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Review Article

Capsaicin-like agonists

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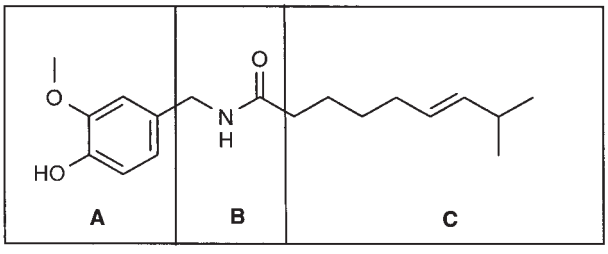
Introduction

Capsaicin is the primary pungent component of hot chili peppers and related plants of the Capsicum family. While chili peppers have been mainly used as a spice, their medicinal properties have a long history in the treatment of gastric disorders and, when applied topically, for the relief of pain and inflammation (1). The compound was first isolated in 1876 (2), and the structure was determined as *N*-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide [**1**] in 1919 (3). The first total synthesis was described in 1955 (4).

Capsaicin exhibits a broad spectrum of biological actions which have been intensively studied in recent years (5, 6), following the pioneering work of Szolcsanyi and Jansco-Gabor (7). Besides showing excitatory effects on the cardiovascular and respiratory systems, capsaicin induces pain and irritation upon topical application to the skin. This algesic response is, however, followed by a period of desensitization, not only to subsequent applications of capsaicin itself, but, importantly, also to application of other noxious stimuli. When applied at higher concentrations (supramicromolar), capsaicin is neurotoxic, particularly in neonates (8), and it also exhibits a range of channel blocking "nonspecific" effects (9).



Capsaicin [**1**]

In contrast to the properties of capsaicin observed at high concentration, the effects observed on a subset of peripheral sensory neurons (C-fiber polymodal nociceptors) and their functional sequelae are most easily explained by the interaction of capsaicin with a specific receptor (9). Several lines of evidence now point to a non-selective cation channel present only on this subset of sensory neurons as the prime candidate for this receptor. Confirmation of this awaits the cloning and expression of this (at present) operational molecular complex, and the connected questions of the endogenous ligand [protons? (10)], and the location of the capsaicin binding site are consequently still hypothetical (however, see footnote). In this context, the report of a proton-gated cation channel involved in acid-sensing in dorsal root ganglia (11) is provocative. Is this multi-subunit membrane channel related to the long sought capsaicin receptor?

Two other agents have recently contributed to our increased understanding of the mechanism of action of capsaicin and of the putative receptor. These are resiniferatoxin (RTX) and capsazepine. Resiniferatoxin is a diterpene natural product structurally related to the phorbol esters which has the properties of a high-affinity capsaicin analog and consequently has had a significant impact as a biological tool on attempts to characterize the receptor. Attempts to exploit its therapeutic potential have also been carried out, albeit with little success, and these are described in more detail below. Capsazepine is a close structural analog of capsaicin which, however, exhibits the properties of an antagonist, competitive with capsaicin and RTX for the receptor. A brief description of the design and characterization of this molecule and a hypothetical explanation for agonism and antagonism are presented below.

The excitatory and analgesic properties of capsaicin can be explained by its interaction with this putative receptor. The initial excitatory effect is caused by interaction

of capsaicin with its binding site on the receptor-ion channel complex on the sensory neuron membrane and the subsequent ligand-gated ion flux. In whole animals this effect is perceived as an initial algesic response mediated by Ca^{2+} (and other cations) entry through the channel. The immediate effect of this increased intracellular Ca^{2+} is to depolarize and hence excite the neuron. The subsequent analgesic effect is, however, also mediated by the raised intracellular Ca^{2+} levels, which ultimately lead to block of voltage-gated calcium channels in the C-fibers and thereby to inhibition of noxious signal transmission in the spinal cord.

These specific agonist effects of capsaicin on the sensory neurons which are involved in nociception thus lead to the hypothesis that capsaicin analogs have potential as mechanistically novel analgesic agents. Separation of the excitatory effects from the analgesic effects would, however, be a prerequisite for a useful agent and this requirement has been the stimulus for much of the structure-activity work described below.

This hypothesis that capsaicin has therapeutic potential as an analgesic agent is supported by data from animal and human studies. In a variety of animal models of acute and chronic pain, low doses of capsaicin, given subcutaneously, induce short-lasting analgesic and anti-inflammatory effects (12, 13): however, the small therapeutic window between these effects and the excitatory side effects, such as hypothermia, bronchoconstriction, increased GI mobility and hypotension, preclude development of capsaicin as a systemic agent in man. A further problem for systemic use is the low oral bioavailability of capsaicin.

Consequently, attention has focussed on the topical and local application of capsaicin in clinical studies, and in the past 8 years there have been several studies in neuropathic and inflammatory pain, pruritis, psoriasis, vasomotor rhinitis, bladder dysfunction and cluster headache (14). In all of these studies there has been some evidence for improvement of the condition, but the initial excitatory effects of capsaicin treatment are also manifest by these routes of application. This leads to high patient dropout rates and underscores the inadequacies of capsaicin as a therapeutic agent.

The focus, therefore, of medicinal chemistry efforts to improve this situation have been to establish the structural basis for the excitatory effects *versus* the analgesic properties and then to attempt to modulate these by structural modification. The importance of appropriate assays is paramount in this process, and recent progress has been a consequence of improved *in vitro* readouts which have been shown to correlate with analgesic responses in the whole animal. This is reflected in the chronological review of the medicinal chemistry developments in the field which are outlined below.

Capsaicin-related structures

Following the determination of the structure of capsaicin in 1919 (3), a series of analogs with variations in the aliphatic side chain and in the aromatic ring were made and tested on the human tongue to measure their relative pungency (15). This bioassay revealed among other results that omega-phenylalkyl derivatives retain pungent properties, a result which has had important repercussions in more recent times.

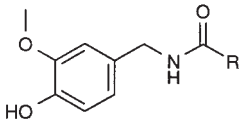
Another *in vivo* assay, the rat eye-wipe test, was used by Szolcsanyi and Jancso-Gabor (7) in their seminal SAR study of synthetic capsaicin analogs which paved the way for all future studies and resulted in a hypothetical schematic representation of binding to the putative receptor based on this measure of pungency. The general features of this picture survive intact to the present day.

Further analogs of capsaicin were made by the Glaxo group (16) in an attempt to separate the antinociceptive properties of these compounds from their attendant hypothermic effects. This was unsuccesful and led to the suggestion that the various actions of capsaicin, both desired and undesired, may be mediated by a common mechanism on the primary afferent nerve.

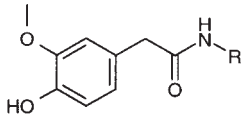
The studies described above provided the platform for the major efforts in the 1980s from two groups, one at Procter and Gamble and the other from Sandoz, which aimed to separate the excitatory effects of capsaicin analogs from the antinociceptive properties of these molecules. These studies were complimentary in that the former group developed SAR from *in vivo* data, whereas the latter group made use of *in vitro* assays established as predictive of analgesia in animal models. The conclusions reached by the two groups within the different structural sets which were made, using their different screening approaches, were broadly in concert. Many of the compounds made in these studies have only been reported in the patent literature without attendant biological data; however, the SAR data summarized below is now widely available in the primary literature.

A comprehensive variation of the alkyl chain of capsaicin (C-region, [**1**]) has been described by Janusz *et al.* (17), who used the mouse and rat hot plate assays to monitor antinociceptive effects and the croton oil inflamed mouse ear assay to record antiinflammatory effects of the compounds made. Measurements of acute toxicity and pungency were also made on selected compounds to establish a therapeutic index, relative to capsaicin, of the beneficial antinociceptive and antiinflammatory effects. It was established that two series of long-chain unsaturated alkyl substituted analogs, vanillylamides [**2**, R = long-chain alkyl] and homovanillic acid amides [**3**, R = long-chain alkyl], were more potent, less pungent and less acutely toxic than capsaicin. This is in contrast to the short-chain alkyl substituted derivatives which were active systemically, but not orally, and which were toxic and highly pungent.

Antinociceptive potency clearly correlated positively with increasing chain length (a measure of lipophilicity as well as size), and also, observationally, with lower melting point. It was proposed that these properties could be related to ease of transport through membrane barriers, a point which will be discussed below with regard to the putative capsaicin receptor.



[**2**]



[**3**]

From these observations two compounds containing long unsaturated side chains in the C-region were identified which exhibited an optimal balance of properties. These were NE-19550 ([**2**], R = $COC_{17}H_{33}\Delta_{9,cis}$), subsequently known as Olvanil, and NE- 28345 ([**2**], R = $COC_{18}H_{35}\Delta_{9,cis}$).

Olvanil was extensively studied by the originators (18) and others (19). Comparison with capsaicin in various animal models established that olvanil was less excitatory than capsaicin but that it retained analgesic activity. The analgesic effects were not blocked by naloxone and prostaglandin synthesis was not blocked, which established that olvanil does not act by an opioid-like mechanism or possess NSAID-like properties. The mechanism of action, however, remains elusive. It was proposed that differences in bioavailability may account for the different balance of properties between capsaicin and olvanil (17); nonetheless, olvanil maintains significant excitatory effects which ultimately led to the conclusion that this molecule was not suitable for further development as a clinical candidate.

The approach towards the goal of separation of analgesic properties from excitatory side effects taken by the Sandoz group was based on the use of *in vitro* assays. These were established as being predictive of analgesia in animal models using capsaicin and other closely related analogs. This approach enabled removal of some of the obfuscating *in vivo* effects and allowed a much higher throughput of compounds for SAR generation. The details of this have been described (20-25), so only the conclusions are presented here.

Systematic exploration of each of the structural regions of capsaicin (designated as A, aromatic ring; B, bond region and C, hydrophobic side chain in [**1**]), while holding the others constant in turn, enabled a modular picture of the structural requirements for analgesic effects to be built. Full agonists in the calcium flux assay with EC_{50} less than 1 μm were found to retain analgesic activity, whereas less potent agonists, partial agonists and antagonists did not.

In region A (20), compounds with the natural substitution pattern (3-methoxy-4-hydroxy) and the corresponding catechols were the only active entities among a large number of variants made. The poor *in vivo* activity of the catechols led to the conclusion that the natural substitution pattern was optimal.

Variation of the amide bond linker region B (21) established that the thiourea replacement was most potent. An operational model was developed to explain the activity of this and other variants of the parent amide which was based on multiple H-bond interactions between the ligands and the putative capsaicin receptor. This is shown schematically in Figure 1.

For activity in this model, compounds must make multiple hydrogen bonding interactions at the B_2 and B_3 positions, ideally in a *trans*-coplanar configuration. This is illustrated for the most potent analog, the thiourea [**4**] in Figure 2.

While this model explains the available data on the B-region analogs, it is important to note that there is no independent experimental evidence for this proposed interaction.

Further studies on the relative dispositions of the A- and B-regions led to the discovery of the novel capsaicin antagonist, capsazepine (22). Constraint of these two regions was achieved by the synthesis of saturated B-region ring systems of varying size [**5**].

Compounds with 5- and 6-membered rings retained agonist potencies similar to their unconstrained counter-

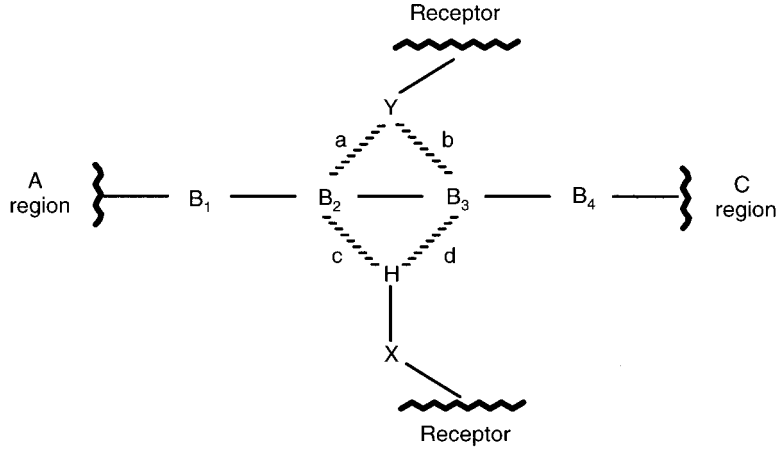


Fig. 1. Potential interactions of B region moieties of capsaicin agonists with the receptor model. B_1-B_4 represent component structural units of the B region and a-d represent potential dipolar interactions of these with structural units X-H and Y on the receptor.

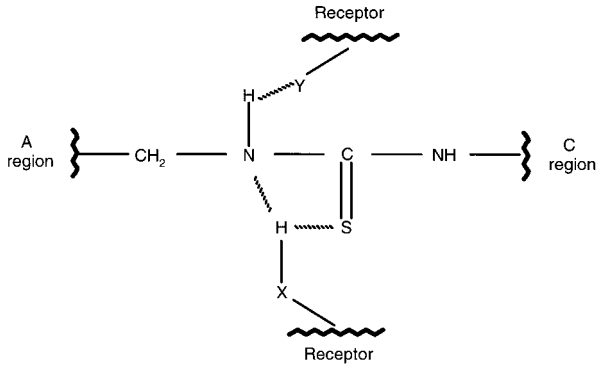


Fig. 2. Potential dipolar interactions between thiourea [**4**] and the receptor model.

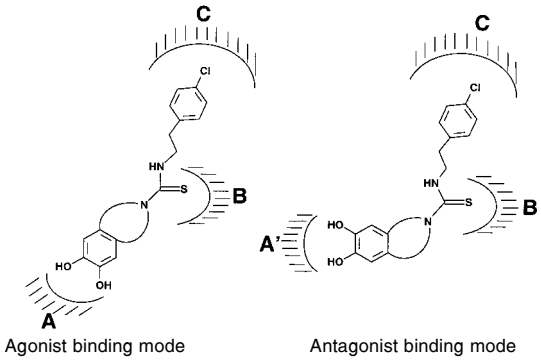


Fig. 3. Postulated different binding modes for capsaicin agonists and antagonists to the receptor.

parts, but the 7-membered ring benzazepine [**5**] (n=3, capsazepine) was totally inactive as an agonist and was shown to be a moderately potent competitive antagonist in competition studies with capsaicin and RTX.

A rationale for this striking difference manifested by this small structural change has been proposed based on an extensive study of the conformational properties, experimental and theoretical, of representative agonists and antagonists. This rationale defines different binding modes for agonists and antagonists with the putative receptor (Fig. 3).

Modification of the hydrophobic side chain C-region of capsaicin (23) established the requirement for a hydrophobic group of limited size for high potency. From indications in the early literature (13), it became clear that an aromatic ring could be accommodated in the binding site. It seems that an extended conformation of a linear two carbon spacer unit attaching the B-region to the aromatic ring is optimal (*i.e.*, ethylphenyl or *E*-ethenylphenyl, [**6**]). *para*-Substitution of the aromatic ring is tolerated and a good correlation between $X\pi_{para}$ and potency was determined.

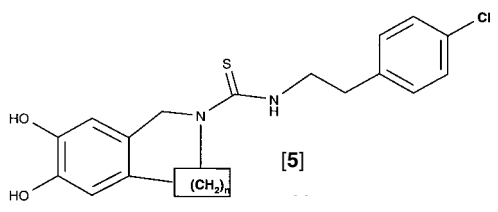
The size limitation on the C-region binding site was established from the potencies of a homologous series of thioureas [**7**]. As the chain length increases in this series potency increases to a plateau at a chain length of 8-12 carbon atoms and thereafter drops off.

If the assumption is made that the phenylalkyl compounds [**6**] and the aliphatic thioureas [**7**] occupy the same site, it would appear that the hydrophobicity of the C-region substituent governs potency to a certain size limit, but that moieties larger than this limit, whatever their hydrophobicity, are inactive on the reasonable grounds that they cannot fit in the site. It is important to point out that olvanil does not fit this model. The only explanation we can offer is that this molecule in some way has an alternative binding mode.

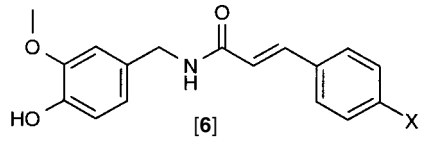
A QSAR study of capsaicin agonism based on the biological data supplied in these papers has been published (26). A process called MULTICASE analysis was carried out on the data set, which showed that log P was a primary determinant of agonist activity. Could this be related to rate of penetration through the cellular membrane? (see below). A series of biophores (structural fragments) important for activity were also determined. These results and the proposal of a 3D model of the pharmacophore are very similar to the "nonquantitative" model studies described above.

The strategy behind the modular approach of the Sandoz group was to exploit the information gathered on each region by combining the optimal features of each region into a molecule which should exhibit high potency. This was achieved and has been described recently (24).

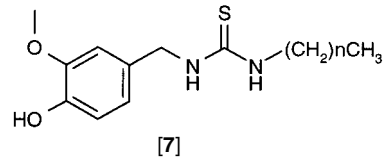
Combination of the most potent substituents from each region, namely the natural substitution pattern in the A-region, the thiourea moiety for the B-region and the *para*-substituted phenylalkyl group for the C-region led to a set of compounds, exemplified by [**8**], which, with the exception of the natural product RTX, are the most potent capsaicin agonists so far described. These compounds also fit into the schematic models for interaction with the receptor described above.



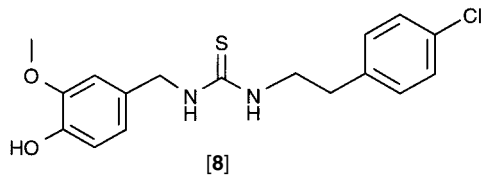
[**5**]



[**6**]



[**7**]



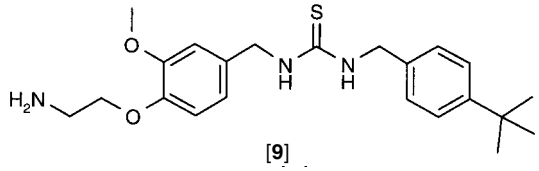
[**8**]

Pharmacological evaluation of [**8**] quickly established (24) that, although it was a potent analgesic, it was poorly active by the oral route and that it retained sufficient excitatory properties to preclude further development as a systemic analgesic agent. These properties were also observed in several close analogs.

The medicinal chemistry goal, therefore, was to retain the enhanced potency of this series of compounds but to reduce the attendant excitatory effects and to improve the oral biovailability. With regard to the latter problem, some preliminary metabolism studies on [**8**] showed that it was rapidly metabolized to the *O*-glucuronide and, presumably, was thereafter rapidly excreted. This could explain the poor oral bioavailability of the molecule. As described above, earlier SAR studies (20) had established that removal or simple alkylation of the phenol group greatly reduced potency, but further modification of the *O*-substituent revealed that an aminoethyl attachment to the phenol, alone among a variety of substituents placed at this position, gave a molecule which retained significant potency in the calcium flux assay. All attempted modifications to the aminoethyl group led to compounds with reduced potency (24). This same substituent was also identified by the Procter and Gamble group (27) and was attached to the aliphatic vanillylamides, represented by olvanil [**2**]. The resulting molecule was claimed to have improved water solubility, reduced irritant effects and to have retained the antinociceptive properties of the parent compound.

This modification of the phenolic group in the thiourea series had been carried out in an attempt to improve oral activity. This indeed was achieved (24), but fortuitously the therapeutic window between the desired analgesic properties and the unwanted side effects (manifested by bronchoconstriction and pungency) also widened considerably. This was particularly evident with compound [**9**].

A possible explanation for this may lie in the rate of excitation of the sensory neuron by such molecules (28). From electrophysiological studies there is evidence that the capsaicin binding site lies on the inside of the plasma membrane. It is, therefore, plausible that rate of penetration of capsaicin-like agonists through the membrane could govern the degree of excitation if this is caused by interaction of the analogs with the receptor leading to calcium entry, as described above. It might be anticipated

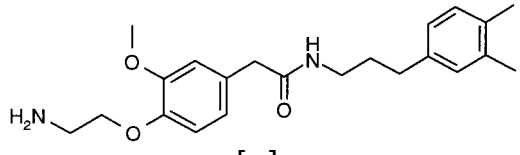


[**9**]

that the partially ionized aminoethyl substituent could modulate the rate of passage of the compounds through the plasma membrane lipid bilayer more slowly than the more excitatory parent phenols.

The pharmacological profile of molecules such as [**9**] enable their development as potential clinical candidates. They still retain excitatory properties, however, which may indeed be a necessary prerequisite for analgesic effects consequent on receptor activation and calcium entry. It remains to be seen whether the balance of such properties in these molecules will make them effective therapeutic agents.

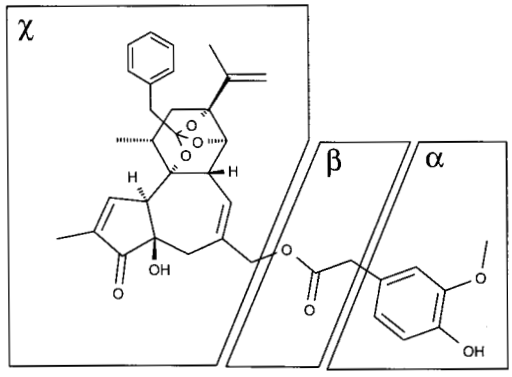
A group from Korea have also described antinociceptive vanilloid-like phenylacetamides culminating in the identification of KR-25018 [**10**], which showed nonnaloxone reversible analgesic properties in acute and chronic inflammatory pain models at a level comparable with the effects of morphine (29).



[**10**]

Resiniferatoxin and analogs

Resiniferatoxin (RTX, [**11**]) is a tricyclic diterpene isolated from *Euphorbia resinifera* (30). Although closely related structurally and biogenetically to the phorbol esters, RTX has a different biological profile (31) in that it does not cause tumor promotion and binds only weakly to protein kinase C, in contrast to the phorbol esters. The biological profile of RTX is of a high-affinity capsaicin analog as illustrated by its ability to induce hypothermia, to cause neurogenic inflammation and to elicit pain in animal models in a similar way to capsaicin itself. The difference between RTX and capsaicin is one of potency, particularly for the first two properties mentioned above, where RTX induces neurogenic inflammation and hypothermia at concentrations 3-4 orders of magnitude



[**11**]

lower than those of capsaicin. This is also manifest at the cellular level, where RTX was 200-fold more potent than capsaicin in inducing Ca^{2+} influx in rat neonatal dorsal root ganglia cells (32). Finally, in binding to rat dorsal root ganglia membranes [^{3}H]-RTX exhibited a dissociation constant (K_d) of 0.27 nM, whereas capsaicin was only able to displace this specific binding with a K_i of 7 μM (33).

These properties of RTX have been put to use in attempts to characterize the receptor. Radiation inactivation analysis of the RTX binding site suggested a molecular mass of 270 ± 25 kD consistent with the size of other ligand-gated channels (34). In an attempt to exploit the potency of RTX, a synthetic photoaffinity labelled analog of RTX was made which irreversibly inhibited [^{3}H]-RTX binding to DRG neurons after irradiation (35). This reagent has also been used in cloning strategies, but so far, unsuccessfully.

Besides the pharmacological similarities between RTX and capsaicin there are also structural ones, and various SAR studies have been carried out to explore these boundaries (36-39). The results of the early studies (36, 37), testing a series of resiniferanol derivatives using mouse ear reddening as a measure of irritancy, were mirrored by the more extensive study described below and are subsumed into this discussion. Szallasi and Blumberg (38) studied RTX in comparison to several closely related natural products, with and without the unusual *ortho*-ester unit, and showed this to be an important determinant of "vanilloid" effects in a variety of *in vivo* tests.

The Sandoz group (39) extended the modular approach applied to capsaicin to RTX by splitting the latter into three separate pieces [**11**]. Systematic variation of each of these pieces in comparison with those of capsaicin established SAR similarities with and differences from the earlier studies. It is clear that parallel structural changes in the two series of compounds lead to different biological consequences as measured by the Ca^{2+} flux assay. In particular, they are as follows.

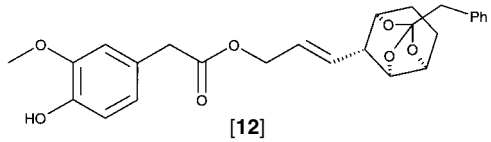
1) The very tight structural requirements in the A-region (4-OH,3-MeO) for potency of capsaicin analogs is not seen for RTX in the α-region, which is much more forgiving of structural variation.

2) The homovanillyl ester group in the β-region of RTX is more potent than amide and thiourea variants, which is the inverse of that observed in the B-region of capsaicin.

3) Modifications to the diterpene portion (χ) of RTX suggest that the functionalized 5-membered ring is important for high potency, but that this moiety is constrained in a particular conformation by the *ortho*-ester unit. If this constraint is not present (as in the closely related phorbol esters), potency is reduced.

This latter result is supported by the lack of activity of the simplified RTX analog [**12**]. This fragment of RTX which overlays the backbone of RTX without the 5-membered ring unit shows that the correct orientation of the *ortho*-ester unit alone is insufficient for high potency (40).

It would seem from these accumulated studies that the structural similarities between RTX and capsaicin are



[**12**]

superficial in that the SAR profiles of the two series are not closely in parallel. This situation is further complicated by the work of Blumberg's group who have described SAR differences within the two structural classes for the Ca^{2+} uptake assay and the [^{3}H]-RTX binding assay (41). The suggestion was made from this data that the two assays are readouts for distinct subclasses of receptor. Very recently (42), these proposals have been elaborated by Blumberg, in a series of elegant but complex experiments which showed that desensitization caused by RTX in the Ca^{2+} uptake assay (induced by capsaicin or RTX itself) correlated quantitatively with [^{3}H]-RTX binding but did not correlate with the ability of RTX to cause Ca^{2+} uptake *per se*. In contrast, capsaicin caused desensitization of Ca^{2+} uptake with a potency corresponding exactly to its ability to cause Ca^{2+} uptake. These results were interpreted as providing extra support for distinct receptor subtypes which primarily recognize RTX (R-type) and capsaicin (C-type), respectively. The provocative corollary to this classification is that the R-type receptor, which mediates pharmacological desensitization without effecting Ca^{2+} uptake, may be a more important therapeutic target than the C-type receptor. It is likely that this latter receptor has been the target of the capsaicin analogs described above.

This hypothesis is speculative and is open to the alternative interpretation that these subtypes could simply be different binding sites on the same operational ion-channel molecule. Further resolution must await confirmation by Blumberg's group of the preliminary report of the identification of a non-neuronal cell line showing only the C-type receptor and the consequent molecular biological sequelae; however, this exciting proposal opens up new avenues of exploration for the medicinal chemist.

Conclusions

The medicinal chemical goal to exploit capsaicin agonists as therapeutic products has not yet been achieved. Separation of the functional desensitization of the sensory neurons which underlies the analgesic effects from the excitatory effects caused by these compounds still seems a worthwhile goal, although it may be that the two sets of properties are mechanistically inextricable. The future requirement will be for the correct balance of the two in an orally active molecule which should lead to a highly innovative type of analgesic agent for use in chronic pain with minimal side effects. This would add significant value to the present armamentarium of currently used products. The impetus for this would include cloning and full characterization of the capsaicin receptor (see footnote). Besides providing a molecular basis for drug design, this

advance would shed light on the curious question of why the hot constituent of chili peppers can relieve pain.

Footnote

The capsaicin receptor has now been cloned (43)! An expression-cloning strategy using a cDNA library from rodent DRG messenger RNA and utilizing the Ca^{2+} flux assay to monitor capsaicin responses gave an individual clone that conferred capsaicin responses in a nonneuronal (HEK293) cell line and showed capsaicin-like electrophysiological responses when expressed in *Xenopus oocytes*. This clone has been extensively characterized as the "vanilloid receptor subtype 1" (VR1), which exhibits all of the pharmacological and electrophysiological characteristics of the previously described "operational" receptor and seems to be somewhat related to store-operated calcium channels. Importantly, the receptor is sensitive to noxious heat, although this has not yet been established at the single channel level. This strongly suggests it may be the molecular entity that mediates thermal nociception. These exciting results provide a critical piece of the capsaicin puzzle and undoubtedly will form the springboard for a new phase of the story.

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