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TRPV1 activation is not an all-or-none event: TRPV1 partial agonism/antagonism and its regulatory modulation

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Abstract

TRPV1 has emerged as a promising therapeutic target for pain as well as a broad range of other conditions such as asthma or urge incontinence. The identification of resiniferatoxin as an ultrapotent ligand partially able to dissect the acute activation of TRPV1 from subsequent desensitization and the subsequent intense efforts in medicinal chemistry have revealed that TRPV1 affords a dramatic landscape of opportunities for pharmacological manipulation. While agonism and antagonism have represented the primary directions for drug development, the pharmacological complexity of TRPV1 affords additional opportunities. Partial agonism/partial antagonism, its modulation by signaling pathways, variable desensitization, and slow kinetics of action can all be exploited through drug design.

Keywords

resiniferatoxin; capsaicin; partial agonist; partial antagonist; TRPV1; efficacy; signaling pathway

Background

The vanilloid receptor TRPV1 provides a paradigm for the impact of natural products on drug discovery. Capsaicin, the pungent ingredient in hot peppers, demonstrated the existence of a critical pathway controlling pain, thermosensation, and neurogenic inflammation [1-3]. Characterization of capsaicin action showed that these responses reflected the activation by capsaicin, acting through a relatively non-selective calcium channel, of C-fiber sensory neurons. Prolonged exposure to capsaicin diminished or abrogated these C-fiber responses as a function of duration and dose, suggesting a therapeutic strategy for intervention. The availability of capsaicin then provided a drug lead to evaluate the therapeutic potential of this approach, defining a long list of pathological conditions for which capsaicin represented a potential treatment. This experience likewise afforded abundant evidence of the limiting toxicity of capsaicin, namely the extent of initial pain arising from the C-fiber stimulation. Together, these advances provided a mature appreciation of the therapeutic potential and limiting side effects of capsaicin analogs. Unfortunately, the initial efforts in medicinal chemistry to realize this potential proved to be disappointing [4-7]. Derivatives yielded only limited dissociation of desensitization from the induction of acute pain and achieved little gain in potency.

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This situation changed with the identification of another natural product, resiniferatoxin, which functioned as a capsaicin analog. Resiniferatoxin, one of the irritant constituents in the medicinal preparation *Euphorbia* [8], prepared from the dried sap of *Euphorbia resinifera*, afforded two conceptual breakthroughs [9]. First, being up to four orders of magnitude more potent than capsaicin, resiniferatoxin unambiguously demonstrated that great potential remained unrealized for improvement in the potency of vanilloid analogs through medicinal chemistry. Second, resiniferatoxin was not simply an ultrapotent analog but was able to dramatically dissect categories of capsaicin response. In particular, while resiniferatoxin was up to four orders of magnitude more potent than capsaicin for inducing desensitization – the therapeutically useful action of vanilloid agonists – it was only a single order of magnitude more potent for inducing acute pain – the limiting toxicity. Resiniferatoxin thus demonstrated that great potential likewise remained for improvement of the therapeutic index of vanilloid agonists through medicinal chemistry. Finally, radiolabeled resiniferatoxin permitted the demonstration and pharmacological characterization of a capsaicin receptor [10, 11], the existence of which had been postulated years before based on vanilloid structure activity analysis [12]. The cloning of this receptor TRPV1 [13], the first member of the TRPV class of TRP channels, provided the final impetus for the intense effort in vanilloid medicinal chemistry which has now advanced to the stage of moving multiple promising drug candidates into clinical trials [14-18].

The natural products such as capsaicin or resiniferatoxin focused attention on desensitization of the vanilloid receptor TRPV1 and C-fiber sensory neurons as a therapeutic strategy [14]. The identification of a vanilloid antagonist, capsazepine, was of great importance because it demonstrated that it was possible to achieve competitive vanilloid antagonism [19, 20]. Likewise, antagonistic activity by 5-iodoresiniferatoxin showed that antagonists could achieve high potency [21]. Because of the reversible nature of antagonism, in contrast to the long duration for desensitization consequent to prolonged agonist action, vanilloid antagonists have received particular attention as potential TRPV1 directed therapeutics [15-18]. Although a theoretical issue had been whether antagonists would have any activity under physiological / pathological conditions, as distinct from the experimental situation of challenge with an exogenous agonist such as capsaicin, this has not proven to be an issue in practice.

As researchers have characterized TRPV1 and the ligands emerging from the vigorous efforts to design and develop drug candidates, it has become clear that the pharmacology of vanilloids is highly complex. Two central issues regarding vanilloid agonists / antagonists are the following: First, antagonism of what? There are multiple stimuli which lead to activation of TRPV1, such as elevated temperature, low pH, exogenous ligands like capsaicin, endogenous ligands such as anandamide, and various signaling pathways. It turns out that antagonists may differ in their ability to antagonize these various stimuli. Closely tied to this issue is the question, very relevant for drug development, of what pattern of antagonism correlates with the utility of an antagonist as a drug affecting physiological conditions. Second, and linked to the above, antagonism need not be an all-or-none affair. Rather, some compounds may be partial agonists / partial antagonists, and their efficacy (degree of partiality) can depend on the stimulus that they are antagonizing. Superimposed on this receptor behavior in response to a ligand is the influence of the TRPV1 milieu. TRPV1 is a site of integration of a wide array of signaling systems, reporting on the influence of external, potentially noxious conditions on the cell. The action of TRPV1 ligands is thus not simply determined by the TRPV1- ligand pair but rather reflects the integration of the influence of the ligand and all those other modulators on TRPV1. In addition, TRPV1 activation leads to changes in internal calcium, which itself represents a powerful influence on cellular signaling, feeding back upon TRPV1 function. Finally, different ligands enter the cell and consequently activate TRPV1 with different kinetics, so

that the response as a function of time is likewise dependent on the specific ligand. Such complexity, which of course is overlaid on the usual complexity of drug absorption / metabolism, is at one level problematic. On the other hand, it provides the potential basis for establishing a level of drug selectivity that would otherwise be unobtainable.

Antagonism (full or partial) to various stimulatory modalities

Studies from our group illustrate the patterns of behavior observed. As part of our effort to understand the structure activity of vanilloid ligands, we characterized in detail two derivatives, designated KJM429 and JYL1421 (also designated SC0030) [22]. Using Chinese hamster ovary cells expressing TRPV1, both compounds fully antagonized the calcium uptake stimulated either by capsaicin or by resiniferatoxin, where both of these agonists were present at their approximate EC_{50} 's. Consistent with their functioning at the agonist binding site rather than as channel blockers, both compounds likewise inhibited the binding of [3H]resiniferatoxin. To distinguish whether the action was competitive, the IC_{50} values for inhibition of calcium uptake were evaluated as a function of agonist concentration. Both for capsaicin and for resiniferatoxin, the two antagonists showed increasing IC_{50} values in the presence of increasing agonist concentrations. Parameters derived from Schild plots gave consistent K_i values for antagonism and consistent K_d values for the agonists. A different picture emerged when calcium uptake was stimulated by elevated temperature (44 °C). Here JYL1421 led to full inhibition of the stimulated uptake, whereas KJM429 afforded only partial inhibition. Similar behavior was observed upon stimulation of calcium uptake by acidification to pH 6.0. Additional acidification to pH 5.5 yielded a further change in behavior, with KJM429 now stimulating uptake and JYL1421 only inhibiting uptake partially.

Several clear conclusions emerged from these studies. First, antagonism to one TRPV1 activator need not imply antagonism to all activators. This necessarily implies that the receptor undergoes somewhat different conformational changes depending on the specific activator. These different conformations are differently affected by ligand binding at the vanilloid binding site. Secondly, these results themselves demonstrate that full antagonists of vanilloid action may have partial antagonism or even enhanced response for stimulation by temperature or low pH.

As part of their extensive efforts to develop TRPV1 antagonists, Gavva and coworkers characterized an extensive series of compounds for their effects on TRPV1 stimulation by capsaicin and low pH [23]. They introduced the term group A antagonists for those compounds that blocked stimulation by both capsaicin and low pH and group B antagonists for those which blocked response to capsaicin but not low pH. Antagonist behavior depends not only on the ligand but also on the specific receptor structure. Thus, capsazepine behaved as a group A antagonist in the guinea pig but as a group B antagonist in the rat [24, 25].

Partial antagonism to vanilloid action, as distinct from partial antagonism to other stimulatory modalities, was demonstrated for two vanilloids closely related to KJM429 and JYL1421 [26]. Compounds were evaluated both for agonism as well as for the ability to antagonize stimulation by capsaicin. JYL827 yielded 6.8% agonism relative to capsaicin; JYL1511 yielded 17.4% agonism. Levels of antagonism were complementary, 93.9% and 84.1%, respectively. As might be expected, the level of partial agonism observed depended on the level of receptor expression. Thus, the extent of partial agonism for JYL827 could be varied from 0 to 23% and for JYL1522 from 17% to 59%, presumably reflecting the influence of receptor number on the level of calcium uptake, as the level of TRPV1 expression was varied under conditions of limiting activated receptors. Additionally, the partial agonists / partial antagonists showed that their patterns of activity were different for other stimulatory conditions. Both yielded enhanced agonism at reduced pH or elevated

temperature. TRPV1 is known to be subject to modulation by multiple signaling pathways, such as protein kinase C, protein kinase D, or protein kinase A. The protein kinase C activator phorbol 12-myristate 13-acetate (PMA) enhanced the extent of partial agonism, from 6.8% to 17.1% for JYL827 and from 17.4% to 27.8% for JYL1511. Effects on antagonism were reciprocal. Dramatically, a combination of the three stimulatory factors – phorbol ester, heat, and low pH - converted the two partial agonists / partial antagonists into almost complete agonists, with agonism reaching 89% for JYL827 and 98% for JYL1511.

A critical concept emerging from these studies is that the response to vanilloids with partial agonist / partial antagonist properties depends very much on the other influences impinging on the receptor. The attractive but speculative model is that it may be possible to design vanilloids that act on TRPV1 only when the TRPV1 is in a specific environment in which it is subjected to modulation by the right combination of modulators. The above studies illustrate this behavior for partial agonists, where the extent of agonism / antagonism is influenced. In this instance, a therapeutic strategy could be to have an antagonist shift to agonism at a specific site, thereby yielding selective desensitization.

Abundant evidence indicates that TRPV1 receptor levels and signaling are impacted by physiological / pathological conditions. TRPV1 levels are elevated in inflammatory bowel disease [27], in vulvodynia [28], in chronic cough [29] and in various bladder syndromes [30]. In primary sensory neurons, levels are elevated in response to stimulation with NGF [31]. Inflammatory mediators can sensitize TRPV1, as shown for the proinflammatory chemokine CCL3 [32]. As illustrated above, changes in receptor level have the potential to change the pattern of response.

It has long been clear that TRPV1 environment can affect the potency of agonists [33]. Thus, heat, acidity, or phorbol ester activation of protein kinase C can sensitize TRPV1 to stimulation by lower doses of capsaicin. A distinct issue is whether the structure activity relations for ligands are shifted in parallel by the TRPV1 environment or whether their structure activity relations are differentially altered. This question was addressed in the case of the influence of protein phosphatases [34]. A series of 8 structurally diverse agonists were evaluated for stimulation of calcium uptake in the absence or presence of cyclosporin A. Cyclosporin A is an inhibitor of the protein phosphatase 2B calcineurin, which in turn influences the state of phosphorylation of TRPV1 among other targets. The 8 agonists were all rendered more potent in the presence of cyclosporin A. Strikingly, however, the extent of sensitization varied from a negligible 1.1-fold for resiniferatoxin to 7.8 fold for DA-5018. The important conclusion was that the signaling environment in which TRPV1 resides can modulate not only its sensitivity to ligands but also its structure activity relations. As above, these findings argue that it should be possible to optimize ligands for TRPV1 in a particular environment, thereby increasing specificity.

In the same study, a series of seven antagonists was examined. The K_i values of the antagonists were increased in the presence of cyclosporin A, albeit with only a modest 1.4-2.5 fold change in relative potencies. The partial antagonist JYL-1511 was also examined. Not surprisingly, its extent of partial agonism increased in the presence of the phosphatase inhibitor. Finally, two other phosphatase inhibitors, okadaic acid and calyculin A, which have different selectivities for protein phosphatases, gave different patterns of sensitization of the agonists than did cyclosporin A. Once again, these findings argue that multiple signaling environments can give distinct structure activity relations for TRPV1.

Taken together, these studies have three conceptual lessons for development of drugs targeted to TRPV1. First, it will be important to screen and assess the structure activity relations of the products of medicinal chemistry efforts using TRPV1 under conditions that

mimic different signaling environments. Second, the structure activity relations of TRPV1 in various animal models need to be compared with those in various cellular systems to better understand which of these screening systems are most predictive. Third, the complicated matrix of patterns of agonism / partial agonism/ antagonism versus various co-stimulatory influences needs to be assessed.

The influence of various conditions on the extent of partial agonism observed may contribute to some of the conflicts in the literature about whether compounds are complete or partial agonists /antagonists. For example, anandamide has been shown to be either a partial [35] or a complete agonist [36], depending on the report and the conditions. In any case, a further factor may be that the behavior is dose dependent, reflecting either a further site of interaction or else the presence of multiple binding sites for ligand on the TRPV1 tetramer. This is perhaps best illustrated for 5-iodo-resiniferatoxin, one of the first antagonists described [21]. High concentrations of 5-iodoresiniferatoxin were described as causing elevated calcium levels in a TRPV1 dependent manner, although the maximum level described remained somewhat less (77%) than that induced by capsaicin [37]. What was very striking, however, is that the doses used were very high, perhaps 10-30 μM for agonism, compared to the pM to nM concentrations at which 5-iodoresiniferatoxin has been found to be an antagonist. Other complications were that the dose response curves were very shallow and that the ability of capsazepine to inhibit the 5-iodoresiniferatoxin induced current, as determined by electrophysiology, suggested very weak affinity for the 5-iodoresiniferatoxin. Furthermore, it would be expected that phorbol diterpene based compounds such as iodoresiniferatoxin will cause membrane perturbing effects at μM concentrations, which might have secondary effects on TRPV1. In any case, these findings raise the issue that vanilloids at high doses may show additional patterns of behavior not seen at lower doses.

Other partial agonists / antagonists

Because partial agonism has not been a particular focus of interest, reports of partial efficacy of agonists have often not been pursued to determine if the basis is simply an incomplete dose response curve, perhaps occasioned by insolubility of the compound, or in fact represents partial agonism / partial antagonism. For example, a large series of vanillamides were prepared incorporating fatty and terpenoid acids [38]. Modest variations in maximal response were observed. In contrast to 5-iodoresiniferatoxin, which was a potent TRPV1 antagonist, the 6-iodoRTX derivative possessed substantial agonist activity [39]. Its efficacy on hTRPV1 was 50% compared to capsaicin, whereas on rTRPV1 it was a full agonist, suggesting that in the human it functioned as a partial agonist.

The dramatic effect of halogenation of resiniferatoxin in converting it from a potent agonist to a potent antagonist [21] suggested that a similar strategy might be effective for capsaicin related derivatives. Appendino prepared the 5- and 6- iodo, bromo, and chloro derivatives of nonanoylvanillamide [40, 41]. Using a fluorometric assay to detect elevation in intracellular calcium in HEK293 cells overexpressing hTRPV1, all the compounds were full antagonists, with enhanced potencies in the order 6-halogen > 5-halogen and iodo > bromo > chloro. The antagonism by the 6-halogenated compounds was examined for competition with capsaicin and shown to be competitive. No agonism was observed.

Following up on the above results, we examined the effect of halogenation on multiple potent thiourea containing agonist templates using $^{45}\text{Ca}^{2+}$ as a measure of rTRPV1 functional activity and evaluating the effect of 5- and 6-halogenation on multiple potent thiourea containing agonist templates [42]. Binding affinities, as determined by competition for [^3H]RTX binding, showed very little difference between the 5- and 6-halogenated derivatives. Consistently, the 5- and 6-iodo derivatives were full antagonists. The most

potent had a binding affinity of 13 nM. In contrast to the iodo derivatives, however, the chloro and bromo derivatives showed variable extents of antagonism, together with reciprocal extents of agonism. Although Schild analysis was not performed to confirm the competitive nature of the antagonism, the finding of partial agonism ruled out the interpretation that the partial extent of the antagonism reflected the inability to achieve a fully effective concentration. Within each series, the bromo derivative possessed more antagonist activity than did the chloro derivative and the 6- position conferred more antagonism than did the 5-position for the same halogen and the same template. These studies provide a panel of derivatives for more extensive probing of the biological action of partial agonists.

Influence of lipophilicity on vanilloid response

Vanilloid potency for TRPV1, as assessed by competition for [³H]resiniferatoxin binding, provides a robust measure for structure activity relations. In contrast, functional activity, as assessed by calcium uptake, is more problematic. At the cellular level, different ligands induce different patterns of cellular activation. Response is subject to continuous feedback consequent to the influx of calcium upon receptor activation, which influences the cellular signaling mechanisms in complex ways. The level of TRPV1 expression, potentially generating “spare receptors”, will shift dose response curves. Finally, although the traditional concept of an ion channel gated by an externally applied ligand is that the relevant channel regulates ion fluxes across the plasma membrane, TRPV1 is localized to a significant degree at the endoplasmic reticulum, where it can function to release calcium from internal calcium stores into the cytosol [33]. These different locations for TRPV1 should have different access to externally added ligands and, as described above, by being imbedded in different environments, should be differentially influenced by cellular signaling mechanisms. How important this internally released calcium is to the overall response remains uncertain. In any case, these various mechanisms in the intact cell will complicate efforts to assess vanilloid potency and functional activity.

At the cellular level, different ligands may induce markedly different kinetics of action. The dose response curves for capsaicin and resiniferatoxin, as evaluated by calcium uptake on populations of cells, yielded dramatically different patterns of response [43]. Whereas graded doses of capsaicin gave largely similar kinetics of activation, with graded extents of maximal stimulated response, graded doses of resiniferatoxin gave graded slopes for response versus time, with apparently similar levels of final stimulation. Exploring this phenomenon at the single cell level, monitoring Ca²⁺ uptake as a function of time, we again found a dramatic difference in the pattern of response [44]. Graded doses of capsaicin gave graded responses, with rapid attainment of maximal stimulation in all cases. In contrast, graded doses of resiniferatoxin behaved very differently. Averaged over multiple cells, response increased slowly with time and with a graded rate of increase in response to graded doses of resiniferatoxin. At the individual cell level, however, the pattern was different. Individual cells responded in a largely all-or-none fashion after a variable latent period. Increasing doses of resiniferatoxin reduced the average latent period before response in a graded fashion.

Resiniferatoxin is a diterpene ester structurally related to the phorbol esters, the biochemical pharmacology of which has been extensively studied [45]. Previous studies on phorbol esters have indicated that their rate of entry into the cell can be very slow, ranging up to several hours, as indicated by the translocation of their targets, the protein kinase C isoforms, in response to phorbol ester binding [46]. Using fluorescent phorbol esters, we have been able to image the uptake of the ligands directly, confirming their slow uptake [47]. An important element in this rate of uptake is the lipophilicities of these compounds, which typically have LogP values ranging from 3 – 8. The most hydrophilic compounds are

rapidly taken up; the most hydrophobic are taken up very slowly. The more hydrophilic compounds distribute throughout the internal membranes; the more lipophilic compounds initially occupy the plasma membrane before redissolving in the cytosol and equilibrating with internal membranes.

Using a fluorescent vanilloid, CHK-884, we were able to show that vanilloid uptake could likewise be quite slow, in this case with a half time of 30 min [48]. As with the phorbol esters, localization was almost exclusively to internal membranes. A consequence of slow uptake of ligand should be that the apparent potencies will depend on the incubation time. For CHK-884, a 3-fold enhancement of potency was observed by imaging of the increase in intracellular calcium concentration with a 60 min incubation time as compared to a 5 min incubation time. Olvanil is highly lipophilic, with a calculated LogP of 8.0. By calcium imaging, the potency of olvanil was enhanced 7-fold when assayed with a 60 min incubation time compared to a 5 min incubation time. Finally, the antagonist 5-iodo-resiniferatoxin has a calculated LogP of 8.03. Its K_i for inhibition of capsaicin stimulation of TRPV1, as determined by $^{45}\text{Ca}^{2+}$ uptake, shifted from nM with a 5 min incubation time, similar to reported potencies for this compound [21], to the pM range when preincubated with the cells for 1-2 hr before capsaicin challenge.

These findings indicate that the time of incubation for assessment of activity is critical, at least for the more lipophilic ligands. Many of the described screening efforts for structure activity analysis have relied on 3 or 5 min incubations. Such times are inadequate for more lipophilic compounds, such as resiniferatoxin related derivatives or halogenated derivatives. For antagonists, increasing the time of preincubation represents a simple remedy. For agonists or partial agonists, the time dependent effects arising from TRPV1 activation pose an obvious complication.

A second implication, for which the consequences are less clear, is that of the time dependent distribution of the ligand within the cell. Contrary to the simplistic expectation that a channel protein should be located at the plasma membrane, TRPV1 is distributed between two functional compartments – the plasma membrane and the endoplasmic reticulum [33, 49]. Whereas the former will control the influx of calcium into the cell, the latter can release calcium into the cytoplasm from intracellular stores. As shown for the phorbol esters, the more hydrophobic ligands will access these two pools of TRPV1 with different kinetics. Furthermore, as described above, the different membrane and signaling environments of TRPV1 in these two locations have the potential to afford different structure activity relations and thus different selectivity, depending on the specific ligand.

Different kinetics of desensitization

Just as different ligands can show different patterns for activation of TRPV1, so different ligands may show different patterns of desensitization. Once again, this was evident on monitoring the level of intracellular calcium in individual cells upon ligand treatment [44]. Whereas cells responding to resiniferatoxin continued to maintain the stimulated level of calcium over the following 20 min, the elevated level of calcium in response to capsaicin decreased by 50% and that to anandamide decreased by more than 80%. Using electrophysiological measurement and evaluating responses on the second time scale, response to piperine likewise diminished much more rapidly than did that to capsaicin [50]. Camphor has been described as a weak activator of TRPV1, with the extent of activation being potentiated by phorbol ester treatment or phospholipase C activators and with the nature of the activation being distinct from that of capsaicin, since its effect was not antagonized by capsazepine or observed for chicken TRPV1, which is capsaicin insensitive. In any case, camphor was observed as causing more extensive desensitization than did capsaicin [51].

Different extents of cooperativity

Consistent with a tetrameric structure for TRPV1, vanilloids have long been recognized to show cooperativity for both binding and receptor activation. It was therefore striking that phorbol 12-phenylacetate 20-homovanillate (PPAHV) failed to do so either for binding or for induction of calcium uptake [52]. It is perhaps noteworthy that this ligand was unique among compounds examined for its selectivity (500-fold) between rat and human TRPV1, reflecting strong dependence on M547 in TRPV1 for activity. A more extensive series of related phorbol 12,13-diester 20-homovanillate derivatives showed variable degrees of cooperativity, with several showing Hill coefficients of 1.1 or less [53]. *In vivo*, PPAHV showed very weak potency in the eye wiping assay and no effect on induction of hypothermia. In any case, the significance of non-cooperative interaction with TRPV1 has not been explored in detail.

Role of TRPV1 under basal conditions in physiological regulation

A critical issue for the suitability of vanilloid antagonists as therapeutic agents is whether they will have undesired side effects as a consequence of interference with the physiological, as distinct from the nociceptive functions, of TRPV1. Characterization of TRPV1 knock out mice initially suggested that this was not a concern [54, 55]. In particular, core body temperature was not affected [54] or else showed only a minor increase in daily body temperature rhythm [56].

Limited effects have subsequently been observed. For example, there was some desensitization of bladder function [57]. TRPV1^{-/-} mice showed reduced protection from myocardial ischemia-reperfusion injury upon PAR-2 activation [58] and an enhanced renal inflammatory response in response to deoxycortisterone-salt induced hypertension [59]. In a model of endotoxin-induced sepsis, the TRPV1^{-/-} mice showed an enhanced endotoxemic response [60]. They displayed reduced sensitivity to sedation and they showed enhanced uncoordination in response to ethanol [61]. Interestingly, the TRPV1 knockout mice were reported to have a significantly enhanced sensitivity to tumor promotion in the two stage mouse skin carcinogenesis protocol [62].

In contrast to the limited effects of TRPV1 knockout in the mouse, in particular the lack of an effect on body temperature, an elevation of body temperature emerged as a significant source of concern in the testing of the newly developed TRPV1 antagonists [63]. Gavva and coworkers reported that a variety of TRPV1 antagonists caused hyperthermia in rats, dogs, and monkeys [64]. Such behavior was observed both for group A and group B antagonists. Likewise, it was observed both for compounds that penetrated the CNS and compounds that did not, suggesting that the hyperthermia reflected interaction at peripheral TRPV1. This conclusion was further supported by studies desensitizing abdominal TRPV1, leading to loss of the hyperthermic response [65]. Interestingly, JYL1421 did not cause hyperthermia in the rat, although it did so in the dog and monkey. Similarly, JYL1421 was described as potentiating activation of TRPV1 by pH 5.0 only in the rat, suggesting that maybe this profile of activities would be linked to lack of hyperthermic response. Implications of this finding were the following: 1) maintenance of hyperthermia was separable from the acute induction of hyperthermia and 2) antagonists with a profile of antagonism only for a subset of TRPV1 activating stimuli might not induce hyperthermia.

Further analysis of the hyperthermic response induced by antagonists strongly argued that it represented inhibition of tonically activated visceral TRPV1 [66, 67]. Consistent with the induction of hyperthermia being a transient response to TRPV1 antagonists, multiple applications of antagonist attenuated the response in animals [68]. Phase 1 clinical studies of AMG517, a lead TRPV1 antagonist, confirmed that hyperthermia was induced in man as

well as in animal models [69]. While the hyperthermia attenuated somewhat with time, it nonetheless proven to be problematic in some individuals.

As part of an effort to surmount the induction of hyperthermia by antagonists, the Amgen group explored the pattern of behavior of antagonists which showed different patterns of behavior for different TRPV1 stimuli. In addition to their type A (blocks capsaicin, heat, and low pH) and type B (blocks capsaicin and heat but not low pH) antagonists, they identified compounds described as type C (blocks capsaicin, does not block heat, and stimulates response to low pH) and type D (blocks capsaicin but stimulated response to both heat and low pH) (In this classification system, low pH means 5.0) [70]. The type C antagonist AMG8562 caused a modest decrease, not an increase, in body temperature in the rat and the type C antagonist AMG7905 caused a marked decrease in body temperature. Interestingly, as was described for JYL1421, AMG8562 behaved differently on human TRPV1 than it did on rat TRPV1; for human TRPV1 it behaved as a type B, not a type A, antagonist. It should be noted that conceptually, the type C and D antagonists really blend into the partial agonists / antagonists, in that they are functioning as agonists under a subset of experimental conditions such as low pH or elevated temperature. Comparison of a more extensive series of compounds suggested that the ability of antagonists to block stimulation of TRPV1 by low pH was the predominant contributor to the induction of hyperthermia in the animal models [71]. These generalizations, again, will presumably prove to be less representative as more compounds are investigated. For example, JYL1421 was an exception in the analysis of Garami, in that it did not cause hyperthermia but rather at very high doses induced hypothermia.

Other studies likewise show that the situation is more complicated. The antagonist A-425619 caused only transient hyperthermia followed by hypothermia, with the duration of the hyperthermia dependent on the time of administration relative to the circadian rhythm of the rats [72]. The pattern did not desensitize, however, upon repeated daily dosing, perhaps reflecting that the duration of action of this antagonist was appreciably less than the intervals between dosing. In contrast, ABT-102 was described as showing almost complete desensitization of the hyperthermic effect by upon repeated dosing, whereas the therapeutic effects were enhanced [73].

Conclusion

The complexities of the biochemical pharmacology of vanilloid antagonists are clearly becoming evident as compounds move from the initial screening to whole animal testing or the clinical setting. Formally, there is a distinction between two classes of behavior. On the one hand, partial agonists / partial antagonists for TRPV1 stimulation by capsaicin cause competitive displacement of capsaicin and lead to a different channel conformation with no or lesser conductance than that of the channel when capsaicin is bound. On the other hand, compounds which fully block TRPV1 stimulation by capsaicin under standard conditions but which show less inhibition or even stimulation for other co-stimulators of the channel such as heat or low pH are considered full antagonists with a different profile of activity. In both cases, however, the situation is really the same. The compounds modulate the extent of channel function and the extent of the modulation is regulated by the integral of other signals impinging on TRPV1, whether temperature, pH, phosphorylation at the numerous binding sites, or associated lipids and proteins. Moreover, it is critical to recognize the influence of time scale on apparent activity. Electrophysiological channel measurements tend to be performed on the time scale of seconds, regulation of calcium levels on the minute time scale, and behavior on the time scale of hours. The multiple feedback pathways regulating TRPV1 function will all have different influences on these different time scales. At a later time point, differences in extent of desensitization will look similar to differences

in extent of agonism. In terms of drug development, desensitization through chronic treatment with agonists and channel blockade upon acute or chronic treatment with antagonists have been considered distinct approaches. As the complexities of the action of TRPV1 ligands become better characterized, it may well be that these two approaches represent the extremes of a continuum of treatment strategies.

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