerves triggers the release of the neuropeptides substance P (SP) and CGRP, initiating a cascade of neurogenic inflammation. CRGP can decrease insulin secretion, and SP can induce insulin resistance, initiating a vicious cycle that can contribute to T2D pathogenesis. (C) Breaking this vicious cycle via a TRPV1 antagonist (blockade) may be expected to improve insulin secretion, as well as insulin sensitivity and blood glucose control. If this holds true, TRPV1 antagonism should be further investigated as a candidate therapeutic avenue for the management of T2D. CGRP, calcitonin gene-related peptide.

Box 2. Clinician's Corner: The Skinny on TRPV1

- TRPV1 (formerly known as the capsaicin receptor or vanilloid receptor VR1) is a founding member of the vanilloid subfamily of transient receptor potential (TRP) channels; it is the prototypical 'thermo-TRP' (heat-activated TRP) channel.
- It is highly expressed on capsaicin-sensitive sensory neurons, and is also present, albeit at much lower levels, in both brain nuclei and various non-neuronal cells in mammals (endothelium, urothelium, keratinocytes, myocytes, mast cells, etc.) [9].
- Its main function appears to be a 'molecular gatekeeper to the pain pathway'. Its debated role is as a thermosensor involved in body temperature regulation and energy expenditure.
- It is a nonselective cation channel with a preference for Ca^{2+} and exists as a homotetramer (but may form heterotetramers with its splice variants or other TRP channels such as TRPA1).
- Characteristic TRPV1 agonists include capsaicin (from hot, chili peppers); resiniferatoxin (in the latex of *Euphorbia resinifera*); painful venoms from jelly fish, spiders, and snakes; it can be activated by changes in pH and noxious heat. The existence of endogenous agonists (endovanilloids) remains controversial. Topical capsaicin (creams and occlusive patches) has been used for decades to relieve pain.
- Intrathecal resiniferatoxin is currently being tested in the clinic to achieve permanent analgesia in cancer patients with intractable pain due to bone metastases. Intravesical resiniferatoxin has been reported to restore continence in patients with overactive bladder [9]. In animal models, TRPV1 antagonists have ameliorated cancer, burns, as well as neuropathic and postinflammatory pain [9].
- First-generation TRPV1 antagonists can cause a febrile reaction and increase the risk for burn injuries, and have therefore been withdrawn from clinical trials. Second-generation TRPV1 antagonists appear to be devoid of these adverse effects. In clinical trials, TRPV1 antagonists have not shown clinical efficacy in patients with migraine or chronic cough, but have shown some clinical efficacy in patients exhibiting pain.

Conflict of Interest

D.X.G. is the inventor, founder, and coowner of Pila Pharma that holds patents on TRPV1 inhibition as a treatment of insulin resistance, obesity, and diabetes.

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Resources

[i]http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf

[ii]https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf

[iii]http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/06/WC500129256.pdf

[iv]https://www.easd.org/virtualmeeting/home.html#!resources/the-novel-trpvi-antagonist-azv1-improves-insulin-sensitivity-in-ob-ob-mice–2

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