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Review Article

Small molecules targeting the vanilloid receptor complex as drugs for inflammatory pain

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Abstract

Inflammatory pain is a condition caused by tissue injury and requires efficient drug treatment. The identification and cloning of the vanilloid receptor TRPV1 represents a significant step in the clarification of the molecular mechanisms underlying transduction of noxious chemical and thermal stimuli by peripheral nociceptors. Because of its central implication in hyperalgesia, the TRPV1 receptor has emerged as a key therapeutic target for inflammatory pain management. Modulators of the TRPV1 receptor complex are considered to be useful and selective painkillers. The challenge, however, is to develop receptor-selective drugs that preserve the physiological activity of TRPV1 receptors while correcting those contributing to pathology. Thus, normal proprioceptive and nociceptive responses that represent a safety mechanism to prevent tissue injury must be preserved.

Introduction

Pain is a condition that requires aggressive treatment and the number of people suffering from pain has increased to approximately half a billion cases per year (1). When poorly treated, pain can be debilitating, resulting in loss of work, productivity, family crisis, depression and suicide (2, 3). Currently, it is estimated that at least 50% of individuals seeking treatment are unsatisfied with their present pain management options. Thus, the economic and medical costs of inadequate pain therapy in the community are vast.

Although pain has been traditionally considered a unique and homogeneous pathology, research has established that pain is an extremely complex and dynamic process involving multiple, interrelated neurotransmitter/neuromodulator systems in the peripheral nervous system. Indeed, as many as 15 neurotransmitters have been implicated in pain-processing pathways (4-6). Two major types of pain are widely recognized, namely neuropathic and inflammatory. Nerve injury due to infection, autoimmune disease, trauma and viral infections are the etiology of neuropathic pain. Damaged fibers ectopically discharge augmenting nerve activity, which induces hyperexcitability at the level of the spinal cord leading to burning-like pain.

In contrast to neuropathic pain, inflammatory pain is produced in response to tissue damage (4, 7). Inflammatory pain involves various responses resulting from peripheral tissue injury and/or inflammation produced by trauma, infection, surgery, burns or diseases with an inflammatory component. In most cases, inflammatory pain is mediated by proalgesic mediators such as protons, histamine, cytokines, prostaglandins, neurokinins, chemokines and ATP (4, 7). These molecules sensitize neurons in the pain pathway (known as nociceptive

neurons or nociceptors), either by directly modulating the sensitivity of membrane receptors or upregulating intracellular signaling cascades. Nociceptors are sensitized by activation of both protein kinase C (PKC) and PKA pathways, which lead to phosphorylation of sensory receptors involved in nociceptive function (8-10). As a result, strong sensory signaling is conveyed to the spinal cord and subsequently to specific brain regions leading to the sensation of pain. This phenomenon is known as peripheral sensitization. In addition, synaptic and metabolic changes at the level of the spinal cord may occur, leading to central sensitization which further augments the pain sensation (4, 6). A major hallmark of inflammatory pain which can be classified as acute or chronic is thermal and mechanical hyperalgesia.

The molecular components and mechanisms involved in inflammatory pain transduction are beginning to be elucidated. The identification and cloning of the transient receptor potential (TRP) V1 channel represented a significant step in the clarification of the molecular mechanisms underlying transduction of noxious chemical and thermal stimuli by peripheral nociceptors. There is growing evidence that peripheral sensitization is largely mediated by excessive excitation of TRPV1 receptors (10, 11). Because of its central implication in hyperalgesia, the TRPV1 receptor has emerged as a key therapeutic target for inflammatory pain management.

The TRP receptor family

The TRP gene family is composed of more than 20 different gene products that form nonselective cation channels involved in diverse physiological functions. TRP channels constitute a family of sensory receptors and are divided into 3 subfamilies: TRPC, TRPV and TRPM that have the common topology of 6 transmembrane segments (S1-S6) with a pore region between the fifth and sixth segment and cytoplasmic N- and C-termini. TRPV and TRPC contain 2-4 ankyrin domains that are thought to interact with the cytoskeleton (12). The first cloned TRP was a TRPC channel from *Drosophila* that is essential for visual transduction. In the worm *C. elegans*, TRPC2 is required for transduction of pheromone responses of vomeronasal neurons. These neurons are G-protein-coupled metabotropic receptors that act via the phospholipase C pathway, releasing Ca^{2+} from the endoplasmic reticulum through inositol receptors (13, 14). In contrast, the TRPV family are ionotropic receptors activated by noxious stimuli. For example, TRPV1 gates in response to noxious temperatures (42 ^{o}C or higher), vanilloids, acidic pH and proalgesic substances (15). Three additional TRPV channels have been cloned that activate at different temperature thresholds: TRPV3 at 33 ^{o}C (16), TRPV2 at 55 ^{o}C and TRPV4 at warm temperatures and in response to osmolarity. Recently, 2 receptors of the TRPM family that respond to cold temperatures have been cloned. TRPM8 and ANKTM1 activate at 25 ^{o}C and 17 ^{o}C, respectively.

The TRPV1 receptor

It has long been known that a subset of peripheral sensory neurons are excited by vanilloids. Studies indicate that vanilloid sensitive nociceptors are quite heterogeneous morphologically, neurochemically and functionally. In general, they are peptidergic, small-diameter neurons that give rise to unmyelinated C-fibers which are mainly associated with Substance P, although some Adelta fibers have also been found to be sensitive to vanilloid derivatives (17). TRPV1 expression has been found in dorsal root and trigeminal ganglia in the dorsal horn of spinal cord and caudal nucleus of spinal trigeminal complex, respectively, by *in situ* hybridization, immunocytochemical analysis and drug binding assays (15, 18). In dorsal root ganglia, more than 50% of the neurons are TRPV1 positive. Immunospecific staining of TRPV1 has also been observed in the plasma membrane, Golgi and small clear vesicles (19). This ubiquitous intracellular distribution may be relevant for inflammation-induced sensitization.

TRPV1 is also present in various brain nuclei, as well as in nonneuronal tissues such as urinary bladder epithelium, smooth muscle and epidermal keratinocytes (20-22). TRPV1 is expressed in most of the afferent nerve fibers in the rodent gastrointestinal tract and some of the vagal afferents. However, it is still unknown whether it plays a role in gut sensation. TRPV1 is found not only in afferent fibers within the urinary bladder but also in submucosa, mucosa and epithelial cells lining the bladder (21, 23). Thus, these observations provide a mechanism for the variety of functions exerted by vanilloids in nonneuronal tissues. For example, vanilloid-mediated TRPV1 refractoriness is effective in visceral hypersensitivity. Resiniferotoxin (RTX) and the capsaicin-analogue SDZ-249665 effectively attenuate inflammatory bladder hyperalgesia and visceral pain responses to intraperitoneal acetic acid in animals (24, 25). Taken together, these findings substantiate the hypothesis that TRPV1 plays a role in neurogenic inflammation and visceral pain.

An expression-cloning strategy using capsaicin as a specific agonist unveiled the molecular identity of TRPV1 (15). Heterologous expression of TRPV1 cDNA results in capsaicin-activated inward currents that recapitulate most of the functional properties of those described for the capsaicin and heat-activated receptors of dorsal root ganglion neurons (15, 18). Receptor functional analysis demonstrated that TRPV1 is a nonselective cation channel activated by vanilloids, that exhibits a time- and Ca^{2+}-dependent outward rectification followed by a long-lasting refractory state, during which the cell does not respond to capsaicin and other stimuli. TRPV1 is also gated at temperatures higher than 42 ^{o}C. In addition, mild extracellular acidic pH potentiates vanilloid receptor activation by noxious heat and vanilloids, while strong acidic conditions (pH < 6) directly activate the channel. A widely held belief is that vanilloids and acidic pH decrease the threshold for heat activation from noxious to nonnoxious

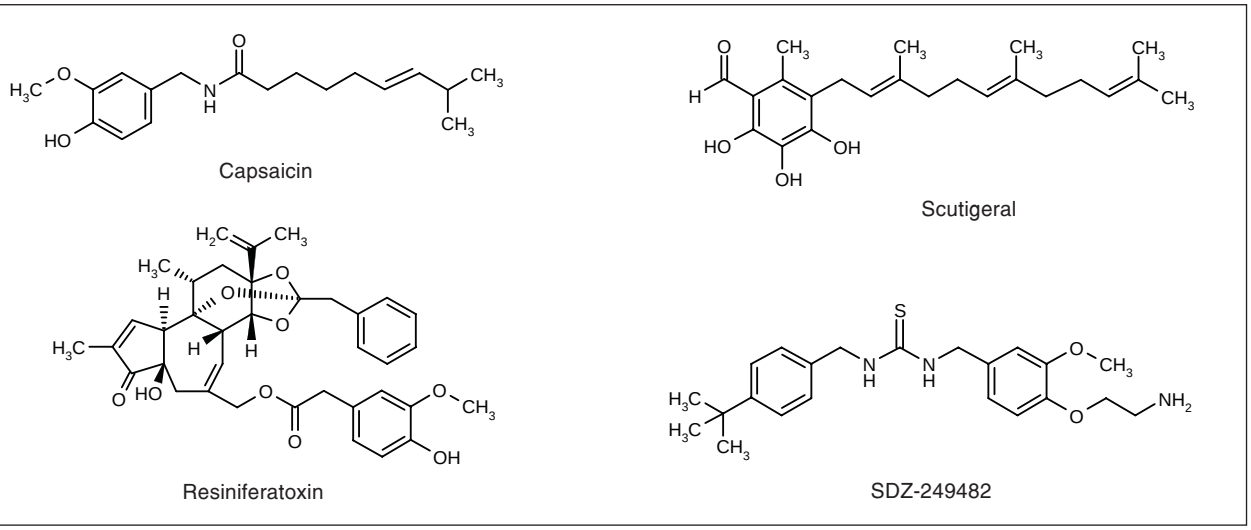


Fig. 1

temperatures (18). Therefore, TRPV1 integrates painful physical and chemical noxious stimuli.

Mice deficient in TRPV1 have been generated to validate TRPV1 as a therapeutic target. Physiological and behavioral studies of the knockout animals indicated a clear role of TRPV1 in thermal hyperalgesia, but not in mechanical hypersensitivity (26, 27). Interestingly, a modest contribution of TRPV1 to thermal nociception was observed, perhaps due to developmental overexpression of other TRPV members that contribute to the final phenotype. These studies also showed that TRPV1 gene ablation does not modify neuropathic pain responses. Similar conclusions were reached by using pharmacological tools. *In vivo* inhibition of TRPV1 activity by noncompetitive antagonists attenuated thermal nociception and hyperalgesia and reduced neurogenic inflammation (28). Taken together, these findings demonstrate that TRPV1 is a widely expressed neuronal receptor that behaves as a gateway for pain transduction. Thus, this receptor is validated as a key therapeutic target for pain management.

Pharmacology of TRPV1 receptors

Because of its involvement in the etiology of inflammatory pain, pharmacological modulation of TRPV1 is a valuable strategy to mitigate pain sensation. Structure-activity relationship (SAR) studies revealed that the TRPV1 receptor is a molecular entity with various potential drug binding sites. These findings prompted research which led to the discovery of different types of receptor modulators that could potentially be of clinical use in the treatment of TRPV1 receptor dysfunction, especially in inflammatory conditions. However, due to the relevant role of this thermosensor in the physiology of nociceptors, complete abrogation of channel activity could lead to unwanted side effects such as general thermal insensitivity. Thus, efforts were aimed at developing different classes of TRPV1 modulators, namely agonists, competitive antagonists and channel blockers, which are discussed below.

TRPV1 agonists

Agonists are molecules that gate the channel. Prolonged exposure of the receptor to the agonist, however, induces its closure by desensitization. Agonist-induced desensitization is dependent on the presence of extracellular Ca^{2+}. Tachyphylaxis is characterized by a profound run-down of TRPV1 responses upon repetitive agonist stimulation. Both desensitization and tachyphylaxis are of therapeutic value because of the involvement of receptor downregulation in these processes. Paradoxically, agonist-induced neuronal death due to sustained receptor activation, especially under ischemic conditions, is also useful for treating specific pain conditions.

The best known TRPV1 agonist family is the vanilloids. Capsaicin is the typical structure of one class of vanilloid compounds known as the capsaicinoids (Fig. 1). There are 3 other known chemical classes of naturally occurring vanilloids: resiniferanoids, unsaturated dialdehydes and triprenyl phenols, whose typical representative structures are resiniferatoxin, isovelleral and scutigeral, respectively (29).

Capsaicin has 3 functional regions: an aromatic A region where a parent homovanillyl (3-methoxy 4-hydroxybenzyl) group is optimal, a B region known as the ester or amide linker and the aliphatic C region where a lipophilic octanyl or *p*-chlorophenetyl moiety is associated with the highest potency. The homovanillyl motif and amide bond regions contain dipolar groups which are implicated in hydrogen bonding interactions. Analysis of the SARs of

numerous capsaicin analogues suggest that these polar regions are essential for maintaining pungency, that could be alternatively expressed as maintaining the excitation of sensory neurons. In contrast, the aliphatic chain in the C region, which has an optimal chain length of 8-10 carbon atoms, is presumed to interact hydrophobically with its receptors (29). Although a receptor model has been suggested based on the SARs of the capsaicin analogues, structural elements in TRPV1 that confer specific interactions with vanilloids have not been characterized. Moreover, unlike other ligand-gated channels that produce fast synaptic transmission, vanilloids are known to act on the TRPV1 receptor from the intracellular site (30). Recently, a region spanning the third transmembrane domain (S3) in TRPV1 was found to be essential for ligand binding, presumably by hydrophobic interactions with capsaicin (31). Complementary regions in TRPV1, predominantly those located in both cytosolic tails, have also been implicated in structuring the agonist binding site (30).

Vanilloid compounds have been considered potential analgesics and have been extensively used in medicine. Capsaicinoids are traditionally indicated for relieving toothache and capsaicin is a standard ingredient in a variety of over-the-counter drugs used worldwide to relieve muscle ache. Desensitization to capsaicin has a clear therapeutic potential for ameliorating urinary bladder overactivity, uremic itching associated with renal failure, urinary incontinence and inflammatory bowel disease. Additionally, capsaicin-induced neurotoxicity has already been shown to be useful in the treatment of neuropathic pain such as herpes zoster-related neuropathic pain, diabetic neuropathy and postmastectomy pain (29).

A major shortcoming of the therapeutic use of capsaicin is the burning sensation and irritation that it produces. SAR studies of vanilloids have led to the development of more potent, orally active, vanilloid-like molecules such as SDZ-249482 (Fig. 1). However, these compounds also exhibit side effects such as irritation. Thus, a drug with long-lasting desensitization and no pungency promises to be of great clinical value. Naturally occurring and synthetic nonpungent capsaicin analogues such as capsiate, olvanil, glycerol nonivamide and scutigeral, have been described. However, their low efficacy prevents their clinical development. On the other hand, clinical trials with resiniferatoxin (RTX), a potent capsaicin analogue isolated from *Euphorbia resinifera*, are ongoing (Fig. 1), with preliminary results suggesting that such vanilloids may in fact be useful (29).

The specific binding of RTX to the capsaicin binding site in dorsal root ganglia has been demonstrated with labeled [^{3}H]-RTX. The pharmacophoric groups of RTX have not yet been clearly defined, although SAR studies suggest that the C_{20}-homovanillic moiety, the C_3-keto group and the orthoester phenyl group on ring C are crucial structural elements responsible for the extremely high potency of RTX. RTX is being developed as an ultrapotent sensory neuron desensitizing agent for the treatment of urinary urge incontinence and pain associated with diabetic neuropathy, and it appears to be superior to capsaicin in terms of tolerability (27). The unquestionable therapeutic potential of RTX is seriously hampered by its limited availability from natural sources and the complexity of its chemical synthesis, as shown by the more than 44 reaction steps required for its total synthesis. Nonetheless, clinical trials with RTX are ongoing.

TRPV1 endogenous ligands

Because TRPV1 is chemically gated, the search for endogenous substances that gate the channel was initiated. There is cumulative evidence that endogenous vanilloid receptor agonists (endovanilloids) exist and play a key role in the development of inflammatory hyperalgesia (32), and there is no doubt that these agents modulate the sensitivity of vanilloid receptors to thermal stimuli.

The first endogenous compound thought to be an endovanilloid was anandamide, a polyunsaturated fatty acid (*N*-arachidonoylethanolamine), activating both native and recombinant rat TRPV1 receptors (Fig. 2) (33). However, anandamide is also considered an endocannabinoid since it activates both cannabinoid CB1 and CB2 receptors at concentrations lower than those needed to activate TRPV1.

Capsaicin-like substances are structurally similar to some eicosanoids, particularly those derived from the action of 5-lipoxygenase and 12-lipoxygenase and, in fact, arachidonic acid has been shown to be capable of activating the rat TRPV1, with more potent activity noted for 12-hydroperoxy-eicosatetraenoic acid (12-HPETE) and leukotriene B_4 (LTB_4) (Fig. 2) (34). The binding site on TRPV1 is thought to appear only by slow, passive diffusion. Eicosanoids are believed to function as intracellular endovanilloids, acting at TRPV1 receptors in the cells where they are synthesized.

Recent studies have led to the identification of another compound, *N*-arachidonoyl-dopamine (NADA), that has been proposed to act as a brain endovanilloid since it is relatively abundant in some brain areas where TRPV1 is also present (Fig. 2) (35). NADA is an endogenous anandamide analogue that is several times more potent than anandamide on TRPV1, yet still capable of activating CB1 receptors. Thus, the identification and characterization of endovanilloids may provide important clues to the development of innovative analgesic and antiinflammatory drugs.

TRPV1 antagonists

Competitive and noncompetitive vanilloid antagonists are the 2 major classes of TRPV1 modulators being explored for therapeutic application. The first class specifically comprises compounds that bind to the agonist binding site and lock the channel in the closed, nonconductive state. Noncompetitive antagonists, however, interact with additional drug binding sites located on the receptor

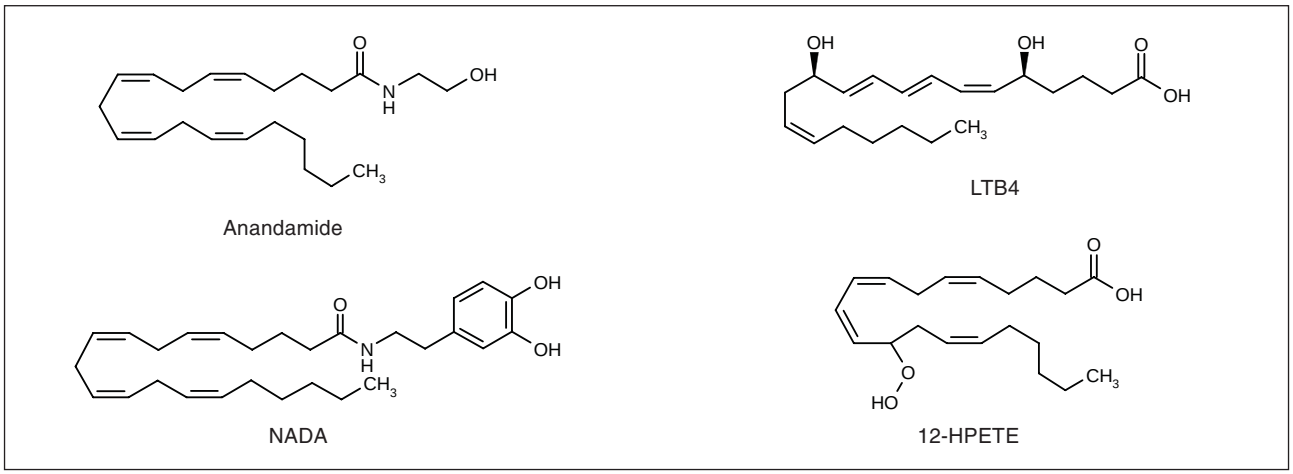


Fig. 2.

structure. Of special interest are those acting as open channel blockers because they specifically recognize the overactivated species, *i.e.*, pathological receptors.

TRPV1 competitive antagonists

The first competitive TRPV1 antagonist identified was capsazepine which was prepared by introducing a saturated 7-membered rigid ring system, which maintained a virtually orthogonal conformation between each *N*-substituent in the thiourea structure (Fig. 3). Tetrahydrobenzazepine and tetrahydroisoquinoline thiourea derivatives were prepared as antagonists by replacing the *p*-chlorophenethyl group with 3-acyloxy-2-benzylpropyl groups and all are competitive vanilloid antagonists with a potency ranging from 0.2-4 μM. When administered in animal models of inflammatory pain, these compounds exhibit analgesic activity, although their therapeutic index is still low (36). In addition, they exhibit limited blocking activity on heat-activated channels present on the neuronal surface.

Recently, a derivative of RTX, 5-iodo-RTX, was shown to be a highly potent antagonist (IC_{50} = 3.9 nM) of TRPV1 (Fig. 3). This compound exhibited analgesic activity *in vivo* and is currently in preclinical studies. A major drawback is the complexity and cost of its chemical synthesis from RTX and its limited oral activity (37).

The search for better competitive vanilloid antagonists continues to be a major focus of pharmaceutical companies and we should witness in the near future increasing pipelines of lead compounds for analgesic drug discovery.

TRPV1 noncompetitive antagonists

The first noncompetitive TRPV1 antagonist introduced as a functional capsaicin antagonist was ruthenium red, which is an inorganic polyamine that binds to the

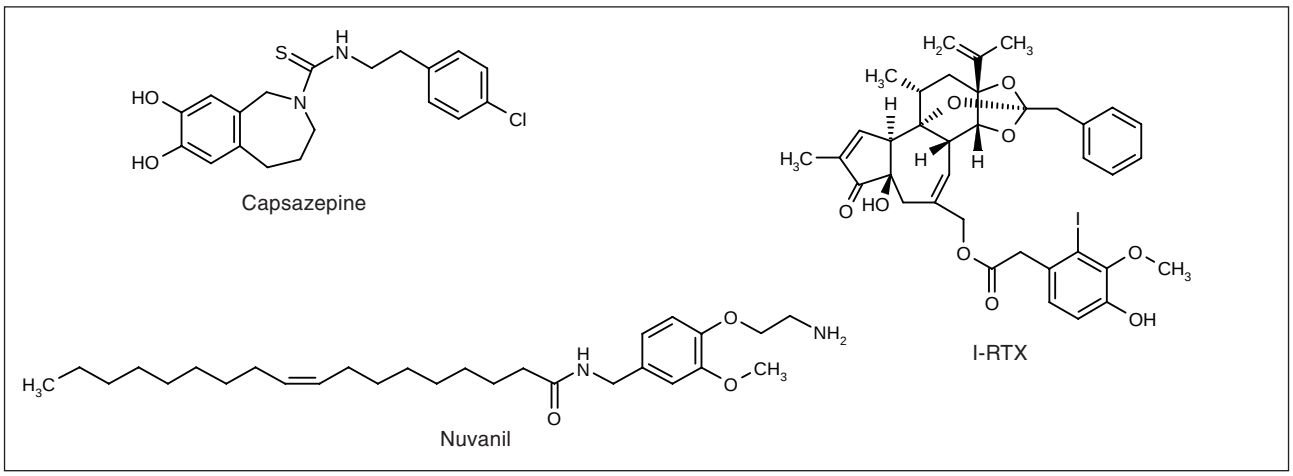
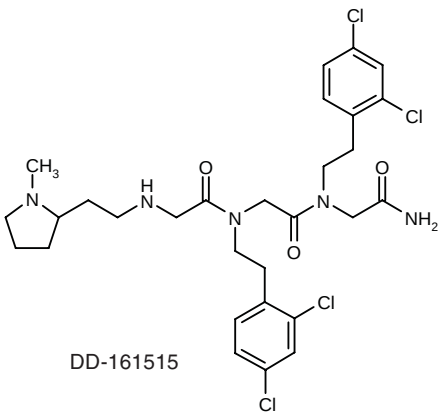


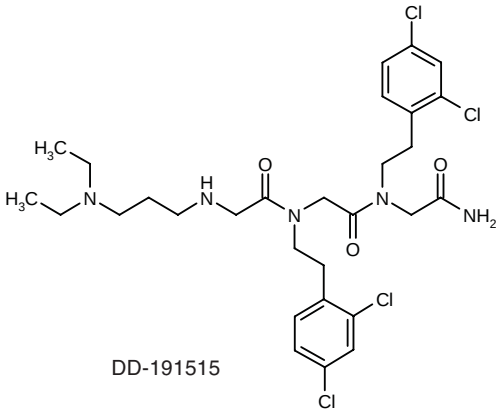
Fig. 3.



DD-161515

$[(NH_3)_5RuORu(NH_3)_4ORu(NH_3)_5]^{6+} \cdot 6Cl^-$

Ruthenium red



DD-191515

$Ac\text{-}Arg\text{-}Arg\text{-}Arg\text{-}Arg\text{-}Trp\text{-}Trp\text{-}CONH_2$

Fig. 4

pore region of the channel (Fig. 4) (29). The therapeutic use of ruthenium red is highly precluded because of its unspecific blocking activity.

Arginine-rich hexapeptides such as RRRRWW-NH_2 noncompetitively block recombinant TRPV1 channels expressed in *Xenopus* oocytes, with submicromolar efficacy (Fig. 4) (38). It was recently reported that they may also act via a competitive mechanism (39). Apart from the specific mechanism, their development as analgesic drugs is prevented due to their pharmacologically unsafe profile.

A novel class of noncompetitive antagonists of TRPV1 was identified from the screening of a peptidomimetic-based library (28) (Fig. 4). Peptidomimetic molecules such as *N*-alkylated glycines (also known as peptoids) constitute a family of nonnatural compounds that exhibit interesting biological properties (40). Peptoids result from the shift of the substituent present at α-carbon atom in amino acids to the adjacent nitrogen atom. Although peptoids are isomers of peptides, they have different structural features, such as a higher degree of conformational freedom and the absence of CO–NH hydrogen bonds. These properties modify the steric interactions leading to the secondary structure present in peptides. Moreover, contrary to what occurs in peptides, peptoid backbones are achiral and protease resistant.

With regard to TRPV1, the screening of a library of trimers of *N*-alkylglycines resulted in the identification of two molecules referred to as DD-161515 {*N*-[2-(2-(*N*-methyl-2-pyrrolidinyl)ethyl]-glycyl-*N*-(2,4-dichlorophenethyl)glycyl-*N*-(2,4-dichlorophenethyl)glycinamide} and DD-191515 {[*N*-[3-(*N*,*N*-diethylamino)propyl-glycyl-*N*-(2,4-dichlorophenethyl)glycyl-*N*-(2,4-dichlorophenethyl)glycinamide} that selectively block TRPV1 channel activity with micromolar efficacy, thus rivaling that of vanilloid-related inhibitors (Fig. 4). These compounds appear to be noncompetitive TRPV1 antagonists that recognize a receptor site distinct from that of capsaicin. Intraperitoneal administration of both compounds in mice significantly attenuated thermal nociception as measured using the hot plate test. It is noteworthy that these compounds also eliminated pain and neurogenic inflammation evoked by intradermal injection of capsaicin into hindpaws and thermal hyperalgesia due to nitrogen mustard-induced tissue irritation. In contrast, responses to mechanical stimuli were not modified by either compound. Modulation of sensory nerve fiber excitability appears to underlie the peptoid analgesic activity. Thus, it appears that these trialkylglycine-based, noncompetitive TRPV1 antagonists may likely be developed into analgesics to treat inflammatory pain. Further progress in this exciting field is being eagerly pursued and new and more potent and selective hits for drug development are being identified and characterized. For example, hydantoin-based compounds have been identified as potent blockers of TRPV1 channels. Notably, lead compounds that produce analgesia without affecting normal nociceptive responses have been developed. The clinical validation of this sort of TRPV1 antagonist is eagerly awaited.

Hybrid cannabinoid-TRPV1 modulators

Since TRPV1 is expressed in several brain regions and is next to CB1 receptors, it is possible that some of the neurobehavioral effects associated with endogenous compounds are due to the combined activation of both receptor types (32). The activation of these receptors often leads to similar pharmacological actions (*e.g.*, antinociception, hypothermia, vasodilation, hypokinesia and inhibition of cancer cell growth). Although via different mechanisms, compounds capable of simultaneously

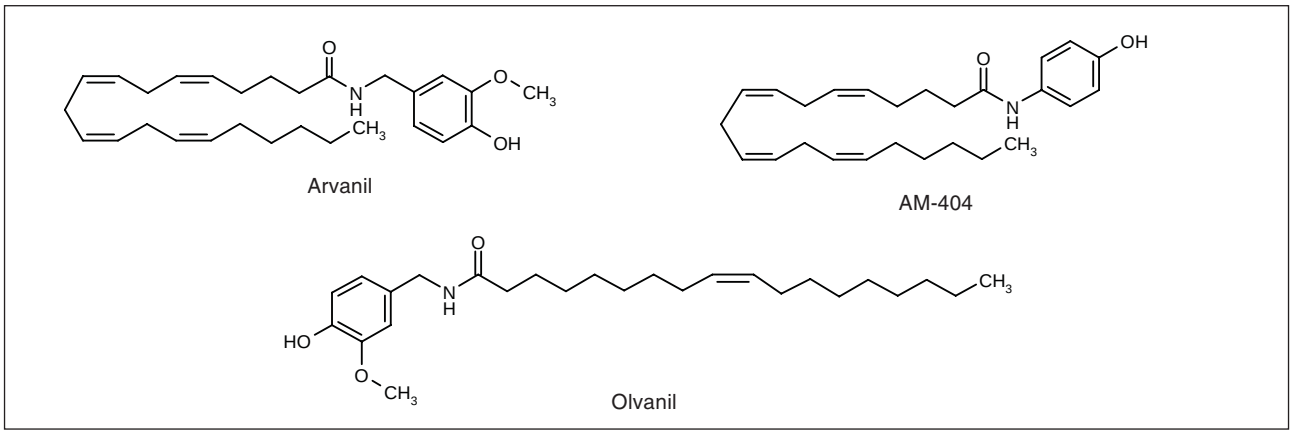


Fig. 5

activating 2 or more of these targets may have great therapeutic potential. These compounds are known as hybrid agonists and include agents such as arvanil (*N*-[3-methoxy-4-hydroxy-benzyl]-arachidonamide) (Fig. 5). This compound has affinity for CB1 receptors that is comparable to that of AEA and it also activates TRPV1 receptors more potently than anandamide or capsaicin (41). Thus, arvanil is much more potent than either anandamide or capsaicin as an antiproliferative agent for human breast cancer cells that are sensitive to both CB1 and TRPV1 receptor antagonists, as a cannabimimetic agent in the mouse tetrad model, as a spinal analgesic and as a relaxant of mouse vas deferens. However, the efficacy of arvanil is not well balanced between TRPV1 and CB1 receptors and, hence, new hybrid CB1/TRPV1 agonists with possible therapeutic significance should be developed. Of great interest is that TRPV1 and cannabinoid CB1 receptors have overlapping ligand recognition properties which may also have far-reaching implications for vanilloid therapy.

The receptor complex as drug target

It is known that ion channels are not isolated entities in the cell membrane but rather components of multiprotein complexes that play a key role in physiology and pathology. These macromolecular assemblies are high order signaling networks that balance external and internal signals giving rise to a response. Dysfunction of protein networks thus has deleterious consequences. These complexes are composed of a plethora of proteins that include receptor, adaptor, signaling, cytoskeletal and novel proteins. Biogenesis, distribution and clustering of receptors and their colocalization with signaling proteins influence efficient transduction of external cues by these synaptic signaling assemblies. Thus, the synaptic protein complex contains a variety of potential drug targets. The molecular identity and organization of the TRPV1 signaling complex is still elusive. Rapidly advancing progress in this area is uncovering the molecular identity of these elements which, in turn, will be validated as therapeutic targets for drug intervention. Because most of these targets are protein complexes, the real challenge ahead is the discovery and development of small molecules that modulate protein-protein interactions, an as yet unmet goal of current drug discovery.

Conclusions

The unquestionable involvement of the TRPV1 receptor in pain transduction has initiated interest in the development of antagonists of this neuronal receptor for pain management. Although an apparently easy task for current drug discovery platforms, there is a real challenge to develop antagonists that preserve the physiological activity of TRPV1 receptors while correcting overactive receptors. This would ensure normal proprioceptive and nociceptive responses that are required to prevent tissue injury. Therefore, physiological constraints have to be included in the development and optimization of TRPV1 receptor antagonists to ensure their clinical utility as analgesics.

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