British Journal of Pharmacology (2004) 141, 1313–1320
www.nature.com/bjp

REVIEW

Activation and sensitisation of the vanilloid receptor: role in gastrointestinal inflammation and function

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1 The exquisite specific excitatory and desensitising actions of capsaicin on a subpopulation of primary sensory neurons have been instrumental in identifying the roles of these neurons in nociception, reflex responses and neurogenic inflammation.

2 Structure activity studies with capsaicin-like molecules have suggested that a 'receptor' should mediate the effects of capsaicin on sensory neurons. The cloning of the vanilloid receptor-1 (VR1) has confirmed this hypothesis.

3 VR1 (TRPV1) belongs to the transient receptor potential (TRP) family of channels, and its activation by various xenobiotics, noxious temperature, extracellular low pH and high concentration of certain lipid derivatives results in cation influx and sensory nerve terminal excitation.

4 TRPV1 may dimerise or form tetramers or heteromers with PLC-γ and TrkA or even with other TRPs. TRPV1 is markedly upregulated and/or 'sensitised' under inflammatory conditions *via* protein kinase C-ε-, cAMP-dependent PK- and PLC-γ-dependent pathways or by exposure to dietary agents as ethanol.

5 TRPV1 is expressed on sensory neurons distributed in all the regions of the gastrointestinal tract in myenteric ganglia, muscle layer and mucosa. There is evidence of TRPV1 expression also in epithelial cells of the gastrointestinal tract.

6 High expression of TRPV1 has been detected in several inflammatory diseases of the colon and ileum, whereas neuropeptides released upon sensory nerve stimulation triggered by TRPV1 activation seem to play a role in intestinal motility disorders.

7 TRPV1 antagonists, which will soon be available for clinical testing, may undergo scrutiny for the treatment of inflammatory diseases of the gut.
British Journal of Pharmacology (2004) **141,** 1313–1320. doi:10.1038/sj.bjp.0705768

Keywords: TRPV1; capsaicin; substance P; CGRP; IBS; gastrointestinal inflammation

Abbreviations: GERD, gastro-oesophageal reflux disease; CGRP, calcitonin gene-related peptide; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; SOCE, store-operated calcium entry; SP, substance P; TRP, transient receptor potential; TRPV1, transient receptor potential vanilloid-1

Transient receptor potential (TRP) family of channels

Capsaicin, the hot principle contained in the plans of the genus *Capsicum*, is a powerful stimulus for a specific subset of primary sensory neurons in experimental animals and in humans. There is a large body of evidence indicating that the excitatory effect of capsaicin on sensory neurons is due to its ability to increase the open state of a channel previously defined as the 'capsaicin receptor'. The recent cloning of this molecular entity has revealed that it consists (Caterina *et al*., 1997) of a 426 amino-acid protein, which has been firstly termed vanilloid receptor-1 (VR1). The VR1 was soon recognised to belong to the TRP family of ion channels. TRPs have been subdivided into three main subclasses: TRPC, TRPM and TRPV. The capsaicin-activated TRPV1 belongs to the latter group (Montell *et al*., 2002). TRPP (PKD2-like channels, PKD2 is mutated in polycystic kidney disease), TRPML (mucolipidin-like channels, mucolipidin mutations are responsible for some lysosomal-like disorders) and TRPN (NOMPC-like channels, NOMPC is required for mechano-sensory function in flies) are additional and newly proposed subtypes of TRPs. More recently, an additional TRP-like channel, which responds to cold temperature ($<15^{\circ}$C), but not to menthol has been cloned (Peier *et al*., 2002) and termed ANKTM1. Sensory neurons that express TRPV1, but not neurons that express the other putative cold receptor, TRPM8, also seem to express the ANKTM1 (McKemy *et al*., 2002; Peier *et al*., 2002).

There is a series of hypotheses that consider TRPs as sensors of diverse stimuli and other hypotheses that do not, but instead propose that they act downstream of various G-protein-coupled receptors, most probably *via* the phospholipase C pathway as intracellular ion regulators (Montell, 1997;

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Advance online publication: 29 March 2004

Clapham, 2003). This alternative hypothesis considers TRP as receptor-operated channels. However, the major challenge of this theory is to identify a receptor-activated messenger that directly binds and activates the channel (Clapham, 2003). This possibility has been proposed on the basis of the findings obtained with the TRPL in the *Drosophila* (Hardie, 2003) and the mammalian TRPV1 (Chuang *et al*., 2001; Prescott & Julius, 2003). These channels are inhibited by PIP_2 binding and activated by PIP_2 hydrolysis. However, in contrast to this hypothesis, constitutive activity of TRPM7 is increased by PIP_2 binding and reduced by PIP_2 hydrolysis (Runnels *et al*., 2002). An additional theory proposes that TRPs regulate the so-called capacitance Ca^{2+} entry or store-operated Ca^{2+} entry (SOCE). SOCE should be a mechanism and a channel(s) that links Ca^{2+} store depletion with Ca^{2+} entry. Although a consistent number of papers have suggested a link between TRPs and SOCE, conclusive proof that one or more TRPs are the selective mechanism that mediates SOCE is still lacking (Clapham, 2003). A series of lipid derivatives, including arachidonic acid metabolites, have been claimed to gate TRPs. A recent example of this assumption derives from the observation that the endocannabinoid anandamide and its metabolite arachidonic acid activate TRPV4 in an indirect way involving the cytochrome *P*450 epoxygenase-dependent formation of epoxyeicosatrienoic acids (Watanabe *et al*., 2003).

Vanilloid receptor (TRP vanilloid-1, TRPV1)

Like many other ion channels the TRPV1 possesses six putative transmembrane domains, with a proposed pore region between transmembrane domains five and six. TRPV1, as all TRPs, is thought to have cytoplasmic N- and C-termini. TRPV1, once activated by vanilloid molecules allows the influx of cations, as Ca^{2+} and Na^{+}. TRPV1 mRNA is highly expressed in a subset of primary sensory neurons with A-δ and C fibres that respond to chemical, mechanical and thermal stimuli and, therefore, they are classified as polymodal nociceptos. TRPV1 mRNA is also expressed in diverse areas of the central nervous system, including the limbic system (e.g. hippocampus, central amygdala and both medial and lateral habenula), striatum, hypothalamus, centromedian and paraventricular thalamic nuclei, substantia nigra, reticular formation, locus coeruleus, cerebellum and inferior olive (Mezey *et al*., 2000). There is also evidence that mRNA and protein of TRPV1 are produced and expressed in non-neuronal cells, including the epithelial cells of the urothelium (Birder *et al*., 2001), keratinocytes (Inoue *et al*., 2002) and epithelial cells of the palatal rugae (Kido *et al*., 2003). The finding that TRPV1 is expressed in several brain nuclei is of great importance in that it places TRPV1 in a much broader perspective than pain perception with regard to its neural functions. Less clear is the physiological and pathophysiological function, if any, of TRPV1 in non-neuronal cells.

TRPV1 channels localise both to the plasma membrane and to intracellular membranes in human embryonic kidney (HEK) 293 cells, whereas it localised predominantly to the plasma membrane in neuronal-derived F-11 cells. However, the function, if any, of TRPV1 expressed at intracellular sites is unknown (Jahnel *et al*., 2001). Distinct sensory functions arise from different combinations of TRPV proteins as OSM-9 and OCR-2, including olfaction, osmosensation, mechanosensation and chemosensation. Thus, alternative combinations of TRPV proteins may direct different functions in distinct subcellular locations (Tobin *et al*., 2002). Although the expression of TRPV1 in recombinant systems suggests that the channel monomer is sufficient for the pore function, there is evidence that in recombinant systems it undergoes biochemical dimerisation (Rosenbaum *et al*., 2002). This phenomenon is particularly intriguing given that functional channels are almost certainly tetramers (Rosenbaum *et al*., 2002). In addition, there is evidence that TRPV1 is capable of forming a specific ternary complex with phospholipase C-γ and the neurotrophin trkA receptor. Finally, the observation that some members of the TRP family can heterodimerise increases interest in the possibility that TRPV1, VRLs or ECACs might assemble with each other or even with TRPs to form novel heteromeric receptors (Gunthorpe *et al*., 2002). Whether different affinities of antagonists may result from diverse channel heteromerisation is, however, a matter of debate.

The ionic event, triggered by TRPV1 gating, results in an excitatory effect on terminals of primary sensory neurons with the subsequent depolarisation of the nerve fibre and the initiation of action potential propagation. Orthodromic conduction of action potentials triggers reflex responses, including cough, voiding of the urinary bladder and in the gut contribute to peristalsis. Ca^{2+} influx into the nerve endings, driven either by antidromic conduction of action potential or directly by TRPV1 gating, causes the local release of neuropeptides, including calcitonin gene-related peptide (CGRP) and the tachykinins, substance P (SP) and neurokinin A (NKA). Activation of CGRP receptors and tachykinin (NK_1, NK_2 and NK_3) receptors on effector cells, particularly at the vascular levels, causes a series of inflammatory responses, collectively referred to as neurogenic inflammation (Geppetti & Holzer, 1996).

TRPV1 is a thermosensor, activated by a moderate noxious temperature between 42 and 53°C (Caterina *et al*., 1997). Studies on the cloned channel (Tominaga *et al*., 1998) confirmed previous indication that the capsaicin receptor is stimulated by low extracellular pH (pH 6–5) (Geppetti *et al*., 1991; Bevan & Geppetti, 1994; Geppetti & Holzer, 1996). Additional stimuli of the TRPV1 include elevated concentrations (in the μM range) of the endocannabinoid, anandamide (Zygmunt *et al*., 1999), or the lipoxygenase metabolites of arachidonic acid, LTB_4 or 12-HPETE (Hwang *et al*., 2000). More recently, *N*-arachidonoyl-dopamine has been recognised as a TRPV1 stimulant, apparently more potent than anandamide (Huang *et al*., 2002; Harrison *et al*., 2003). The finding that lipid derivatives gate the channel and, therefore, are considered as the endogenous ligands of TRPV1 could explain another contradictory observation: TRPV1 is highly expressed in central terminals of primary sensory neurons in laminas I and II of the dorsal horn of the spinal cord and medulla oblongata. However, in these anatomical regions it is unlikely that temperature may rise or pH may fall to levels adequate for TRPV1 gating. The observation that the endocannabinoid, anandamide, activates TRPV1 in the spinal cord (Tognetto *et al*., 2000) suggests the existence of endogenous messengers at central synapses, and the possibility of a physiological role of the channel expressed on central terminals of nociceptors, possibly modulating pain transmission.

Genetic studies have defined that TRPV1 is not required for appropriate temperature sensing. However, mice lacking

TRPV1 show unpaired development of thermal hyperalgesia (Davis *et al*., 2000) and altered urinary bladder function with a higher frequency of low-amplitude, nonvoiding bladder contractions (Birder *et al*., 2002). More recent pharmacological studies (Walker *et al*., 2003) have shown that the TRPV1 antagonist, capsazepine, reverses mechanical hyperalgesia in models of inflammatory and neuropathic pain, in guinea-pigs. This indicates a role of TRPV1 in hyperalgesic conditions driven not only by thermal but also by mechanical stimuli. This latter study underlies the importance of species differences in the evaluation of the efficacy of TRPV1 antagonists. Further support for the hypothesis for a role of TRPV1 in mechanical hyperalgesia derives from the use of novel channel antagonists (Lee *et al*., 2003; Pomonis *et al*., 2003).

Activation of TRPV1 and neurogenic inflammatory responses

The ability of vanilloid molecules to cause robust stimulation of the TRPV1 on sensory neurons and, thus, cause the activation of neurogenic inflammatory and reflex responses has been already mentioned. Neurogenic inflammation consists of a series of responses that mainly occur at the vascular level, but that are also present in other tissues and organs in a species-dependent manner: neurogenic inflammation causes a variety of non-vascular responses (in parenthesis the neuropeptide involved), including chonotropic and inotropic positive responses in the heart (CGRP), contraction of the iris sphincter muscle (SP/NKA), contraction of the smooth muscle of the ureter, bladder neck and urethra (SP/NKA), and relaxation of bladder dome (CGRP), secretion from seromucous glands (SP/NKA) and other effects. Species-related variations in neurogenic inflammatory responses are clearly illustrated by the motor effect produced by sensory nerve activation and tachykinins in the airways. The release of SP/NKA from TRPV1-expressing nerve terminals causes direct bronchoconstriction in the guinea-pig, indirect and nitric oxide/prostanoid-mediated bronchodilatation in the rat and mouse. In humans, as in guinea-pigs, activation of both NK_2 and, in part, NK_1 receptors mediates a robust bronchoconstriction in human isolated bronchi (Amadesi *et al*., 2001). Vascular neurogenic inflammatory responses are present virtually in all organs and tissues and include arteriolar vasodilatation (CGRP), plasma protein extravasation and leucocyte adhesion to the vascular endothelium of postcapillary venules (SP/NKA) (Geppetti & Holzer, 1996).

In the intestine, the role of tachykinins and CGRP is more complex because in addition to the extrinsic nerve supply, substantially represented by TRPV1-positive nerve fibres, tachykinins and CGRP are also expressed and released from intrinsic gastrointestinal neurons. Non-sensory CGRP/tachykinins contribute to the overall functions of these neuropeptides. A major role in the regulation of the motor function in the gut has been recognised as being due to the tachykinins, *via* the activation of all of the three tachykinin receptors, NK_1, NK_2 and NK_3. A large part of their contractile effect originates from direct stimulation of tachykinin NK_1 and/or NK_2 receptors present on smooth muscle cells/cells of Cajal of the circular and longitudinal muscle layers (Holzer & Holzer-Petsche, 2001). In addition, indirect contractile responses can be evoked by tachykinins through stimulation of tachykinin receptors (mainly of the NK_3 type) present on intestinal neurons, from which either acetylcholine and tachykinins themselves are released (Patacchini *et al*., 2000). It is worth mentioning that the release of inhibitory transmitters (e.g. nitric oxide) has also been reported upon stimulation of tachykinin NK_1, NK_2 and NK_3 receptors.

The role of tachykinin NK_2 receptor in visceral hyperalgesia in the gut is suggested by the inhibitory effect of NK_2 receptor antagonists in animal models triggered by inflammation (McLean *et al*., 1997) or pretreated with trinitrobenzenesulphonic acid (TNBS) or previously subjected to restraint stress (Toulouse *et al*., 2000). The results suggest that the tachykinin NK_2 receptor is a main target mediating visceral allodynia/hyperalgesia. The role of tachykinin NK_2 receptors is further supported by the observation that an NK_2 receptor antagonist prevents the increased expression of either *c-fos* and *c-jun* proto-oncogene markers in the spinal cord and dorsal root ganglia neurons from rats pretreated with TNBS, and the hypersensitivity of single spinal cord neurons responding to colorectal distension (Laird *et al*., 2001). However, it is not known if these inflammatory mechanisms are mediated by tachykinins released from sensory TRPV1-espressing nerve terminals or from intrinsic neurons of the gut.

Neurogenic inflammation plays a role in somatic and visceral inflammation in different mammal species. In humans, there is little doubt that at the somatic level neurogenic inflammation exits: the flare response produced by the application of irritants (capsaicin, histamine, etc.) to the human skin is limited or abolished by either local anaesthetics or by repeated application of topical capsaicin (capsaicin desensitisation), a procedure that results in the transient defunctionalisation of terminals of TRPV1-expressing neurons. Less clear is, however, whether neurogenic inflammation plays a pathophysiological role at the visceral level. Data obtained in rodents or other mammal species have not been convincingly replicated in human experimental settings or in human diseases. With the relevant exception of the release of authentic CGRP from slices of the human iris and ciliary body (Geppetti *et al*., 1992), there is no neurochemical evidence that capsaicin is able to release measurable quantities of sensory neuropeptides from visceral human tissues *in vitro*, as, in contrast, occurs in a variety of tissues obtained from other mammal species. The key observation of the release of sensory CGRP from a human anatomical area innervated by the first division of the trigeminal nerve is interestingly associated with the findings that CGRP immunoreactivity is increased in the blood taken from extra- and intracranial circulation during attacks of migraine or cluster headache (Goadsby *et al*., 1990; Fanciullacci *et al*., 1995). Finally, a recent randomised and placebo-controlled trial showed that the intravenous injection of BIBN4096BS, a peptoid with high affinity for the CGRP receptor (Doods *et al*., 2000) and that does not cross the blood–brain barrier, reduces significantly the pain and other symptoms associated with migraine attack (Olesen *et al*., 2004). This finding supports the hypothesis that sensory CGRP is released from terminals of primary sensory neurons and exerts a pathophysiological role in human disease.

TRPV1 antagonists

TRPV1-expressing neurons and neurogenic inflammation have been proposed to play a major role in a large variety of

diseases, including migraine and cluster headache, asthma and chronic obstructive pulmonary disease, perennial and allergic rhinitis, osteoarthritis, cystitis and overactive bladder syndrome, postherpetic neuralgia and postmastectomy pain and many others diseases. A role of these neurons, TRPV1 channel and neurogenic inflammation has also been proposed in some intestinal diseases that will be discussed below. Evidence for the involvement of TRPV1-expressing neurons in human disease originates from studies performed in animal models. There are also novel data obtained in humans by means of morphological localisation and semiquantitative assay of TRPV1 and desensitisation of sensory nerve terminals by repeated capsaicin application. While the use of TRPV1 antagonists is currently limited to animal models, results with capsaicin desensitisation in humans must be regarded with caution for two reasons. The first is due to the pungent action associated with capsaicin treatment, an action that makes it difficult to blind appropriately, and control clinical trials. The second derives from the fact that capsaicin desensitisation results from the specific action of the drug on TRPV1, but produces its effects (including the beneficial effect) by the defunctionalisation of the entire nerve terminals, which no longer responds, not only to TRPV1 agonists (protons, lipid derivatives, capsaicin, etc.) but also to all the other stimuli that act on different channels/receptors expressed on the nerve terminal. Thus, in this latter case, TRPV1 is of critical importance for the production of the capsaicin effect, but cannot offer any firm conclusion on the role of TRPV1 with regard to the pathological condition under investigation. A detailed description of TRPV1 antagonists currently used in experimental animals and that may undergo scrutiny in clinical trials is reported in Table 1.

A critical issue for the definition of the therapeutic potential of receptor antagonists is the understanding of whether endogenous agonists exist and under which circumstances receptors are activated by their agonists. Accordingly, in the TRPV1 field the search for endovanilloid molecules has been active for many years. There is no doubt that apart from certain physicochemical agents (temperature and protons), molecules with the ability to activate the TRPV1 exist. However, so far requirements for defining these molecules as endovanilloids have not been satisfactory fulfilled. Specifically, the demonstration that putative agonists, such as *N*-arachidonoyl-dopamine, anandamide, 12-HTEPE or LTB_4, can be released in the vicinity of the receptor in quantities adequate to activate the receptors has not been given. However, a number of examples suggest that TRPV1 undergoes remarkable regulation under a large variety of conditions. In particular, upregulation of TRPV1 is often produced after exposure to proinflammatory agents, thus suggesting the general hypothesis that under inflammatory circumstances, a 'sensitised' TRPV1 can be activated by agonist concentrations much lower than those tested under conventional experimental conditions.

TRPV1 sensitisation and upregulation

As with other TRP channels, also for TRPV1, there are examples that its expression can be upregulated or that its activity can be 'sensitised'. One clear indication of TRPV1 upregulation derives from the observation that TRPV1 protein expression in cell bodies of dorsal root ganglion (DRG)

Table 1 IC_{50} (nM) values of diverse TRPV1 antagonists

14 (Jerman *et al.*, 2000)	Ruthenium Red (ammoniated ruthenium oxychloride)
912 (Rigoni *et al.*, 2003)[a]	Capsazepine (*N*-[2-(4-chlorophenyl)ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2H-2-benzazepine-2-carbothioamide)
0.071 (Rigoni *et al.*, 2003)[a]	Iodo-resiniferatoxin (6,7-deepoxy-6,7-didehydro-5-deoxy-21-dephenyl-21-(phenylmethyl)-daphnetoxin,20-(4-hydroxy-5-iodo-3-methoxybenzeneacetate)
638.6 (Appendino *et al.*, 2003)	6-iodo-nordihydrocapsaicin (6-iodo-nordihy 8-methyl-*N*-vanillyl-*trans*-6-nonenamide)
7.5 (Gunthorpe *et al.*, 2003)	SB 366791 (*N*-(3-methoxyphenyl)-4-chlorocinnamide)
100 (Himmel *et al.*, 2002)	I-R_4W_2 (l-enantiomers of the arginine-rich hexapeptide)
4.8–58 (Sun *et al.*, 2003)[b]	4-(2-pyridyl)piperazine-1-carboxamides
7.8 (Lee *et al.*, 2003)[c]	*N*-[2-(3,4-dimethylbenzyl)-3-pivaloyloxypropyl]-*N*′-[3-fluoro-4-(methylsulphonylamino)benzyl] thiourea
9.2 (Wang *et al.*, 2002)[c]	JYL1421 ([*N*-(4-tert-butylbenzyl)-*N*′-[3-fluoro-4-(methylsulphonylamino)benzyl]thiourea])
6–35 (Valenzano *et al.*, 2003)	BCTC (*N*-(4-tertiarybutylphenyl)-4-(3-cholorphyridin-2-yl)tetrahydropyrazine -1(2H)-carbox-amide)

NB: IC_{50} values are referred to the inhibition of capsaicin-induced calcium uptake in: endogenous TRPV1 in rat DRG neurons. [a]Human TRPV1 HEK293 cell line. [b]Rat TRPV1 HEK293 cell line. [c]rVR1 CHO cells.

neurons is increased by inducing inflammation in their peripheral receptive areas. This upregulation is mediated by NGF and p38MAP kinase and results in an increased TRPV1 protein transportation to the peripheral endings of sensory neurons and in a parallel increase in heat hypersensitivity (Ji *et al*., 2002). A large body of evidence has accumulated on TRPV1 sensitisation. Anandamide has been shown to sensitise TRPV1 to other channel agonists. Anandamide also causes lowering of the threshold temperature to TRPV1 stimulation, an effect that is mediated by a protein kinase C (PKC)-ε dependent pathway (Premkumar & Ahern, 2000). More interestingly, activation of the bradykinin B_2 has been found to result in TRPV1 sensitisation by diverse intracellular mechanisms, including PKC-ε (Premkumar & Ahern, 2000; Sugiura *et al*., 2002), and PLC-γ-dependent displacement of phosphatidylinositol-4,5-bisphosphate from TRPV1 binding (Chuang *et al*., 2001), and 12- and 5-lipoxygenase metabolites production (Shin *et al*., 2002; Carr *et al*., 2003). PKC-dependent TRPV1 sensitisation seems to be promiscuously used by different stimuli, as in addition to anandamide, heat and protons also sensitise the channel by this enzymatic pathway (Vellani *et al*., 2001). Also, cAMP-dependent PK (PKA) seems to be involved in TRPV1 sensitisation (De Petrocellis *et al*., 2001), as capsaicin responses in sensory neurons exhibit a robust potentiation by PKA, and PKA activation reduces TRPV1 desensitisation and directly phosphorylates TRPV1 (Bhave *et al*., 2002). The observation that prostaglandins may induce/sensitise the cough response (Costello *et al*., 1985) and more relevantly, that one major adverse effect of angiotensin-converting enzyme (ACE or kinase II) inhibitors is cough (Israili & Hall, 1992), suggests that bradykinin accumulation *via* a PKC-dependent pathway or, indirectly through prostanoid release, and a PKA-dependent pathway may lead to TRPV1 sensitisation and a consequent exaggeration of the cough response.

It has been recently shown that ethanol (Trevisani *et al*., 2002) and other alcohols (M. Trevisani, S. Harrison and P. Geppetti, unpublished observation) cause responses apparently mediated by TRPV1. Indeed, intracellular Ca^{2+} mobilisation in rat DRG neurons in culture and SP/CGRP release from slices of rat dorsal spinal cord are all effects produced by exposure to 0.3–3% ethanol, and these effects are inhibited by the TRPV1 antagonist, capsazepine. The observation that HEK cells did not respond to ethanol, but transfection of these cells with the human TRPV1 conferred to them the ability to respond to capsaicin and ethanol, in a capsazepine-sensitive manner, demonstrated that ethanol can be considered as a TRPV1 agonist. Electrophysiological experiments with HEK cells transfected with human TRPV1 have clarified that currents produced to the TRPV1 agonists, anandamide and protons were potentiated by ethanol (by about 10-fold and by about 50-fold, respectively). More importantly, it was shown that ethanol lowered the threshold temperature to stimulate the TRPV1 by about 8°C. TRPV1 is usually activated at 43°C. However, electrophysiological data allow to conclude that in the presence of ethanol TRPV1 activation already occurs at the physiological temperature of 37°C (Trevisani *et al*., 2002). This observation gives a mechanistic explanation for the burning painful sensation that follows ethanol exposure to wound and mucosal surfaces. This study also shows that exogenous agents of widespread dietary use and therefore of epidemiological importance for gastrointestinal and other diseases may cause a dramatic regulation of TRPV1 activity (Figure 1).

Gastrointestinal inflammation and function and TRPV1

TRPV1-like immunoreactivity has been documented on nerves within myenteric ganglia and interganglionic fibre tracts throughout the gastrointestinal tract. TRPV1 nerves have also been observed within the muscle layers and had an irregular profile, with varicose-like swellings along their lengths. Blood vessels within the gastrointestinal wall had TRPV1-immunoreactive nerve fibres associated with them. TRPV1-like nerves and other immunopositive cells were also observed within the mucosa. (Ward *et al*., 2003). Of interest for the present discussion is the recent finding that a rat gastric epithelial cell line (RGM-1) and cells of the rat gastric wall express TRPV1 mRNA. However, it is not clear whether the relative amount of expression of TRPV1 in gastric epithelial cells and if the channel expressed in this cell type exerts, if any, a physiological and physiopathological role (Kato *et al*., 2003).

The findings described in the previous paragraph with regard to protons and ethanol and TRPV1 activation/sensitisation also offer a possible explanation for those patients with gastro-oesophageal reflux disease (GERD), who experience burning pain during reflux episodes and, occasionally, when they drink alcoholic beverages or eat hot foods or liquids. The sensation of burning pain could be triggered following the stimulation of TRPV1 by each individual stimulus (acidic media, alcohol, high temperature), but all these stimuli may synergise and the burning pain may be exaggerated in a proinflammatory environment, where TRPV1 function has been upregulated. With regard to ethanol, there is some experimental evidence supporting this hypothesis. In the rat, exposure of the oesophageal wall to ethanol causes a remarkable increase in plasma extravasation, an effect that, being blocked by the TRPV1 antagonist, capsazepine, and by the NK_1 receptor antagonist, SR 140333, is due to the activation of sensory nerve terminal *via* a TRPV1-dependent mechanism, the release of SP/NKA and the activation of NK_1

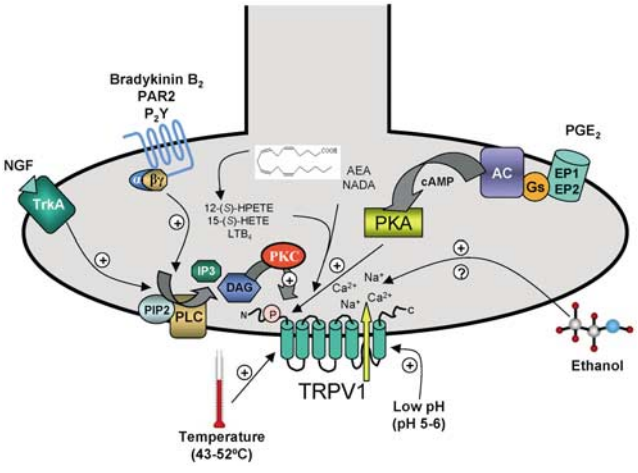

Figure 1 Schematic diagram of some of the stimuli and intracellular pathways that contribute to the sensitization TRPV1 function in terminals of primary sensory neurons.

receptor on endothelial cells of postcapillary venules of the oesophagus (Trevisani *et al*., 2002). In this case, it is possible that ethanol sensitises TRPV1 to the 'physiological' temperature of 37°C to elicit sensory nerve activation and neurogenic plasma extravasation. Whether this neurogenic inflammatory effect occurs in humans is, however, not known.

The discovery of high-affinity channel ligands, as resiniferatoxin (Szallasi & Blumberg, 1999), and the cloning of the human TRPV1 (Hayes *et al*., 2000) with the subsequent availability of specific antisera have allowed detailed investigation of the presence and distribution of the channel in human tissues in health and disease. With regard to the gastrointestinal tract, major advancements have been carried out in the field of inflammatory bowel disease (IBD): in intestinal specimens taken from patients with Chron's disease and ulcerative colitis, immunoblotting and immunostaining revealed a greatly increased density of TRPV1 than in colonic tissues from control subjects (Yiangou *et al*., 2001). Upregulation of TRPV1 immunoreactivity of TRPV1 in colonic nerve fibres of patients with active IBD, suggests that drugs that antagonise endogenous inflammatory substances that activate this receptor could lead to new therapies for gastrointestinal pain and dysmotility.

Previous observation has shown that in Hirschsprung's disease (HSCR), hypertrophic nerves in aganglionic bowel are mainly of extrinsic origin and may contain sensory elements. More recent immunostaining studies detected fibres and nerve fascicles, but not somata, positive for TRPV1 in all regions of the bowel in specimens from control subjects, with few weakly immunostained fibres in the mucosa/lamina propria (Facer *et al*., 2001). TRPV1 immunoreactivity was found to be intense in hypoganglionic and aganglionic bowel, whereas normoganglionic regions of HSCR were similar to controls. Thus, the presence of TRPV1 immunoreactivity in aganglionic HSCR bowel indicates that sensory nerves may form a significant proportion of the hypertrophic innervation in this condition (Facer *et al*., 2001).

Faecal urgency and incontinence associated to rectal hypersensitivity is a distressing and inadequately treated condition. The mechanism that results in the symptoms that characterise the disease is unknown. The two observations that: (a) in specimens from patients with rectal hypersensitivity, TRPV1-positive nerve fibres were increased in muscle, submucosal and mucosal layers as compared to control specimens, and that; (b) the thresholds for heat and distension significantly correlated with expression of TRPV1-positive nerve fibres, suggest that faecal urgency and rectal hypersensitivity could result from increased numbers of polymodal sensory nerve fibres expressing TRPV1. Again, in this condition TRPV1 antagonists could afford some benefit for the control symptoms and perhaps they may positively modify the natural course of the disease.

Specific studies addressing the link between irritable bowel disease and TRPV1 in animal models are lacking, and a few papers have associated chilli pepper and irritable bowel syndrome (Shah *et al*., 2000; Agarwal *et al*., 2002; Schmulson *et al*., 2003). However, the possibility that a syndrome, characterised by enhanced perception of visceral events that span from diarrhoea to constipation and results in symptoms as abdominal pain and discomfort, is associated with altered function of TRPV1 and TRPV1-expressing sensory neurons is conceivable. Further mechanistic studies in experimental animals and both observational and interventional clinical investigation are required to address this interesting hypothesis.

This work was supported in part by a grant from ARCA, Padua and MURST, Rome.

References

AGARWAL, M.K., BHATIA, S.J., DESAI, S.A., BHURE, U. & MELGIRI, S. (2002). Effect of red chillies on small bowel and colonic transit and rectal sensitivity in men with irritable bowel syndrome. *Indian J. Gastroenterol.,* **21,** 179–182.

AMADESI, S., MOREAU, J., TOGNETTO, M., SPRINGER, J., TREVISANI, M., NALINE, E., ADVENIER, C., FISHER, A., VINCI, D., MAPP, C., MIOTTO, D., CAVALLESCO, G. & GEPPETTI, P. (2001). NK1 receptor stimulation causes contraction and inositol phosphate increase in medium-size human isolated bronchi. *Am. J. Respir. Crit. Care Med.,* **163,** 1206–1211.

BEVAN, S. & GEPPETTI, P. (1994). Protons: small stimulants of capsaicin-sensitive sensory nerves. *Trends Neurosci.,* **17,** 509–512.

BHAVE, G., ZHU, W., WANG, H., BRASIER, D.J., OXFORD, G.S. & GEREAU, R.W.T. (2002). cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. *Neuron,* **35,** 721–731.

BIRDER, L.A., KANAI, A.J., DE GROAT, W.C., KISS, S., NEALEN, M.L., BURKE, N.E., DINELEY, K.E., WATKINS, S., REYNOLDS, I.J. & CATERINA, M.J. (2001). Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.,* **98,** 13396–13401.

BIRDER, L.A., NAKAMURA, Y., KISS, S., NEALEN, M.L., BARRICK, S., KANAI, A.J., WANG, E., RUIZ, G., DE GROAT, W.C., APODACA, G., WATKINS, S. & CATERINA, M.J. (2002). Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. *Nat. Neurosci.,* **5,** 856–860.

CARR, M.J., KOLLARIK, M., MEEKER, S.N. & UNDEM, B.J. (2003). A role for TRPV1 in bradykinin-induced excitation of vagal airway afferent nerve terminals. *J. Pharmacol. Exp. Ther.,* **304,** 1275–1279.

CATERINA, M.J., SCHUMACHER, M.A., TOMINAGA, M., ROSEN, T.A., LEVINE, J.D. & JULIUS, D. (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature,* **389,** 816–824.

CHUANG, H.H., PRESCOTT, E.D., KONG, H., SHIELDS, S., JORDT, S.E., BASBAUM, A.I., CHAO, M.V. & JULIUS, D. (2001). Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. *Nature,* **411,** 957–962.

CLAPHAM, D.E. (2003). TRP channels as cellular sensors. *Nature,* **426,** 517–524.

COSTELLO, J.F., DUNLOP, L.S. & GARDINER, P.J. (1985). Characteristics of prostaglandin induced cough in man. *Br. J. Clin. Pharmacol.,* **20,** 355–359.

DAVIS, J.B., GRAY, J., GUNTHORPE, M.J., HATCHER, J.P., DAVEY, P.T., OVEREND, P., HARRIES, M.H., LATCHAM, J., CLAPHAM, C., ATKINSON, K., HUGHES, S.A., RANCE, K., GRAU, E., HARPER, A.J., PUGH, P.L., ROGERS, D.C., BINGHAM, S., RANDALL, A. & SHEARDOWN, S.A. (2000). Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature,* **405,** 183–187.

DE PETROCELLIS, L., HARRISON, S., BISOGNO, T., TOGNETTO, M., BRANDI, I., SMITH, G.D., CREMINON, C., DAVIS, J.B., GEPPETTI, P. & DI MARZO, V. (2001). The vanilloid receptor (VR1)-mediated effects of anandamide are potently enhanced by the cAMP-dependent protein kinase. *J. Neurochem.,* **77,** 1660–1663.

DOODS, H., HALLERMAYER, G., WU, D., ENTZEROTH, M., RUDOLF, K., ENGEL, W. & EBERLEIN, W. (2000). Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. *Br. J. Pharmacol.,* **129,** 420–423.

FACER, P., KNOWLES, C.H., TAM, P.K., FORD, A.P., DYER, N., BAECKER, P.A. & ANAND, P. (2001). Novel capsaicin (VR1) and purinergic (P2X3) receptors in Hirschsprung's intestine. *J. Pediatr. Surg.,* **36,** 1679–1684.

FANCIULLACCI, M., ALESSANDRI, M., FIGINI, M., GEPPETTI, P. & MICHELACCI, S. (1995). Increase in plasma calcitonin gene-related peptide from the extracerebral circulation during nitroglycerin-induced cluster headache attack. *Pain,* **60,** 119–123.

GEPPETTI, P., DEL BIANCO, E., CECCONI, R., TRAMONTANA, M., ROMANI, A. & THEODORSSON, E. (1992). Capsaicin releases calcitonin gene-related peptide from the human iris and ciliary body *in vitro*. *Regul. Pept.,* **41,** 83–92.

GEPPETTI, P., DEL BIANCO, E., PATACCHINI, R., SANTICIOLI, P., MAGGI, C.A. & TRAMONTANA, M. (1991). Low pH-induced release of calcitonin gene-related peptide from capsaicin-sensitive sensory nerves: mechanism of action and biological response. *Neuroscience,* **41,** 295–301.

GEPPETTI, P. & HOLZER, P. (1996). *Neurogenic Inflammation*. Boca Raton FL: CRC Press.

GOADSBY, P.J., EDVINSSON, L. & EKMAN, R. (1990). Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Ann. Neurol.,* **28,** 183–187.

GUNTHORPE, M.J., BENHAM, C.D., RANDALL, A. & DAVIS, J.B. (2002). The diversity in the vanilloid (TRPV) receptor family of ion channels. *Trends Pharmacol. Sci.,* **23,** 183–191.

HARDIE, R.C. (2003). Regulation of TRP channels *via* lipid second messengers. *Annu. Rev. Physiol.,* **65,** 735–759.

HARRISON, S., DE PETROCELLIS, L., TREVISANI, M., BENVENUTI, F., BIFULCO, M., GEPPETTI, P. & DI MARZO, V. (2003). Capsaicin-like effects of *N*-arachidonoyl-dopamine in the isolated guinea pig bronchi and urinary bladder. *Eur. J. Pharmacol.,* **475,** 107–114.

HAYES, P., MEADOWS, H.J., GUNTHORPE, M.J., HARRIES, M.H., DUCKWORTH, D.M., CAIRNS, W., HARRISON, D.C., CLARKE, C.E., ELLINGTON, K., PRINJHA, R.K., BARTON, A.J., MEDHURST, A.D., SMITH, G.D., TOPP, S., MURDOCK, P., SANGER, G.J., TERRETT, J., JENKINS, O., BENHAM, C.D., RANDALL, A.D., GLOGER, I.S. & DAVIS, J.B. (2000). Cloning and functional expression of a human orthologue of rat vanilloid receptor-1. *Pain,* **88,** 205–215.

HOLZER, P. & HOLZER-PETSCHE, U. (2001). Tachykinin receptors in the gut: physiological and pathological implications. *Curr. Opin. Pharmacol.,* **1,** 583–590.

HUANG, S.M., BISOGNO, T., TREVISANI, M., AL-HAYANI, A., DE PETROCELLIS, L., FEZZA, F., TOGNETTO, M., PETROS, T.J., KREY, J.F., CHU, C.J., MILLER, J.D., DAVIES, S.N., GEPPETTI, P., WALKER, J.M. & DI MARZO, V. (2002). An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc. Natl. Acad. Sci. U.S.A.,* **99,** 8400–8405.

HWANG, S.W., CHO, H., KWAK, J., LEE, S.Y., KANG, C.J., JUNG, J., CHO, S., MIN, K.H., SUH, Y.G., KIM, D. & OH, U. (2000). Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. *Proc. Natl. Acad. Sci. U.S.A.,* **97,** 6155–6160.

INOUE, K., KOIZUMI, S., FUZIWARA, S., DENDA, S. & DENDA, M. (2002). Functional vanilloid receptors in cultured normal human epidermal keratinocytes. *Biochem. Biophys. Res. Commun.,* **291,** 124–129.

ISRAILI, Z.H. & HALL, W.D. (1992). Cough and angioneurotic edema associated with angiotensin-converting enzyme inhibitor therapy. *Ann. Intern. Med.,* **117,** 234–242.

JAHNEL, R., DREGER, M., GILLEN, C., BENDER, O., KURRECK, J. & HUCHO, F. (2001). Biochemical characterization of the vanilloid receptor 1 expressed in a dorsal root ganglia derived cell line. *Eur. J. Biochem.,* **268,** 5489–5496.

JI, R.R., SAMAD, T.A., JIN, S.X., SCHMOLL, R. & WOOLF, C.J. (2002). p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. *Neuron,* **36,** 57–68.

KATO, S., AIHARA, E., NAKAMURA, A., XIN, H., MATSUI, H., KOHAMA, K. & TAKEUCHI, K. (2003). Expression of vanilloid receptors in rat gastric epithelial cells: role in cellular protection. *Biochem. Pharmacol.,* **66,** 1115–1121.

KIDO, M.A., MUROYA, H., YAMAZA, T., TERADA, Y. & TANAKA, T. (2003). Vanilloid receptor expression in the rat tongue and palate. *J. Dent. Res.,* **82,** 393–397.

LAIRD, J.M., OLIVAR, T., LOPEZ-GARCIA, J.A., MAGGI, C.A. & CERVERO, F. (2001). Responses of rat spinal neurons to distension of inflamed colon: role of tachykinin NK2 receptors. *Neuropharmacology,* **40,** 696–701.

LEE, J., KANG, M., SHIN, M., KIM, J.M., KANG, S.U., LIM, J.O., CHOI, H.K., SUH, Y.G., PARK, H.G., OH, U., KIM, H.D., PARK, Y.H., HA, H.J., KIM, Y.H., TOTH, A., WANG, Y., TRAN, R., PEARCE, L.V., LUNDBERG, D.J. & BLUMBERG, P.M. (2003). *N*-(3-acyloxy-2-benzylpropyl)-*N*′-[4-(methylsulfonylamino)benzyl] thiourea analogues: novel potent and high affinity antagonists and partial antagonists of the vanilloid receptor. *J. Med. Chem.,* **46,** 3116–3126.

MCKEMY, D.D., NEUHAUSSER, W.M. & JULIUS, D. (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature,* **416,** 52–58.

MCLEAN, P.G., PICARD, C., GARCIA-VILLAR, R., MORE, J., FIORAMONTI, J. & BUENO, L. (1997). Effects of nematode infection on sensitivity to intestinal distension: role of tachykinin NK2 receptors. *Eur. J. Pharmacol.,* **337,** 279–282.

MEZEY, E., TOTH, Z.E., CORTRIGHT, D.N., ARZUBI, M.K., KRAUSE, J.E., ELDE, R., GUO, A., BLUMBERG, P.M. & SZALLASI, A. (2000). Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. *Proc. Natl. Acad. Sci. U.S.A.,* **97,** 3655–3660.

MONTELL, C. (1997). New light on TRP and TRPL. *Mol. Pharmacol.,* **52,** 755–763.

MONTELL, C., BIRNBAUMER, L., FLOCKERZI, V., BINDELS, R.J., BRUFORD, E.A., CATERINA, M.J., CLAPHAM, D.E., HARTENECK, C., HELLER, S., JULIUS, D., KOJIMA, I., MORI, Y., PENNER, R., PRAWITT, D., SCHARENBERG, A.M., SCHULTZ, G., SHIMIZU, N. & ZHU, M.X. (2002). A unified nomenclature for the superfamily of TRP cation channels. *Mol. Cell,* **9,** 229–231.

OLESEN, J., DIENER, H.C., HUSSTEDT, I.W., GOADSBY, P.J., HALL, D., MEIER, U., POLLENTIER, S., LESKO, L.M. & BIBN 4096 BS CLINICAL PROOF OF CONCEPT STUDY GROUP. (2004). Calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine. *N. Engl. J. Med.,* **350,** 1073–1075.

PATACCHINI, R., MAGGI, C.A. & HOLZER, P. (2000). Tachykinin autoreceptors in the gut. *Trends Pharmacol. Sci.,* **21,** 166.

PEIER, A.M., MOQRICH, A., HERGARDEN, A.C., REEVE, A.J., ANDERSSON, D.A., STORY, G.M., EARLEY, T.J., DRAGONI, I., MCINTYRE, P., BEVAN, S. & PATAPOUTIAN, A. (2002). A TRP channel that senses cold stimuli and menthol. *Cell,* **108,** 705–715.

POMONIS, J.D., HARRISON, J.E., MARK, L., BRISTOL, D.R., VALENZANO, K.J. & WALKER, K. (2003). *N*-(4-tertiarybutylphenyl)-4-(3-cholorphyridin-2-yl)tetrahydropyrazine-1(2H)-carbox-amide (BCTC), a novel, orally effective vanilloid receptor 1 antagonist with analgesic properties: II. *in vivo* characterization in rat models of inflammatory and neuropathic pain. *J. Pharmacol. Exp. Ther.,* **306,** 387–393.

PREMKUMAR, L.S. & AHERN, G.P. (2000). Induction of vanilloid receptor channel activity by protein kinase C. *Nature,* **408,** 985–990.

PRESCOTT, E.D. & JULIUS, D. (2003). A modular PIP2 binding site as a determinant of capsaicin receptor sensitivity. *Science,* **300,** 1284–1288.

ROSENBAUM, T., AWAYA, M. & GORDON, S.E. (2002). Subunit modification and association in VR1 ion channels. *BMC Neurosci.,* **3,** 4.

RUNNELS, L.W., YUE, L. & CLAPHAM, D.E. (2002). The TRPM7 channel is inactivated by PIP(2) hydrolysis. *Nat. Cell Biol.,* **4,** 329–336.

SCHMULSON, M.J., VALDOVINOS, M.A. & MILKE, P. (2003). Chili pepper and rectal hyperalgesia in irritable bowel syndrome. *Am. J. Gastroenterol.,* **98,** 1214–1215.

SHAH, S.K., ABRAHAM, P. & MISTRY, F.P. (2000). Effect of cold pressor test and a high-chilli diet on rectosigmoid motility in irritable bowel syndrome. *Indian J. Gastroenterol.,* **19,** 161–164.

SHIN, J., CHO, H., HWANG, S.W., JUNG, J., SHIN, C.Y., LEE, S.Y., KIM, S.H., LEE, M.G., CHOI, Y.H., KIM, J., HABER, N.A., REICHLING, D.B., KHASAR, S., LEVINE, J.D. & OH, U. (2002). Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia. *Proc. Natl. Acad. Sci. U.S.A.,* **99,** 10150–10155.

SUGIURA, T., TOMINAGA, M., KATSUYA, H. & MIZUMURA, K. (2002). Bradykinin lowers the threshold temperature for heat activation of vanilloid receptor 1. *J. Neurophysiol.,* **88,** 544–548.

SZALLASI, A. & BLUMBERG, P.M. (1999). Vanilloid (Capsaicin) receptors and mechanisms. *Pharmacol. Rev.,* **51,** 159–212.

TOBIN, D., MADSEN, D., KAHN-KIRBY, A., PECKOL, E., MOULDER, G., BARSTEAD, R., MARICQ, A. & BARGMANN, C. (2002). Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in *C. elegans* neurons. *Neuron,* **35,** 307–318.

TOGNETTO, M., AMADESI, S., HARRISON, S., CREMINON, C., TREVISANI, M., CARRERAS, M., MATERA, M., GEPPETTI, P. & BIANCHI, A. (2000). Anandamide excites central terminals of dorsal root ganglion neurons *via* vanilloid receptor-1 (VR-1) activation. *J. Neurosci.,* **21,** 1104–1109.

TOMINAGA, M., CATERINA, M.J., MALMBERG, A.B., ROSEN, T.A., GILBERT, H., SKINNER, K., RAUMANN, B.E., BASBAUM, A.I. & JULIUS, D. (1998). The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron,* **21,** 531–543.

TOULOUSE, M., COELHO, A.M., FIORAMONTI, J., LECCI, A., MAGGI, C. & BUENO, L. (2000). Role of tachykinin NK2 receptors in normal and altered rectal sensitivity in rats. *Br. J. Pharmacol.,* **129,** 193–199.

TREVISANI, M., SMART, D., GUNTHORPE, M.J., TOGNETTO, M., BARBIERI, M., CAMPI, B., AMADESI, S., GRAY, J., JERMAN, J.C., BROUGH, S.J., OWEN, D., SMITH, G.D., RANDALL, A.D., HARRISON, S., BIANCHI, A., DAVIS, J.B. & GEPPETTI, P. (2002). Ethanol elicits and potentiates nociceptor responses *via* the vanilloid receptor-1. *Nat. Neurosci.,* **5,** 546–551.

VELLANI, V., MAPPLEBECK, S., MORIONDO, A., DAVIS, J.B. & MCNAUGHTON, P.A. (2001). Protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. *J. Physiol.,* **534,** 813–825.

WALKER, K.M., URBAN, L., MEDHURST, S.J., PATEL, S., PANESAR, M., FOX, A.J. & MCINTYRE, P. (2003). The VR1 antagonist capsazepine reverses mechanical hyperalgesia in models of inflammatory and neuropathic pain. *J. Pharmacol. Exp. Ther.,* **304,** 56–62.

WARD, S.M., BAYGUINOV, J., WON, K.J., GRUNDY, D. & BERTHOUD, H.R. (2003). Distribution of the vanilloid receptor (VR1) in the gastrointestinal tract. *J. Comp. Neurol.,* **465,** 121–135.

WATANABE, H., VRIENS, J., PRENEN, J., DROOGMANS, G., VOETS, T. & NILIUS, B. (2003). Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature,* **424,** 434–438.

YIANGOU, Y., FACER, P., DYER, N.H., CHAN, C.L., KNOWLES, C., WILLIAMS, N.S. & ANAND, P. (2001). Vanilloid receptor 1 immunoreactivity in inflamed human bowel. *Lancet,* **357,** 1338–1339.

ZYGMUNT, P.M., PETERSSON, J., ANDERSSON, D.A., CHUANG, H., SORGARD, M., DI MARZO, V., JULIUS, D. & HOGESTATT, E.D. (1999). Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature,* **400,** 452–457.

(Received February 10, 2004
Revised March 1, 2004
Accepted March 2, 2004)