28 Mendelson, W. B. *et al.* (1987) *Psychopharmacology* 93, 226–229
29 Limmroth, V. *et al.* (1996) *Br. J. Pharmacol.* 117, 99–104
30 Moskowitz, M. A. *et al.* (1998) *Am. Assoc. Stud. Headache Syllabus*
31 Data, J. *et al.* (1998) *Am. Assoc. Stud. Headache Syllabus*
32 Devaud, L. L., Purdy, R. H. and Morrow, A. L. (1995) *Alcohol Clin. Exp. Res.* 19, 350–355
33 Finn, D. A., Roberts, A. J. and Crabbe, J. C. (1995) *Alcohol Clin. Exp. Res.* 19, 410–415
34 Devaud, L. L. *et al.* (1996)*J. Pharmacol. Exp. Ther.* 278, 510–517
35 Reddy, D. S. and Kulkarni, S. K. (1997) *Eur. J. Pharmacol.* 337, 19–25
36 Budziszewska, B., Jaworska-Feil, L. and Lason, W. (1996) *Eur. Neuropsychopharmacol.* 6, 135–140
37 Reddy, D. S. and Kulkarni, S. K. (1997) *Methods Find. Exp. Clin. Pharmacol.* 19, 395–405
38 Rogawski, M. A. and Porter, R. J. (1990) *Pharmacol. Rev.* 42, 223–285
39 Dhuna, A. *et al.* (1991) *Neurotoxicology* 12, 621–626
40 Rogawski, M. A. (1996) in *Neurotherapeutics: Emerging Strategies* (Pullan, L. and Patel, J., eds), pp. 193–273, Humana Press
41 Frye, C. A. (1995) *Brain Res.* 696, 113–120
42 Schumacher, M., Robel, P. and Baulieu, E. E. (1996) *Dev. Neurosci.* 18, 6–21
43 Monaghan E. P. *et al.* (1997) *Epilepsia* 38, 1026–1031
44 Lader, M. (1995) in *$GABA_A$ Receptors and Anxiety: From Neurobiology to Treatment* (Biggio, G., Sanna, E. and Costa, E., eds), pp. 135–152, Raven Press
45 Baldessarini, R. J. (1996) in *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (9th edn) (Hardman, J. G. *et al.*, eds), pp. 399–430, McGraw-Hill
46 Miczek, K. A. *et al.* (1997) in *Recent Developments in Alcoholism* (Vol. 13) *Alcoholism and Violence* (Galanter, M., ed.), pp. 139–171, Plenum Press
47 Britton, K. T. *et al.* (1991) *J. Pharmacol. Exp. Ther.* 258, 124–129
48 Wieland, S. *et al.* (1997) *Psychopharmacology* 134, 46–54
49 Lancel, M. *et al.* (1996) *Am. J. Physiol.* 271, E763–E772
50 Friess, E. *et al.* (1997) *Am. J. Physiol.* 272, E885–E891
51 Hill-Venning, C. *et al.* (1996) *Neuropharmacology* 35, 1209–1222
52 Sear, J. W. (1998) *Eur. J. Anaesthesiol.* 15, 129–132
53 Bradley, W. G. *et al.* (1968) *Br. Med. J.* 3, 531–533
54 Lundberg, P. O. (1968) *Acta Neurol. Scand.* 45, 309–326
55 Chen, T. and Leviton, A. (1994) *Headache* 34, 107–110
56 Emmett-Oglesby, M. W. *et al.* (1990) *Psychopharmacology* 101, 292–309
57 Marshall, F. H. *et al.* (1997) *Pharmacol. Biochem. Behav.* 58, 1–8
58 Vanover, K. E. *et al.* (1999) *Psychopharmacology* 141, 77–82
59 Finn, D. A. *et al.* (1997) *Pharmacol. Biochem. Behav.* 56, 261–264
60 Winger, G. *et al.* (1996) *Behav. Pharmacol.* 7 (Suppl. 1), 121

Chemical names

CCD3693: 3α-hydroxy-3β-trifluoromethyl-19-nor-5β-pregnan-20-one

Co21068: 3β-(4-acetylphenyl)ethynyl-3α,21-dihydroxy-5β-20-one-21-hemisuccinate Na

Co26749: 3α,21-dihydroxy-3β-trifluoromethyl-19-nor-5β-pregnan-20-one

Co30593: 3β-ethenyl-3α-hydroxy-5α-pregnan-20-one

Co60549: 3α,21-dihydroxy-3β-trifluoromethyl-19-nor-5β-pregnan-20-one, 21-hemisuccinate

Co87071: 3α,21-dihydroxy-3β-trifluoromethyl-5β-pregnan-20-one,21-hemisuccinate

ORG20599: 21-chloro-3α-hydroxy-2β-(4-morpholinyl)-5α-pregnan-20-one methanesulphonate

ORG21465: 3α-hydroxy-2β-(2,2-dimethylmorpholin-4-yl)-5α-pregnane-11,20-dione

Capsaicin, protons and heat: new excitement about nociceptors

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The past few years have witnessed a remarkable progress in understanding the neurobiology of pain. Important advances have been made particularly in the field of peripheral signal transduction in nociceptors. Membrane receptors have been identified for capsaicin, a pungent ingredient of chilli peppers, protons (i.e. acidic solutions) and for heat, three stimuli that specifically excite nociceptors. Of particular interest appears to be the first cloned capsaicin receptor, VR1, which has been suggested to serve as an integrator of these three nociceptive stimuli. These findings not only give new insights into the molecular machinery of nociceptor activation and sensitization, but can also provide a rational basis for pharmacological research aiming for a new class of peripherally acting analgesics, which should selectively interfere with nociceptor activation.

Improving the treatment and the prevention of acute and especially chronic pain is an intriguing challenge for modern medicine. Most of the currently available analgesics fall into two groups. They either belong to the aspirin-like drugs, which mainly block prostaglandin production by inhibiting the cyclooxygenases, or to the narcotic analgesics, such as morphine, which activate opioid receptors. Because prostaglandins and opioid receptors are widely distributed in the body, these drugs can cause a number of serious side-effects. None of the currently available drugs is known to block nociceptor-specific receptors or enzymes, although such compounds might be well suited peripheral analgesics with possibly less side-effects.

The term nociceptor was introduced by Sherrington, who defined nociceptors as those primary afferent neurones that can be activated by harmful or potentially harmful stimuli and then give rise to the sensation of pain[1]. Among the many chemical and physical stimuli that excite nociceptors, low extracellular pH, noxious heat and capsaicin have attracted particular interest owing to their specific and persistent activation of nociceptors[2,3]. Putative receptors of these stimuli are therefore promising targets for new peripherally acting analgesics. The molecular cloning of the capsaicin receptor and of proton-gated ion channels has set a milestone in the search for such compounds. The capsaicin receptor is at present probably one of the most attractive targets for such a new approach.

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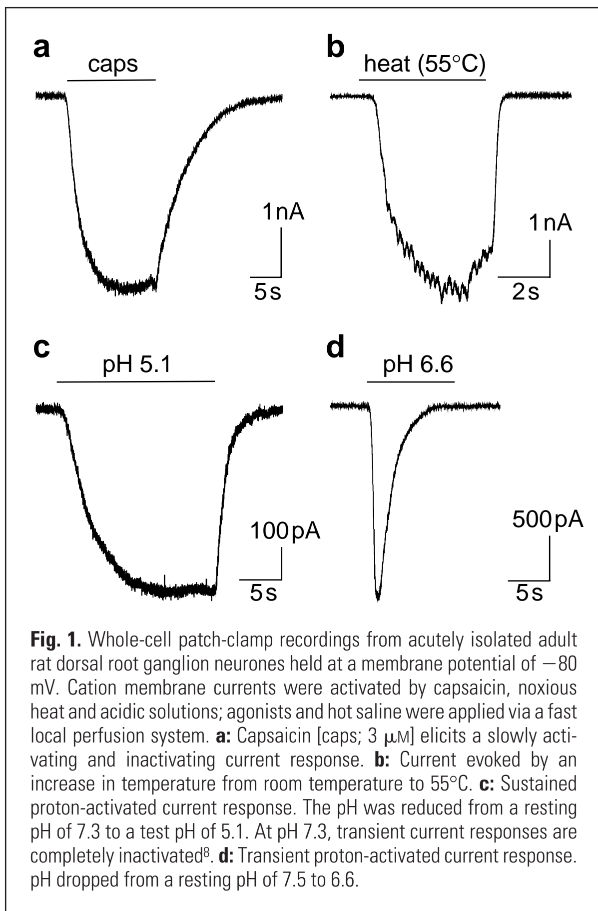

Fig. 1. Whole-cell patch-clamp recordings from acutely isolated adult rat dorsal root ganglion neurones held at a membrane potential of −80 mV. Cation membrane currents were activated by capsaicin, noxious heat and acidic solutions; agonists and hot saline were applied via a fast local perfusion system. **a:** Capsaicin [caps; 3 μM] elicits a slowly activating and inactivating current response. **b:** Current evoked by an increase in temperature from room temperature to 55°C. **c:** Sustained proton-activated current response. The pH was reduced from a resting pH of 7.3 to a test pH of 5.1. At pH 7.3, transient current responses are completely inactivated[8]. **d:** Transient proton-activated current response. pH dropped from a resting pH of 7.5 to 6.6.

Capsaicin receptors

For more than 20 years capsaicin has been well known as a very selective activator of thinly or unmyelinated nociceptive afferents[4]. Within the past few years it has been revealed that capsaicin directly opens a cation channel in the plasma membrane of nociceptive nerve fibres[5,6]. The current generated by this channel can be studied in the small, presumed nociceptive, dorsal root ganglion (DRG) or trigeminal ganglion neurones[5–8], which probably represent the cell bodies of these nerves[9]. An example of such a current response is shown in Fig. 1a and important characteristics are summarized in Table 1 (Refs 5, 8, 10–18). The selectivity of capsaicin is also reflected in the tissue distribution of its receptors, which has been assessed using the naturally occurring irritant tricyclic diterpene resiniferatoxin (RTX), another extremely potent activator of these receptors[19]. RTX and capsaicin are structurally quite different molecules but share a common vanilloid moiety and the capsaicin receptor has therefore also been termed the vanilloid receptor[20]. Binding studies using radioactively labelled RTX revealed that expression of capsaicin receptors in the rat nervous system is very specific and limited to the membranes of both somatic and visceral primary sensory neurones (for details see Table 1)[10].

There is much evidence to indicate that both capsaicin and RTX are specific activators of nociceptors and can cause pain under experimental conditions, but neither of them occurs endogenously in vertebrates. The nature of the physiological activator(s) of capsaicin receptors has therefore been a long-standing issue and only recently results have been obtained providing sufficient information allowing speculation about which pain states involve the activation of capsaicin receptors or, in other words, what types of pain could be treated with capsaicin receptor antagonists or channel blockers.

Physiologcial activators of capsaicin receptors

Of the many stimuli that activate cation currents in primary sensory neurones, low extracellular pH and noxious heat have been proposed as physiological activators of capsaicin receptors. Currents activated by these stimuli (for examples see Fig. 1) are co-expressed with capsaicin sensitivity[2] and, as shown in Tables 1 and 2, share many properties.

Local tissue acidosis (i.e. low extracellular pH) frequently occurs under inflammatory or ischaemic conditions

Table 1. Electrophysiological and pharmacological properties of native ion channels activated by capsaicin or noxious heat and of VR-1

	Native capsaicin receptors	Refs	Heat-activated cation currents	Refs	VR-1 (Ref. 26)
Tissue distribution	DRG, trigeminal and nodose ganglia; nucleus of the solitary tract, spinal sensory nucleus of the trigeminal nerve; spinal cord dorsal horn (RTX-binding), small DRG and trigeminal neurones (capsaicin-induced currents)	5, 8, 10–12	Small DRG neurones	15–17	Small DRG and trigeminal neurones (VR-1 mRNA)
Activators	Capsaicin (EC_{50} =1.1 μM), RTX (EC_{50} = 2–10 nM)	5	Heat (≥42°C)	15, 16	Capsaicin (EC_{50} = 712 nM), RTX (EC_{50} = 39 nM), heat (≥45°C)
Blockers	Capsazepine (IC_{50} = 148 nM), ruthenium red	13, 14	Extracellular mM Cs^+, capsazepine and ruthenium red	18[a]	Capsazepine (IC_{50} = 238.5 nM), ruthenium red
Single-channel conductance	45.3 pS (at negative potentials), 80.0 pS (at positive potentials)	5	34.8 pS (at –60 mV)	15	35.4 pS (at negative potentials), 76.7 pS (at positive potentials)
Ionic selectivity	Nonselective cation channel PCa/PNa = 1.48	5, 8	Nonselective cation channel PCa/PNa = 1.28	16	Nonselective cation channel PCa/PNa = 9.6

[a]But see Ref. 16. DRG, dorsal root ganglion; RTX, resiniferatoxin.

Table 2. Electrophysiological and pharmacological properties of native and artificially expressed proton-activated ion channels

	Sustained proton-activated current[8,23]	Transient proton-activated current[8,37,38,42]	ASIC (Ref. 40)	ASIC/MDEG1 (Ref. 43)	DRASIC (Ref. 41)	DRASIC/MDEG2 (Ref. 44)
Tissue distribution	Rat DRG neurones	DRG and trigeminal neurones	DRG neurones, wide expression in the brain	Wide expression in the brain	DRG neurones, absent in the brain	DRG neurones
Activators	pH ≤6.2	pH ≤7.0	pH <6.9; half maximal at pH 6.2	pH ≤6.0; half maximal at pH 4.8	Sustained current: pH ≤4.0; transient current: pH ≤7.0	Sustained current: pH ≤5.0; transient current: pH ≤7.0
Single-channel conductance	n.d.	25 pS	14.3 pS	10 pS	12.6 pS	n.d.
Ionic selectivity	Nonselective cation channel PCa/PNa = 0.55	Na^+ channel PCa/PNa <0.13; PCa/PNa = 0.31	Na^+ channel PCa/PNa = 0.4 PNa/PK = 13	Na^+ channel $Na^+ \approx Li^+ > K^+ > Ca^{2+}$ PCa/PNa = 0.03	Na^+ channel PNa/PK = 11.5	Transient phase: Na^+ channel; sustained phase: nonselective cation channel PCa/PNa not known
Kinetics	Slow activation and inactivation	Fast activation and inactivation	Fast activation and inactivation	Intermediate ($t_{inact} \approx 1$ s)	Two phases, transient and sustained	Two phases, transient and sustained

DRG, dorsal root ganglion; n.d., not determined.

and is often accompanied by pain[3]. Although, in general, a decrease in the extracellular pH reduces neuronal excitability[21], nociceptors represent an exception as they usually show ongoing spike activity when exposed to an acidic environment[22]. The membrane current underlying this excitation was first described by Bevan and Yeats[23]. It exhibits slow activation and inactivation upon extracellular acidification to pH <6.3 and is nonselective for cations. It has therefore been termed 'sustained proton-activated current' to distinguish it from the more widely expressed transient proton-activated current (see below). There is considerable evidence suggesting that the sustained current plays an important role in nociceptive signal transduction. It probably mediates the persistent pain sensation during tissue acidification. Similarities of capsaicin- and sustained proton-activated currents have led to the suggestion that low extracellular pH might function as one endogenous activator of capsaicin-sensitive ion channels[2,24].

A second 'hot' candidate is noxious heat, which has only recently been discovered as an activator of cation currents in DRG neurones[15–18]. About half of the small- but not the large-diameter DRG neurones respond to temperatures above ≈42°C with the specific activation of a cationic membrane conductance, probably due to the opening of ion channels[15]. The threshold of activation is similar to that of heat-sensitive C-fibres, which become activated at temperatures beyond 42°C (e.g. Ref. 25). It appears likely that the channel underlying the current is at least one important signal transduction mechanism of heat sensation.

A major breakthrough in understanding the molecular mode of action of capsaicin and in the search for its physiological activators has come from Julius and colleagues[26,27]. Using an expression cloning assay, which took advantage of the high calcium permeability of the capsaicin receptor channels, this group has cloned an ion channel protein that binds capsaicin and RTX. This clone has been termed VR-1 (for vanilloid receptor 1). When artificially expressed in HEK293 cells or *Xenopus* oocytes, VR-1 responds to capsaicin with a cation current very similar to that of native capsaicin receptors. The tissue distribution of VR-1 as well as its electrophysiological and pharmacological properties leave little doubt that VR-1 corresponds to the native capsaicin receptors expressed in DRG neurones (Table 1). However, the existence of different subtypes of capsaicin receptors has been proposed on the basis of pharmacological binding studies. A C-type receptor expressed in DRG neurones and possibly also in mast cell lines[28] has been suggested to be coupled to $^{45}Ca^{2+}$ uptake through capsaicin-activated ion channels and an R-type receptor was detectable by RTX binding[11,29]. Although these findings and also some electrophysiologcial studies[12,30,31] suggest the existence of more than one capsaicin receptor, the exact nature of those findings has yet to be investigated.

VR-1-transfected *Xenopus* oocytes and HEK293 cells have been used to screen for possible physiological activators. In these cells membrane currents could not only be elicited by capsaicin, but also by noxious heat (>40°C)[26] and low extracellular pH (Ref. 27), while other algogenic stimuli including ATP, 5-HT, acetylcholine, bradykinin, substance P, histamine and glutamate were ineffective[26]. These findings suggest a common signal-transduction mechanism for capsaicin-, acidosis- and heat-sensation. This hypothesis fits the experience that exposure to heat, acids or capsaicin leads to a burning

Box 1. Structure of the capsaicin receptor VR-1 and of proton-gated ion channels

VR-1 c-DNA encodes for a protein of 838 amino acids with six proposed transmembrane regions (Fig. I)[1]. Analysis of the relationship of VR-1 to other cloned receptor channels has revealed that VR-1 is quite unrelated to other ligand-gated channels. The most closely related channel proteins belong to the only recently identified class of store-operated ion channels[2], which permit the so called Ca^{2+} release activated Ca^{2+} current (I_{CRAC}). Despite the structural homology of VR-1 and store-operated channels, VR-1 is not activated by store depletion.

ASIC (Ref. 3) c-DNA codes for a protein of 526 amino acids, which forms a proton-gated ion channel when expressed in *Xenopus* oocytes. ASIC and DRASIC[4], a related protein, belong to the family of amiloride-sensitive Na^+ channels/degenerins. The term degenerin refers to the fact that in *Caenorhabditis elegans* members of this ion channel family cause neurodegeneration when a certain amino acid [e.g. Ala704 in MEC-4 (Ref. 5)] is mutated. The physiological role of this protein is not yet entirely clear, but the non-mutated proteins may be part of a mechanosensor in *C. elegans*[6]. Several human analogues of these ion channels have been cloned recently[7,8]. Hydrophobicity plots suggest that ASIC and DRASIC contain two transmembrane domains. This topology has been experimentally proven for MEC-4 (Ref. 9), a degenerin in *C. elegans*, and for α-NaCh (Ref. 10), an amiloride-sensitive ion channel, which are both closely related to ASIC and DRASIC. MDEG1 (Ref. 11) and MDEG2, a splice variant of MDEG1, are other mammalian analogues of *C. elegans* neurodegenerines. Like ASIC, MDEG1 forms a proton-activated cation channel, and like the neurodegenerines in *C. elegans*, MDEG1 becomes constitutively active when a certain amino acid is mutated. Its splice variant MDEG2 (Ref. 12) is not activated by protons but might play an important role as an accessory subunit of ion channels containing DRASIC or MDEG1 (Ref. 12).

References

1 Caterina, M. J. *et al.* (1997) *Nature* 389, 816–824
2 Parekh, A. B. and Penner, R. (1997) *Physiol. Rev.* 77, 901–930
3 Waldmann, R., Champigny, G., Bassilana, F., Heurteaux, C. and Lazdunski, M. (1997) *Nature* 386, 173–177
4 Waldmann, R. *et al.* (1997) *J. Biol. Chem.* 272, 20975–20978
5 Driscoll, M. and Chalfie, M. (1991) *Nature* 349, 588–593
6 Hong, K. and Driscoll, M. (1994) *Nature* 367, 470–473
7 Garcia-Anoveros, J., Derfler, B., Neville-Golden, J., Hyman, B. T. and Corey, D. P. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 1459–1464
8 Price, M. P., Snyder, P. M. and Welsh, M. J. (1996) *J. Biol. Chem.* 271, 7879–7882
9 Lai, C. C. *et al.* (1996) *J. Cell Biol.* 133, 1071–1081
10 Renard, S., Lingueglia, E., Voilley, N., Lazdunski, M. and Barbry, P. (1994) *J. Biol. Chem.* 69, 12981–12986
11 Bassilana, F. *et al.* (1997) *J. Biol. Chem.* 272, 28819–28822
12 Lingueglia, E. *et al.* (1997) *J. Biol. Chem.* 272, 29779–29783
13 Reichling, D. B. and Levine, J. D. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 7006–7011
14 Waldmann, R. and Lazdunski, M. (1998) *Curr. Opin. Neurobiol.* 8, 418–424

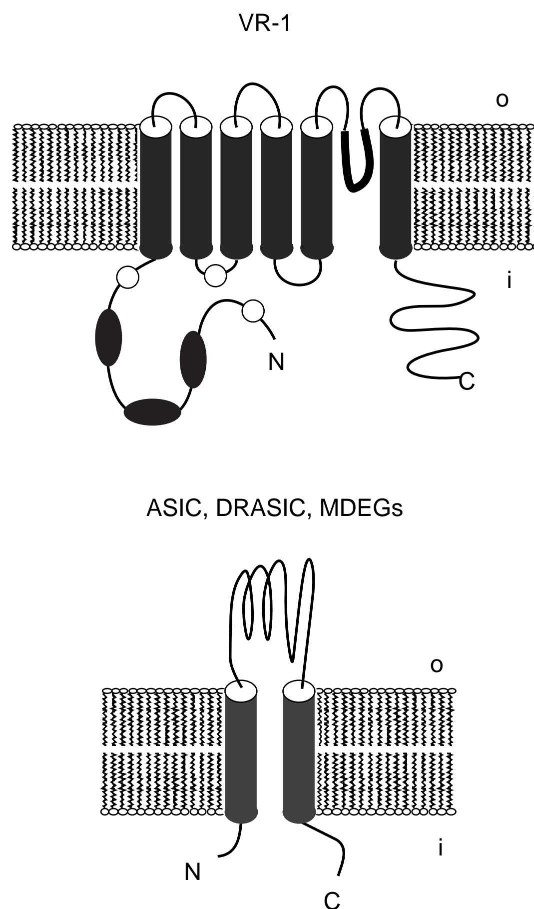

Fig. I. Proposed transmembrane topology of the VR-1 (capsaicin) receptor and of ASIC, a proton-gated ion channel. VR-1 encodes for a protein with seven putative transmembrane domains (TMs), whereas members of the proton-gated ion channel family possess only two TMs (Refs 13, 14). Open circles indicate the conserved site for protein kinase A-dependent phosphorylation of VR-1. Filled ovals indicate ankyrin repeat domains. Inner and outer membrane leaflets are depicted by i and o, respectively. ASIC, acid-sensing ion channel; DRASIC, dorsal-root-specific acid-sensing ion channel; MDEG, mammalian degenerin.

('hot') sensation. Interestingly, low extracellular pH sensitized the VR-1 receptor not only to capsaicin, a phenomenon well known from native capsaicin receptors in DRG neurones[32,33], but also to heat[27]. At a pH of 6.3, significant current activation could be detected at temperatures as low as 35°C (Ref. 27). Thus, low extracellular pH and increased temperature, two conditions frequently found in inflamed tissue, appear to induce a mutual sensitization and thereby favour the activation of capsaicin receptors[27]. This further supports the idea that capsaicin receptors are permanently activated in inflamed tissue and provides a rational basis for the observation that inflammatory pain is significantly relieved by moderate local cooling[34].

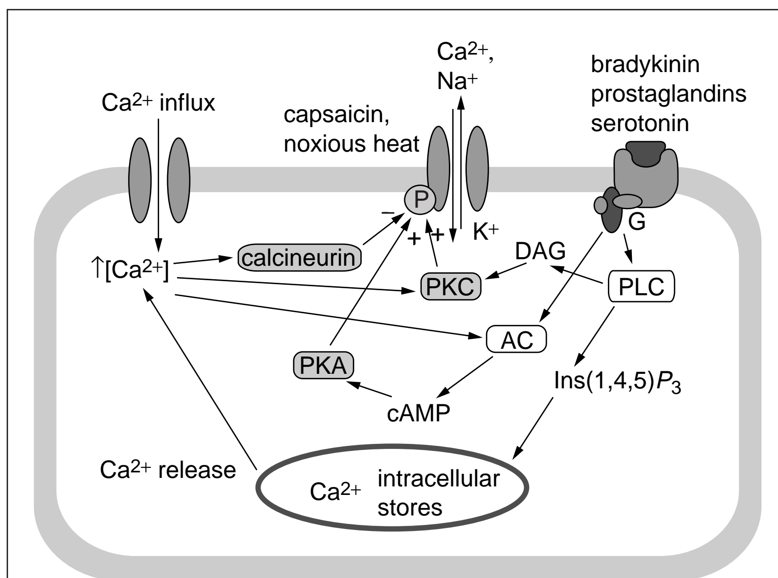

Fig. 2. Schematic diagram of possible modulatory pathways affecting heat sensation. Inflammatory mediators interact with receptors coupled to G proteins and activation of phospholipase C (PLC) or adenylate cyclase (AC). Activation of these receptors is followed by the formation of the second messengers diacyl glycerol (DAG) or cAMP. These activate protein kinase A (PKA) or protein kinase C (PKC) which may phosphorylate capsaicin- or heat-activated ion channels to increase their conductance or open probability. Both pathways can alternatively be activated by increases in intracellular Ca^{2+}, resulting either from Ca^{2+} influx or release. Ca^{2+} ions activate the phosphatase calcineurin, which can dephosphorylate and desensitize the channel. Whether facilitatory or inhibitory actions predominate could depend on the magnitude and the local distribution of intracellular Ca^{2+} signals.

Blockers of capsaicin receptors and potential indications

The idea that the capsaicin receptor functions as an 'integrator of multiple pain-producing stimuli'[27] implies that capsaicin receptor antagonists or channel blockers should have profound antinociceptive effects, especially in inflammatory pain models. To date, however, only very few such compounds are available. Capsaicin-activated membrane currents are inhibited by the rather non-specific cation-channel blocker ruthenium red[35] and by the competitive capsaicin receptor antagonist capsazepine[13]. Both compounds exerted antinociceptive effects in a behavioural study[36], but convincing data on analgesic actions is still lacking. Because capsazepine is a competitive antagonist at the capsaicin binding site[13], it does not necessarily interfere with physiological receptor activation. Assuming that VR-1 *in vivo* serves the transduction of noxious heat and tissue acidosis, potential new antagonists should rather inhibit receptor activation by heat or protons than compete for the capsaicin binding site.

Proton-activated ion channels

There is now considerable evidence that protons are activators of both native and artificially expressed capsaicin receptors, but these receptors are certainly not the only ion channels activated by protons. Ionic currents activated by acidic solutions have been described in a variety of central and peripheral neurones[21]. In rat DRG neurones, protons activate at least two current types with different kinetics, pH dependence of activation and inactivation, and ionic selectivities[8,23]. The first (transient) type is a rather Na^+-selective ion channel that exhibits fast activation and inactivation upon acidification to pH <7.0 and is widely expressed throughout the rat nervous system[37,38]. The physiological function of the transient proton-activated current is not yet known. The other (sustained) type is selectively expressed in nociceptive neurones and often co-expressed with capsaicin sensitivity[2,23]. This current type is in many respects similar to the current activated by capsaicin (see above) and many groups favour the idea that this current mediates the sustained pain sensation during tissue acidification[2,22,23,39]. As outlined above, recent results indicate that capsaicin receptor channels can generate sustained proton-induced currents[27], but there is also considerable evidence suggesting that other ion channels contribute. Currents elicited by the two activators differ significantly in their Ca^{2+} ion conductance[8]. In single-channel recordings, acidic solutions failed to activate single-channel activity in membrane patches which clearly responded to capsaicin[5]. In single-fibre recording experiments, similar percentages of nociceptive nerve fibres are activated by capsaicin and protons, but some fibres are activated only by one agent[22].

Molecular cloning of proton-activated ion channels

Recently, Michel Lazdunski and colleagues have identified several genes encoding for ion channels which can be activated by a drop in extracellular pH. ASIC (Ref. 40) (for acid sensing ion channel) and DRASIC (Ref. 41) (for dorsal root specific acid sensing ion channel) were the first such ion channel proteins to be discovered (see Box and Table 2; Refs 8, 23, 37, 38, 40–44). Meanwhile, a great variety of related subunits and splice variants has been identified, which differ in their tissue distribution and in the properties of the ionic currents they generate. In addition, co-expression of these different subunits results in an even greater variety of currents[45]. Of those, ASIC appears to correspond to the transient proton-activated current, which is unlikely to mediate sustained nociceptor activation. Ion channel heteromers containing DRASIC and MDEG2 (Ref. 43) (see Box 1) are a closer fit to the proton-induced sustained currents in nociceptive DRG neurones (Table 2). However, none of the subunit combinations characterized so far matches the properties of native proton-activated currents exactly.

Blockers of proton-activated currents

Although all these channels are structurally related to amiloride-sensitive ion channels[45], amiloride neither blocks the sustained current component through DRASIC channels[41] nor does it inhibit proton-induced nociceptor discharges[39]. It is therefore not surprising that amiloride has not been reported to exert analgesic effects. At present, no single specific antagonist of proton-activated currents is available and functional expression

of these channels in DRG neurones remains to be demonstrated. However, if it turns out that heteromeric channels containing members of this family of ion channels are sensors of noxious tissue acidosis, blockers might have similar potential indications as capsaicin receptor antagonists or channnel blockers for the treatment of inflammatory pain.

Sensitization of nociceptors

The treatment and prevention of chronic pain syndromes where pain often persists despite the fact that the excitatory stimulus has long disappeared, is an even greater challenge than treating acute pain. Chronic pain syndromes are often caused by increases in the sensitivity to painful stimuli. They can occur as a consequence of ongoing nociceptor activation, for example, during inflammatory diseases, which are often accompanied by profound mechanical and heat hyperalgesia[3]. Mechanical hyperalgesia is commonly accepted to occur as a consequence of activity-dependent facilitation at central synapses. Prevention of central plastic changes in the nociceptive system has attracted much interest and created the idea of preemptive analgesia[46], but there is also growing evidence for similar processes in the peripheral nociceptive system. For example, sensitization to heat is preserved in isolated organs, e.g. skin–nerve preparations[47], where the nociceptive afferent is disconnected from the CNS. Prevention of such a sensitization might be another potential approach to pain therapy.

Like most neuronal receptors and ion channels, the nociceptor-specific receptors not only mediate excitation and acute pain, but appear also be subject to modulatory processes, which could underlie activity-dependent increases in the sensitivity to painful stimuli and chronic pain. Of the many possible intracellular signalling cascades involved in sensitization of nociceptors, most support is currently available for the cAMP/protein kinase A (PKA) system. The amino acid sequence of VR-1, which probably serves as a heat and acid sensor in nociceptors, contains three conserved sites for PKA-dependent phosphorylation (Fig. 2)[26]. Several lines of evidence support the idea of PKA-mediated facilitation of nociceptor responses to heat. (1) Mice carrying a null mutation for the neurone-specific isoform of the type I regulatory subunit (RIβ) of PKA show an unaltered acute pain processing, but clearly reduced PGE_2-induced heat hyperalgesia, which is regularly observed in wild-type mice[48]. (2) Exposure to membrane permeant and stable analogues of cAMP induces heat sensitization in nociceptors[49–51]. (3) Forskolin-induced heat sensitization can be prevented using non-selective PKA inhibitors[52]. A similar role of the cAMP/PKA cascade has been proposed for the PGE_2-induced enhancement of capsaicin-induced membrane currents[53,54], which fits nicely the hypothesis that VR-1 mediates both capsaicin and heat sensitivity. Besides PKA, there is also evidence for a role of PKC in facilitation of heat responses. In several studies, bradykinin has induced strong heat sensitization[15,47,55] via B_2 receptors, a phenomenon which can be attributed to PKC activation[15]. The involvement of phosphorylation in the sensitization process to heat and capsaicin is further supported by the observation that the calcium-dependent phosphatase calcineurin mediates desensitization of capsaicin-activated currents[56,57]. This desensitization is prevented when calcineurin is blocked by a complex of cyclosporin A and cyclophillin[56]. Together, these findings suggest that the phosphorylation status regulates the open probability of capsaicin receptor/VR-1 channels.

Another intriguing aspect of intracellular signalling is that multiple pain producing extracellular stimuli might converge onto only a few intracellular signalling cascades. Inhibitors of such processes might therefore cover a relatively wide range of pain conditions of different origin. Compared to the membrane receptors our knowledge of the intracellular signal transduction pathways that lead to hyperalgesia is rather limited. However, these intracellular cascades may in the future become promising targets for pain therapy provided that nociceptor-specific isoforms of the enzymes exist and that chemical agents selectively affecting these enzymes are found.

Concluding remarks

The molecular cloning of membrane receptors for capsaicin, protons and heat has already greatly advanced our knowledge of nociceptive signal transduction that is at the very beginning of a sequence of events that finally initiate pain sensation. It is clear that these findings will have great impact on the search for new analgesic drugs with an entirely new mode of action and an unprecedented selectivity for nociceptors. Pain, however, is a heterogeneous clinical condition and it is at present not entirely clear which forms of pain can be treated with such compounds, or, whether the block of a single receptor or enzyme is sufficient to treat complex pain states.

Selected references

1 Sherrington, C. S. (1908) *The Integrative Actions of the Nervous System*, Archibald Constable
2 Bevan, S. and Geppetti, P. (1994) *Trends Neurosci.* 17, 509–512
3 Kress, M. and Reeh, P. W. (1996) in *Neurobiology of Nociceptors* (Cervero, F. and Belmonte, C., eds), pp. 258–297, Oxford University Press
4 Szolcsányi, J. (1984) in *Antidromic Vasodilatation and Neurogenic Inflammation* (Chahl, L. A., Szolcsányi, J. and Lembeck, F., eds), pp. 7–25, Hungarian Acad. Sci.
5 Oh, U., Hwang, S. W. and Kim, D. (1996) *J. Neurosci.* 16, 1659–1667
6 Vlachova, V. and Vyklicky, L. (1993) *Physiol. Res.* 42, 301–311
7 Baumann, T. K., Burchiel, K. J., Ingram, S. L. and Martenson, M. E. (1996) *Pain* 65, 31–38
8 Zeilhofer, H. U., Kress, M. and Swandulla, D. (1997) *J. Physiol.* 503, 67–78
9 Baccaglini, P. I. and Hogan, P. G. (1983) *Proc. Natl. Acad. Sci. U. S. A.* 80, 594–598
10 Szallasi, A. *et al.* (1995) *Brain Res.* 703, 175–183
11 Acs, G., Biro, T., Acs, P., Modarres, S. and Blumberg, P. M. (1997) *J. Neurosci.* 17, 5622–5628
12 Liu, L. and Simon, S. A. (1996) *J. Neurophysiol.* 76, 1858–1869
13 Bevan, S. *et al.* (1992) *Br. J. Pharmacol.* 107, 544–552
14 Dray, A., Forbes, C. A. and Burgess, G. M. (1990) *Neurosci. Lett.* 110, 52–59
15 Cesare, P. and McNaughton, P. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 15435–15439
16 Reichling, D. B. and Levine, J. D. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 7006–7011

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17 Kirschstein, T., Busselberg, D. and Treede, R. D. (1997) *Neurosci. Lett.* 231, 33–36
18 Kirschstein, T., Greffrath, W., Buesselberg, D. and Treede, R-D. *J. Physiol.* (in press)
19 Szallasi, A. and Blumberg, P. M. (1989) *Neuroscience* 30, 515–520
20 Szallasi, A. (1994) *Life Sci.* 25, 223–243
21 Akaike, N. and Shinya, U. (1994) *Prog. Neurobiol.* 43, 73–83
22 Steen, K. H. *et al.* (1992) *J. Neurosci.* 12, 86–95
23 Bevan, S. and Yeats, J. (1991) *J. Physiol.* 433, 145–161
24 Liu, L. and Simon, S. A. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 738–741
25 Beck, P. W. and Handwerker, H. O. (1974) *Pflügers Arch. Eur. J. Physiol.* 347, 209–222
26 Caterina, M. J. *et al.* (1997) *Nature* 389, 816–824
27 Tominaga, M. *et al.* (1998) *Neuron* 21, 531–543
28 Bíró, T. *et al.* (1998) *Blood* 91, 1332–1340
29 Acs, G., Lee, J., Marquez, V. E. and Blumberg, P. M. (1996) *Mol. Brain Res.* 35, 173–182
30 Liu, L., Szallasi, A. and Simon, S. A. (1998) *Neurosci.* 84, 569–581
31 Petersen, M., LaMotte, R., Klusch, A. and Kniffki, K. D. (1996) *Neurosci.* 75, 495–505
32 Petersen, M. and LaMotte, R. H. (1993) *Pain* 54, 37–42
33 Kress, M. *et al.* (1996) *Neurosci. Lett.* 211, 5–8
34 Brandner, B. *et al.* (1996) *Anaesthesia* 51, 1021–1025
35 Wood, J. N. *et al.* (1988) *J. Neurosci.* 8, 3208–3220
36 Santos, A. R. and Calixto, J. B. (1997) *Neurosci. Lett.* 235, 73–76
37 Krishtal, O. A. and Pidoplichko, V. I. (1980) *Neuroscience* 5, 2325–2327
38 Konnerth, A., Lux, H. D. and Morad, M. (1987) *J. Physiol.* 386, 603–633
39 Reeh, P. W. and Steen, K. H. (1996) *Prog. Brain Res.* 113, 143–151
40 Waldmann, R., Champigny, G., Bassilana, F., Heurteaux, C. and Lazdunski, M. (1997) *Nature* 386, 173–177
41 Waldmann, R. *et al.* (1997) *J. Biol. Chem.* 272, 20975–20978
42 Kovalchuk, Y. N., Krishtal, O. A. and Nowycky, M. C. (1990) *Neurosci. Lett.* 31, 237–242
43 Lingueglia, E. *et al.* (1997) *J. Biol. Chem.* 272, 29779–29783
44 Bassilana, F. *et al.* (1997) *J. Biol. Chem.* 272, 28819–28822
45 Waldmann, R. and Lazdunski, M. (1998) *Curr. Opin. Neurobiol.* 8, 418–424
46 Katz, J. *et al.* (1992) *Anesthesiology* 77, 439–446
47 Lang, E. *et al.* (1990) *J. Neurophysiol.* 63, 887–901
48 Malmberg, A. B. *et al.* (1997) *J. Neurosci.* 17, 7462–7470
49 Ferreira, S. H. and Nakamura, M. (1979) *Prostaglandins* 18, 179–190
50 Kress, M., Rödl, J. and Reeh, P. W. (1996) *Neurosci.* 74, 609–617
51 Mizumura, K., Koda, H. and Kumazawa, T. (1993) *Neurosci. Lett.* 162, 75–77
52 Lynn, B. and O´Shea, N. R. (1998) *Brain Res.* 780, 360–362
53 Lopshire, J. C. and Nicol, G. (1998) *J. Neurosci.* 18, 6081–6092
54 Pitchford, S. and Levine, J. D. (1991) *Neursci. Lett.* 132, 105–108
55 Koltzenburg, M., Kress, M. and Reeh, P. W. (1992) *Neuroscience* 46, 465–473
56 Docherty, R. J. *et al.* (1996) *Pflügers Arch. Eur. J. Physiol.* 431, 828–837
57 Koplas, P. A., Rosenberg, R. L. and Oxford, G. S. (1997) *J. Neurosci.* 17, 3525–3537

Chimaeric Gα proteins: their potential use in drug discovery

Graeme Milligan and Stephen Rees

Approaches that allow ligand occupancy of a wide range of G protein-coupled receptors to be converted into robust assays amenable to relatively high-throughput analysis are ideal for screening for novel ligands at this class of receptor. Many attempts have been made to design universal ligand-screening systems such that any GPCR can be screened using a common assay end-point. Manipulation of the G protein within the assay system offers the possibility of achieving this. To better understand the domains involved in the interactions between G protein-coupled receptors, G proteins and effector polypeptides and the fine details of these contacts, a wide range of chimaeric G protein α subunits have been produced. **Graeme Milligan and Stephen Rees** discuss the information generated by such studies and the ways in which such chimaeric G proteins can be integrated into assay systems for drug discovery.

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The heterotrimeric G proteins transduce information from the family of seven-transmembrane-domain G protein-coupled receptors (GPCRs) to effector polypeptides; these are either ion channels or enzymes that function to regulate levels of intracellular second messengers[1–3]. Knowledge of the GPCR family continues to grow as novel family members are regularly identified by the examination of DNA and expressed sequence tag (EST) databases for their characteristic sequence and structural features (see http://www.gcrdb.uthscsa.edu/ and related links for information). It is likely that more than 1000 distinct GPCR genes will be encoded by the human genome.

Historically, GPCRs have represented the most important family of target proteins for therapeutic intervention in disease processes with estimates that 30% of clinically prescribed drugs function as either agonists or antagonists at GPCRs (Ref. 4). Because of the burgeoning size of the family, the fact that the natural ligands for many GPCRs remain unknown, and that ligands for GPCRs do not have to enter the cell to function, it is an inescapable fact that the GPCRs will remain major targets for drug discovery for the foreseeable future[4].

G proteins

The family of heterotrimeric G proteins is potentially large. At least 16 distinct genes encode G protein α subunits in mammalian systems with splice variation from at least two genes, which further increases the range of mature proteins. Five distinct β-subunit genes and 12 γ-subunit genes have also been identified[5,6]. As β and γ polypeptides form high-affinity non-dissociating complexes, many (but not all) combinations of which are allowed, there is, at least in theory, a large number of distinct βγ combinations that can be formed[5,6]. Both G protein α subunit and βγ complexes have the capacity to

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