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Miniperspective

Vanilloid Receptor TRPV1 Antagonists as the Next Generation of Painkillers. Are We Putting the Cart before the Horse?

Arpad Szallasi* and Giovanni Appendino

Department of Pathology and Laboratory Medicine, 6 Founders Building, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia, Pennsylvania 19104, and Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Università del Piemonte Orientale, 10131 Novara, Italy

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Introduction

A unique responsiveness to capsaicin (**1**), the irritant principle of hot peppers, is a functional signature of nociceptive neurons. The resulting transient excitation of sensitive pathways is followed by a long-lasting refractory state, traditionally referred to as desensitization, during which the neurons become unresponsive not only to subsequent capsaicin challenges but also to unrelated noxious stimuli such as heat and acids.[1] A membrane recognition site for capsaicin was identified as the vanilloid receptor VR1, recently renamed as TRPV1 (transient receptor potential channel, vanilloid subfamily member 1).[2] Desensitization to capsaicin has a clear therapeutic potential. In fact, capsaicin and its ultrapotent analogue resiniferatoxin (**2a**) have been tried on an empirical basis in diverse disease states ranging from chronic intractable pain through vasomotor rhinitis to the overactive bladder.[1] These clinical trials have been reviewed extensively and will not be dealt with here. Instead, we focus on vanilloid receptor antagonists as an emerging class of novel, analgesic, antiinflammatory agents.

There is mounting evidence that during inflammatory conditions the density of TRPV1 expression is enhanced. Furthermore, endogenous substances, the so-called "endovanilloids", generated during inflammation and acting in concert with low pH and elevated temperature may liberate TRPV1 under the inhibitory control of phosphatidylinositol (4,5)-bisphosphate (PIP_2).[3] These findings have launched massive efforts on part of the pharmaceutical industry to identify novel, orally active TRPV1 antagonists in the hope that a block of the endovanilloid signaling via TRPV1 might be useful in chronic pain and inflammatory hyperalgesia. Furthermore, TRPV1 antagonists should be devoid of neurotoxicity, the most important potential side effect of TRPV1 hyperstimulation (calcium overload) by agonists. The use of the vanilloid antagonist capsazepine (**3**) in animal models of human disease has, however, been so far disappointing, and the discovery of TRPV1 on cells other than nociceptive neurons is raising the inevitable questions: Are TRPV1 antagonists really as safe and effective as originally thought? Are such antagonists really the analgesic drugs of the future, or are we putting the cart before the horse? This review attempts to answer these questions.

Definition of "Vanilloid" and the Place of TRPV1 in the Grand Scheme of Receptors

The name vanilloid is semantically confusing, having different implications in chemistry, pharmacology, and molecular biology. Chemically speaking, a vanilloid is a compound whose structure embodies a 4-hydroxy-3-methoxybenzyl (i.e., vanillyl) moiety. Since capsaicin (**1**) and resiniferatoxin (**2a**) are vanilloids, the receptor at which they interact was logically termed the vanilloid receptor.[1] However, an array of vanilloid receptor agonists was subsequently discovered that lacked any recognizable vanillyl moiety,[4] creating the awkward

* To whom correspondence should be addressed. Phone: (215) 573 3648. Fax: (215) 573 6523. E-mail: szallasa@uphs.upenn.edu.

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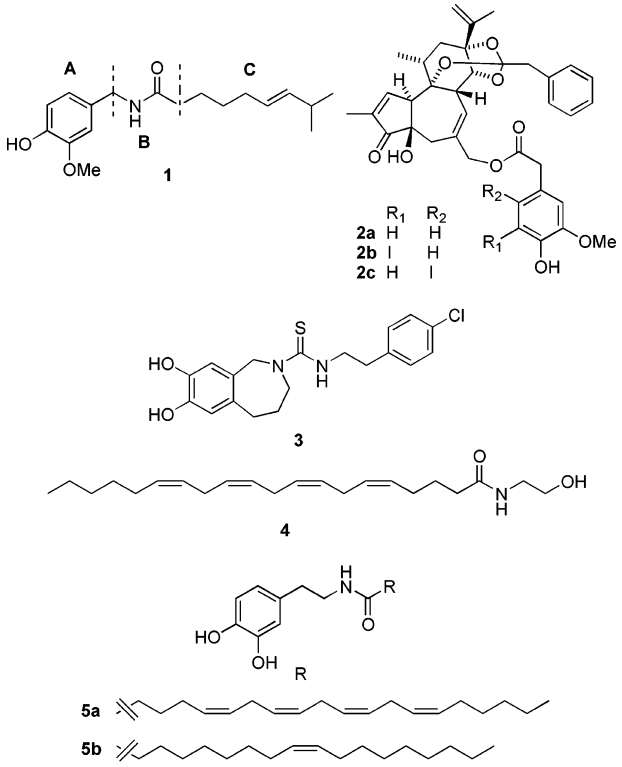


situation of having to define as "vanilloids" even totally aliphatic compounds such as anandamide (**4**). The recent trend of defining receptors at a molecular level (i.e., based on primary amino acid analysis) added a further level of complexity (and uncertainty) to the meaning of the term "vanilloid".

The vanilloid receptor TRPV1 belongs to the large family (currently containing 26 members) of transient receptor potential (TRP) channels that comprise a diverse group of non-voltage-gated cation channels involved in sensory signaling, ranging from thermal and mechanical nociception to vision, taste, olfaction, touch, and osmosensation.[5] The physiologic functions of many remain unknown. These channels are believed to constitute four different subfamilies: C (canonical or classical), V (vanilloid), M (melastatin-related), and P (PKD-type).[6] Membership in a given subfamily does not, however, determine the activating stimuli. This is exemplified by the V and M subfamilies, both of which have members that respond to temperature. By the same reasoning, belonging to the V subfamily does not necessarily imply that the receptor is activated by vanilloids and, conversely, vanilloid-activated receptors may belong to a subfamily of TRP other than V, or a completely distinct receptor family. Predictably, the different meanings of "vanilloid" in chemistry, pharmacology, and molecular biology will remain a source of confusion in the foreseeable future.

Molecular Pharmacology of TRPV1

In a simplistic manner, TRPV1 may be thought of as a molecular integrator of various noxious chemical (e.g., vanilloids and acids) and physical stimuli, most notably heat.[7] However, the pharmacology of TRPV1 is remarkably complex.[1] As a thermal sensor, TRPV1 is activated by temperatures exceeding 43 °C, but the heat threshold is significantly lowered by mild acidification, by endogenous ligands from the fatty acid and eicosanoid pools (lipoxygenase products, anandamide (**4**), *N*-arachidonoyldopamine (NADA, **5a**), *N*-oleoyldopamine (OLDA, **5b**)), and by a structurally heterogeneous family of exogenous ligands.[1,3,7−9] This could result in constant activation of TRPV1 even at body temperature. Conversely, the endogenous lipid phosphatidylinositol (4,5)-diphosphate (PIP_2) has an opposite effect on TRPV1, elevating its threshold of activation and maintaining the receptor in a closed (inactive) state.[8]

TRPV1 is a serpentine protein that has six transmembrane segments flanked by specific ankyrin domains in the cytosolic N-terminal portion.[7] This portion contains consensus phosphorylation sequences for protein kinases. In fact, both protein kinase C and A have been implicated in the sensitization or direct activation of TRPV1 (reviewed in ref 3). Of note, phospholipase C can also activate TRPV1 in an indirect way by cleaving of PIP_2 (reviewed in ref 3). Activation of other membrane receptors by inflammatory mediators such as bradykinin and nerve growth factor can lead to TRPV1 activation via phosphorylation.[8]

The putative channel pore (P-loop) with the proton sensor is located between the last two transmembrane segments, TM5 and TM6, and a vanilloid-binding domain has been identified in the intracellular region linking S2 to S3.[10]

TRPV1 is believed to exist in an oligomeric form, and the heterogeneous quaternary assembly of TRPV1 with other channels (or regulatory proteins) might account for some of the discrepancies observed in cellular assays of vanilloid activity carried out with native and cloned receptors.[1,3]

This complexity foreshadows some inherent problems with TRPV1 antagonists. Since TRPV1 is targeted by heat, protons, and a multitude of "endovanilloids" that most likely interact at distinct recognition sites in a synergistic manner,[3] competitive TPRV1 antagonists may not be able to prevent the activation of the receptor by simply blocking a single class of binding sites. This problem may be circumvented by using a functional antagonist that keeps the channel pore from opening. The feasibility of this approach was first indicated by ruthenium red (see below).

TRPV1 Antagonists: The Beginnings

Historically, the anionic histochemical dye ruthenium red (RR, **6**) was the first (and for decades, the only) vanilloid receptor blocker available.[11] RR is a trinuclear mixed-valence complex that has no effect on capsaicin or resiniferatoxin binding.[1] Its mechanism of action is still unclear but is believed to involve the "clogging" of the channel pore by the large RR molecule.[1,11] While RR served as a useful tool in vitro, its use at the whole animal level was prevented by a powerful proconvulsive activity that may be mediated by a target other than TRPV1.

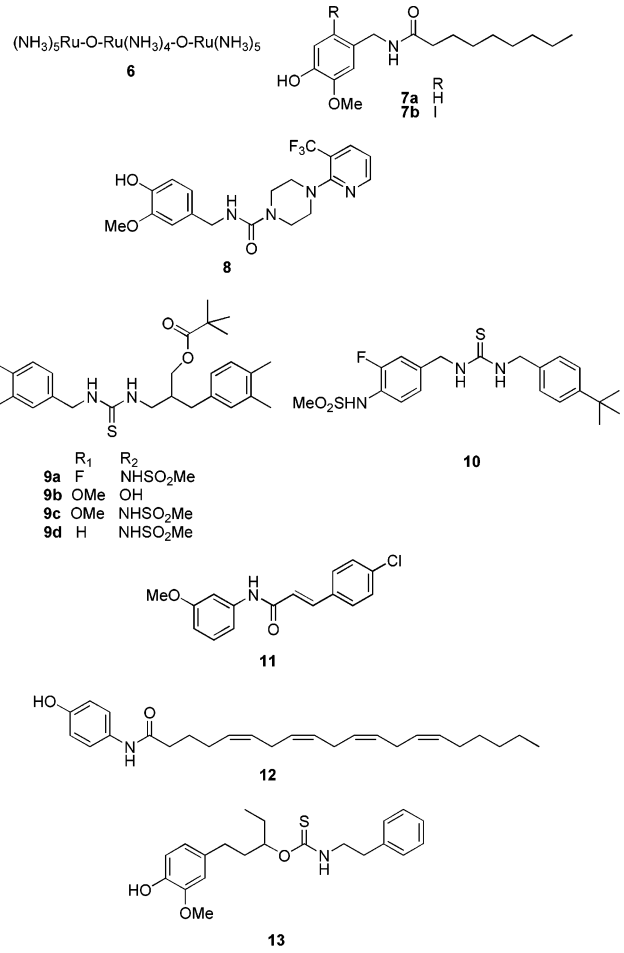
Capsazepine (**3**), the first competitive vanilloid antagonist, emerged from studies by the Sandoz (now Novartis) group that were aimed at assessing the effect of conformational constraint on the lipophilic C-region of capsaicin.[12] In capsazepine (a capsaicinoid of the thiourea type) a propylidene linker between the vanillyl 2-carbon and the amide nitrogen forces the aromatic

ring in an orthogonal orientation compared to the thiourea bond. This constraint has long been considered as the hallmark of vanilloid antagonism, but its relevance for the reversal of biological activity might have been overestimated, since acyclic vanilloids lacking this feature and outperforming capsazepine in terms of inhibition have been discovered.

In animal models, capsazepine has been used with conflicting results to dissect the contribution of VR1 to chronic pain and inflammatory hyperalgesia. Capsazepine has poor metabolic and pharmacokinetic properties in rodents, where it undergoes extensive first-pass metabolism when given orally. In mice, capsazepine blocks the early, but not the late, phase of formalin-induced nociception.[13] In the rat, intradermal injection of capsazepine increased the latency of the paw-withdrawal response evoked by carrageenan, while the inflammation and thermal hyperalgesia by carrageenan remained unaffected.[14] In the guinea pig, capsazepine (1−30 mg/kg sc) produced a 44% reversal of mechanical hyperalgesia by Freund's complete adjuvant (FCA) and an even more profound (80%) reversal of neuropathic pain following partial sciatic nerve ligation.[15] Strikingly, capsazepine had no effect in the rat under similar experimental conditions.[15] The mechanisms underlying these species-related differences are poorly understood, hindering the extrapolation of these findings to humans. A possible explanation involves a combination of pharmacokinetic differences and species-related differences in capsazepine binding to TRPV1. Since capsazepine can also block receptors different from TRPV1,[1] no clear-cut interpretation of these data is possible.

TRPV1 Antagonists Structurally Related to Agonists

A serendipitous discovery at NovoNordisk revealed that the introduction of an iodine atom on the vanillyl moiety of the archetypal TRPV1 agonist resiniferatoxin reverses its pharmacological activity in a way dependent on the location of the halogen.[16] Full reversal of activity takes place upon iodination at C-5 (**2b**),[16] yielding a compound that is 100-fold more potent than capsazepine. Conversely, iodination at C-6 gave only a partial agonist (**2c**).[17] Although scarcity of resiniferatoxin prevented further studies, a systematic investigation on the effect of iodination was carried out with nonivamide (**7a**), the so-called synthetic capsaicin.[18] The results showed that introduction of a halogen (chlorine, bromine, iodine) at both C-5 and C-6 could reverse the vanilloid activity, with higher inhibitory potency for substitution at C-6 compared to C-5. The inhibitory potency (I > Br > Cl) correlates directly with the size of the substituent and inversely with their electronegativity.[18] The 6-iodo derivative **7b** emerged as the most active compound in the series. Taken together, these observations show that halogenation is a powerful means to reverse the activity of vanilloids. Despite the high potency of 5-iodoresiniferatoxin (**2b**) in cellular assays, this compound showed only poor activity in in vivo assays of vanilloid antagonism, including the inhibition of capsaicin-induced flinching in rats.[19] The discrepancy between the in vitro and in vivo activities and the marked species-related differences may reflect poor pharmacokinetic properties.



Antagonistic capsaicinoids could also be obtained by (formal) amidation of vanillamine with a series of 4-(α-pyridyl)piperazine-1-carboxylic acids, as exemplified by Neurogen's urea antagonist **8**.[20] The 4-(α-pyridyl)piperidine-1-carboxamide moiety could also be implanted on various aromatic amines, generating highly potent vanilloid antagonists (as well as thorny proprietary issues; see below). The hallmark of these compounds is the presence of a basic nitrogen atom in the C-region, an interesting modification that makes these compounds the most hydrophilic vanilloid ligands reported so far. The discovery of these ligands further highlights the relevance for the activity of the ring C-region of vanilloids, once simply considered a simple lipophilic tail nonspecifically involved in binding.

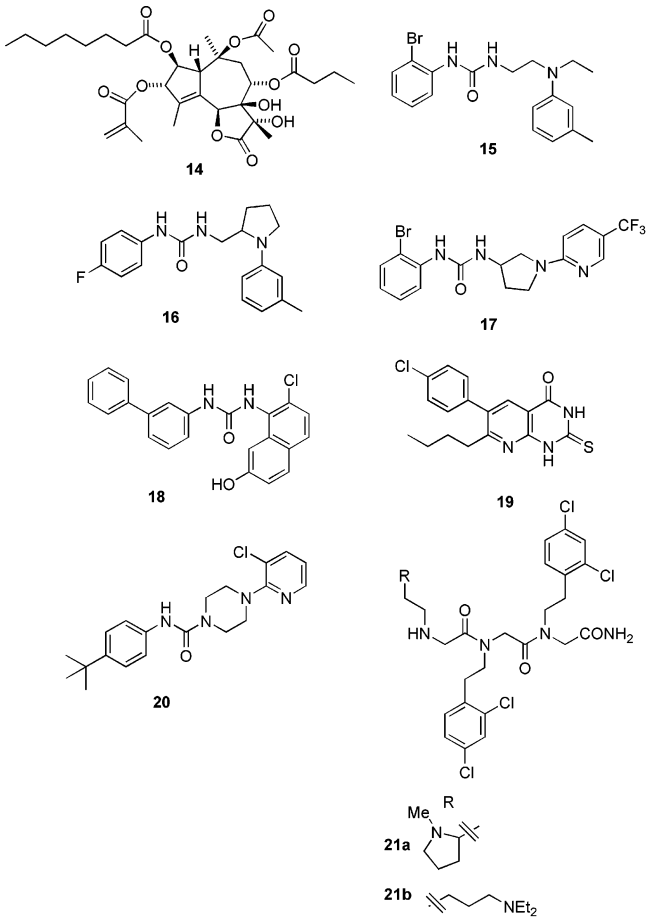
N-(4-Chlorobenzyl)-*N*′-(4-hydroxy-3-iodo-5-methoxybenzyl)thiourea (IBTU) is unusual in that it does inhibit [^{3}H]resiniferatoxin binding at concentrations at which it completely inhibits resiniferatoxin-induced $^{45}Ca^{2+}$ uptake.[21] On the basis of these findings, it was suggested that such antagonists can be designed that antagonize TRPV1 only in defined contexts.

Within the simplified resiniferonol 9,13,14-orthophenylacetate (ROPA) template of 2-benzyl-3-acyloxypropanamine, replacement of the 3-methoxy-4-hydroxyl substitution of the aromatic A-region with a 3-fluoro-4-methansulfonamido functionality could achieve an excellent reversal of activity, as exemplified by **9a**, which substantially maintained the ultrapotency of the parent agonist **9b** (K_i < 10 nM).[22] Simple replacement of the 4-hydroxyl with the sulfonamido group, as in **9c**,

maintained agonistic activity that could be partly reversed by the deletion of the 3-methoxy group, as in **9d**.[22] The ROPA mimic turned out to be redundant for high affinity, and the capsaicinoid-type thiourea **10** eventually emerged from these studies as a powerful in vivo analgesic compound.[23] Of note, compound **10** was inspired by work from the Sandoz (now Novartis) group, where the thiourea and the 4-*tert*-butyl moieties emerged as optimized B and C regions for capsaicin-like activity.[24]

The GlaxoSmithKline lead SB-366791 (**11**)[25] is somewhat related to the inhibitor of anandamide transporter and powerful vanilloid agonist AM-404 (**12**) with an *m*-anisidinyl and a *p*-chlorocinnamate replacing the 4-hydroxyanilinyl and the arachidonyl moieties, respectively.

Branching on the nitrogen α-carbon and bis-homologation of the carbon chain between the guaiacyl moiety and the amide nitrogen to mimic the linker area of capsazepine afforded only weak antagonists whose activity could be increased somewhat by nitrogen-to-oxygen isosteric replacement in the guaiacyl moiety, as exemplified by compound **13**.[26] Despite its low potency, **13** exemplifies the principle that vanilloid receptor affinity can be introduced to structures that deviate considerably from the natural products leads and that there is therefore ample latitude for modification, a situation somewhat reminiscent of that of morphine and opioids.



TRPV1 Antagonists Structurally Unrelated to Agonists

The sesquiterpene lactone thapsigargin (**14**) is the only vanilloid antagonist that has emerged so far from the natural products pool.[27] This complex guaianolide is a powerful inhibitor of the sarcoendoplasmiatic reticulum Ca^{2+}-ATPases (SERCASs), an important class of calcium transporters, and shows tumor-promoting activity and is an extraordinary skin irritant. These unfavorable properties and the lack of a selective bioactivity make thapsigargin a poor lead, but it is not unreasonable to assume that better inhibitors could emerge from the natural products pool or from the chemical modification of thapsigargin. Preliminary data on the vanilloid antagonistic activity of the alkaloid cocaine[28] and the triterpenoid glycosides ginsenosides[29] are still waiting for further confirmation.

Potent vanilloid inhibition was also discovered in compounds bearing no obvious structural resemblance to any class of vanilloid agonists. These compounds have emerged from high-throughput random screening projects and represent the first type of vanilloid ligands whose structures were not inspired (directly or retroactively) from those of natural products. At present, this group of antagonists includes (a) *N*-(haloanilino)carbonyl-*N*′-alkyl-*N*″-arylethylendiamines discovered at SmithKline Beecham and exemplified by structures **15**−**17**,[30−32] (b) *N*-diphenyl-*N*′-naphthylureas discovered at Bayer and exemplified by structure **18**,[33] and (c) pyrido[2,3-*d*]-pyrimidin-4-ones discovered at Novartis and exemplified by **19**.[34]

The powerful vanilloid antagonistic properties of a series of *N*-aryl-4-(2-pyridyl)piperazine-1-carboxamides were discovered, apparently independently, by Johnson & Johnson[35] and Purdue Pharma researchers.[36] These compounds are exemplified by BCTC (**20**) and could be considered as constrained versions of the SmithKline Beecham *N*-(anilino)carbonyl-*N*′-dialkylethylendiamine template of **15**−**17**.[25] BCTC appears to be highly specific for TRPV1 when tested against a panel of 63 receptors, enzymes, and ion channels. It is therefore a better probe than capsazepine for investigating the biology of TRPV1 and also holds potential as a drug. Indeed, BCTC was highly active in animal models of chronic pain from inflammation and from nerve injury and showed good oral bioavailability in rodents. Interestingly, the vanillyl analogue of BCTC (**8**) is currently claimed by Neurogen,[20] making the proprietary state of these compounds rather checkered. Patent matters aside, these compounds and the new "unnatural" templates have the potential to dramatically expand our knowledge of vanilloid pharmacology, complementing the results available from the compounds derived from, or inspired by, the natural leads.

Finally, also worth mentioning are a pair of trimeric and proteolytically stable *N*-alkylglycines (**21a**,**b**) that presumably exert antagonistic activity toward capsaicin because of their channel-blocking properties, acting as a nonmetal analogues of RR.[37]

Genetic and Immunological Approaches to TRPV1 Silencing

Although strictly speaking it is out of the scope of this review, for the sake of completion it should be mentioned that antisense nucleotidic approaches (locked nucleic acids and double-stranded small interfering

RNAs) have been used to inhibit expression of TRPV1 in a manner somewhat similar to that of desensitization.[38] Furthermore, the administration of anti rTRPV1 serum to diabetic mice was shown to ameliorate thermal allodynia and hyperalgesia.[39] Since DNA sequences encoding the human versions of various TRPVs are covered by patents from different companies, any genetic approach to TRPV1 silencing would have to wait until the resolution of these proprietary issues.

New Arguments for the Therapeutic Utility of Selective TRPV1 Antagonists

In the rat, peripheral inflammation was shown to increase TRPV1 levels via p38 MAPK activation by nerve growth factor (NGF).[40] This change is believed to play a pivotal role in maintaining the heat hyperalgesia. In a murine model of diabetic neuropathy, an upregulation of TRPV1 on sensory nerves was described.[41] Of relevance are the findings of increased TPVR1-like immunoreactivity levels in the colon of patients with inflammatory bowel disease[42] and with irritable bowel syndrome characterized by rectal hypersensitivity and fecal urgency.[43]

TRPV1 is coexpressed with the cannabinoid receptor CB1 on sensory neurons (reviewed in ref 3). Anandamide (and possibly also other endovanilloids) exerts opposing actions on TRPV1 and CB1 (reviewed in ref 3). For example, anandamide at concentrations lower than 1 μM blocks the release of the proinflammatory neuropeptide calcitonin gene-related peptide (CGRP) from sensory neurons and thereby exerts an antiinflammatory action.[44] At concentrations over 1 μM, a proinflammatory action (increasing release of CGRP) takes over as activation by anandamide of TRPV1 overcomes the blocking effect of CB1 (ibidem). It is easy to visualize how an overexpressed (see above) and possibly also sensitized TRPV1 becomes dominant over CB1 during inflammatory conditions. If this model holds true, a selective TRPV1 antagonist should be beneficial by blocking the proinflammatory action of anandamide (and/or other endovanilloids) via TRPV1 and thereby restoring the inhibitory influence of anandamide via CB1. In keeping with this, capsazepine was found to ameliorate the experimental colitis induced by dextran sulfate,[45] and ileitis evoked by *C. difficile* toxin A, in the rat.[46]

New Findings Cautioning the Clinical Utility of TRPV1 Antagonists

The old dogma of TRPV1 being a neurochemical signature of primary sensory neurons cannot be maintained in the light of recent findings. TRPV1 is present in various brain nuclei throughout the whole neuroaxis and is also expressed in nonneuronal tissues such as the epidermis (keratinocytes), urothelium, mast cells, fibroblasts, and smooth muscle (reviewed in ref 47). It has been speculated that in the epidermis and urothelium, TRPV1 functions as a protective sensor of noxious stimuli as it does on sensory neurons.[3] A potential indication for TRPV1 antagonists is diabetic neuropathy. Diabetic patients, however, already have compromised tissue protection and are prone to developing necrosis. Thus, the use of TRPV1 antagonists in such patients can be counterproductive.

The role of TRPV1 in the brain remains enigmatic, but several recent reports imply a neuroprotective role for endovanilloids acting on TRPV1. For example, arvanil acting on TRPV1 was shown to protect against oubain-induced excitotoxicy in the rat.[48] Also, TRPV1 agonists exhibited strong antihyperkinetic activity in a rodent model of Huntington's disease.[49] TRPV1 has also been implicated in cognitive functions involving the dopaminergic system.[50] These observations warrant caution as to the clinical use of TRPV1 antagonists passing the blood−brain barrier.

Last, there is good evidence that TRPV1 mediates various important reflex responses, the blockade of which by TRPV1 antagonists may have serious adverse consequences. For instance, TRPV1 plays an important role in gastroprotection, as highlighted by the aggravated ulcer formation in rats administered per os TRPV1 antagonists.[51] Also, anandamide acting on TRPV1 provides an important contribution to the depressor reflex in spontaneously hypertensive rats.[52] These last two effects may complicate the clinical use of TRPV1 antagonists even if they do not cross the blood−brain barrier.

Conclusions

The management of chronic, neuropathic pain with drugs other than narcotic agents is problematic. The cloning of TRPV1 and the recognition of endovanilloid signaling via TRPV1 in pain and inflammatory hyperalgesia have identified a new target for drug development and launched a massive effort on part of the pharmaceutical industry to discover novel TRPV1 antagonists. It is hoped that such antagonists may function as potent analgesic agents by blocking the combined action of heat, protons, and endovanilloids on TRPV1 either by preventing endovanilloid binding or by some direct inhibitory action on TRPV1. The recognition of TRPV1 overexpression during inflammatory hyperalgesia, diabetic neuropathy, inflammatory bowel disease (IBD), and irritable bowel syndrome (IBS) has lent further support to this concept. The last two observations are particularly exciting in that the management of patients with IBD or IBS with existing drugs may be frustrating. Locally active TRPV1 antagonists may provide a relief for such patients with few, if any, potential adverse effect. With regard to orally active TRPV1 antagonists, new findings raise concerns about unforeseen side effects (e.g., gastric ulcer formation, hypertension, central nervous system (CNS) effects). As yet, it is unclear if these findings obtained in experimental animals also hold true in patients. If they do, it remains to be seen whether the beneficial actions of TRPV1 antagonists outweigh their adverse effects.

Biographies

Arpad Szallasi received his M.D. from the University Medical School, Debrecen, Hungary, in 1984 and is a Diplomate of the American Board of Pathology. He also holds a Ph.D. in Pharmacology from the Karolinska Institute, Stockholm, Sweden. He is currently a Fellow in Hematopathology at the University of Pennsylvania, Philadelphia, PA. His research focus is on vanilloid receptors in health and disease.

Giovanni Appendino received his Laurea degree from the University of Torino in 1979 and is currently Professor of Chemistry at Università del Piemonte Orientale, Novara, Italy. His research activity focuses on the chemistry of bioactive

natural products (isolation, chemical modification, and synthesis), for which he received the Rhône Poulenc-Rorer Award of the Phytochemical Society of Europe in 1991.

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