Published in final edited form as:
Trends Pharmacol Sci. 2009 February ; 30(2): 79–84. doi:10.1016/j.tips.2008.10.008.

Role of ionotropic cannabinoid receptors in peripheral antinociception and antihyperalgesia

Armen N. Akopian[1], **Nikita B. Ruparel**[2], **Nathaniel A. Jeske**[3], **Amol Patwardhan**[1], and **Kenneth M. Hargreaves**[1,2,4]

[1] Department of Endodontics, University of Texas Health Science Center at San Antonio, TX 78229, USA

[2] Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, TX 78229, USA

[3] Department of Oral and Maxillofacial Surgery, University of Texas Health Science Center at San Antonio, TX 78229, USA

[4] Department of Pharmacology, University of Texas Health Science Center at San Antonio, TX 78229, USA

Abstract

Despite the wealth of information on cannabinoid-induced peripheral antihyperalgesic and antinociceptive effects in many pain models, the molecular mechanism(s) for these actions remains unknown. Although metabotropic cannabinoid receptors have important roles in many pharmacological actions of cannabinoids, recent studies have led to the recognition of a family of at least five ionotropic cannabinoid receptors (ICRs). The known ICRs are members of the family of transient receptor potential (TRP) channels and include TRPV1, TRPV2, TRPV4, TRPM8 and TRPA1. Cannabinoid activation of ICRs can result in desensitization of the TRPA1 and TRPV1 channel activities, inhibition of nociceptors and antihyperalgesia and antinociception in certain pain models. Thus, cannabinoids activate both metabotropic and ionotropic mechanisms to produce peripheral analgesic effects. Here, we provide an overview of the pharmacology of TRP channels as ICRs.

Introduction

Although cannabinoids have been used for millennia for treating pain and other symptoms, their mechanisms of action remain obscure. With the heralded identification of multiple G-protein-coupled receptors (GPCRs) mediating cannabinoid effects nearly two decades ago, the mystery of cannabinoid pharmacology was thought to be solved [1,2]. However, continued studies demonstrate that many cannabinoid effects cannot be attributed solely to the CB_1 and CB_2 metabotropic GPCRs. For example, several important cannabinoid actions, such as peripherally mediated antihyperalgesia (see Glossary), persist in CB_1- and CB_2-gene knockout animals [3,4]. In addition, the dual generation of both neuroprotective and neurotoxic effects of cannabinoids [5,6] cannot be explained by the presence of known metabotropic cannabinoid

Corresponding author: Akopian, A.N. (akopian@UTHSCSA.edu)..

receptors (MCRs). For example, the CB_1 antagonist rimonabat is neuroprotective in a model of cerebral ischemia, and these effects are independent of actions on MCRs [6]. These and other findings have led to the suggestion of a 'CBx' – an unknown cannabinoid receptor [7]. Here, we focus on the role of transient receptor potential (TRP) channels serving as ionotropic cannabinoid receptors (ICRs) and contributing to the pharmacology of cannabinoids, with an emphasis on their key role in the pain system.

The detection of tissue injury is an essential function of somatosensation and is mediated primarily by a specialized class of peripheral nociceptive afferent neurons. Although tissue injury is detected via peripheral nociceptive neurons, the actual perception of pain occurs in the central nervous system (CNS) and is subject to both stimulatory and inhibitory central modulation [8]. Peripheral nociceptive neurons are classically organized by morphological attributes or conduction velocity properties, although contemporary research has focused on their expression of receptors and ion channels as a functional type of classification [9,10]. The growing recognition of receptors expressed on nociceptors has prompted research on peripheral mechanisms regulating their activity. For example, peripheral administration of cannabinoids into inflamed tissue inhibits nociceptor activity and produces a peripherally mediated antihyperalgesia [11]. Additional analgesic mechanisms are engaged after central administration of cannabinoids. Thus, drugs can modulate peripheral or central targets to produce peripherally or centrally mediated analgesia.

The classical cannabinoid receptors: metabotropic receptors: metabotropic receptors

The first family of cannabinoid receptors identified were the metabotropic GPCRs including CB_1, CB_2, GPR55 and possibly GPR119 and peroxisome-proliferator-activated receptors (PPARs) [1,2,7,12]. The expression pattern for CB_1 (throughout the CNS and heart, gastrointestinal tract, kidney, spleen, liver, lung, testis, uterus and muscle), CB_2 (primarily immune cells and some neurons) and GPR55 (adrenal tissue, ileum, jejunum, frontal cortex and striatum) is broad, implicating a complex modulation of multiple physiologic systems by cannabinoids [13,14]. These MCRs signal primarily via $G_{i/o}$-related pathways, although coupling to G_s and G_q signaling pathways have been reported under certain experimental conditions [15]. Thus, agonist-directed trafficking is observed with these receptors as with other GPCRs. Activation of the MCRs leads to generation of the cannabinoid tetrad of behavior in addition to many other of the classical effects attributed to cannabinoid pharmacology [16]. In general, two approaches have been used experimentally to implicate an observed effect with the MCRs. First, pretreatment with a cannabinoid antagonist is predicted to block the observed effect. However, many of these compounds have inverse agonist activities that complicate interpretation, and other compounds such as AM251 might have known antagonist actions against CB_1 with only recently recognized actions against other receptors (i.e. antagonist or inverse agonist actions at GPR55) [7,14]. A second approach is the use of genetic knockout animals. Interestingly, the knockout studies implicate the existence of non-CB_1 and/or non-CB_2 cannabinoid receptors [17]. It is possible that these effects might be attributed to additional GPCRs activated by cannabinoids. However, the finding that certain cannabinoid actions persist after pretreatment with either pertussis toxin or GDPβS compounds capable of blocking GPCR functions provides strong evidence of a second family of non-MCRs [18,19]. In addition, parallel studies indicate that application of cannabinoids generates a slow inward current in the TRPV1 and TRPA1 ionotropic receptors [20,21]. Taken together, these studies have expanded the scope of cannabinoid research to focus on ionotropic receptor systems.

ICRs regulate sensory neuron activities

Emerging data from several studies has led to the recognition of a second family of cannabinoid receptors, namely the ICRs. The ICRs are either activated or antagonized by cannabinoids in a variety of cell types by nM–μM concentrations of these compounds (Table 1). To date, the known ICRs belong to the TRP family of channels. The TRP channels are a broad family of ligand-gated ion channels that generate an inward flow of cations upon activation. Studies reported to date indicate that cannabinoids gate at least five distinct ICRs (Table 1).

The majority of ICRs are expressed in nociceptive sensory neurons, which can detect and respond to noxious mechanical, thermal and chemical stimuli [22]. Therefore, it could be predicted that activation of sensory neurons, by cannabinoid gating of inward currents generated by these ICRs, could result in nociception and, ultimately, pain perception [23–25]. However, a vast majority of behavioral studies indicate that cannabinoids do not produce nociception [19,23,26] but instead induce a peripherally mediated and efficacious antihyperalgesia and antinociception [27–29]. The interpretation of these data is complex because cannabinoids activate ionotropic receptors and generate inward currents but still produce a profound antihyperalgesia. One possible hypothesis addressing this issue is that partial activation of ICRs does not necessarily generate excitation (i.e. action potential) of nociceptors. From this perspective, it is interesting to note that cannabinoids are not full agonists for TRP channels [4,23,30]. Indeed, cannabinoids typically evoke a slow generation of small inward currents and Ca^{2+} accumulation [4,19,20,30] (Table 1). As a result, cannabinoid-gated responses might not reach the threshold levels required to excite nociceptors. Moreover, slow depolarization of nociceptor membrane potentials might lead to inactivation of voltage-gated channels that, in turn, inhibits the generation of action potentials [31].

Because cannabinoids can trigger peripherally mediated antihyperalgesia and antinociception, it is important to address whether their modulation of ICRs regulates peripheral nociceptor activity. Application of cannabinoids elevates internal Ca^{2+} ($[Ca^{2+}]_i$) levels in nociceptors because known ICRs (TRP channels) are permeable to Ca^{2+} (Table 1). An elevation of $[Ca^{2+}]_i$ can ignite numerous cellular cascades, including induction of Ca_{2+}-dependent kinases, phosphatases and phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) [32–35]. It is well established that these enzymes and lipids can be effective modulators of activities of the TRPV1 and TRPA1 channels [33,34,36–39], which have crucial roles in regulating nociception [40–43]. Thus, one hypothesis is that certain cannabinoids regulate nociceptors by activating Ca^{2+}-permeable channels. As detailed later, the remarkable outcome of this activation leads to a desensitization of nociceptor activities. Recent studies have indeed demonstrated that cannabinoids that selectively activate TRPV1 (e.g. arachidonoylchloro-ethanolamide [ACEA]) or TRPA1 (e.g. WIN55212 or AM1241) induce a homologous and cross-desensitization (i.e. the desensitization of one channel after activation of another channel) of the activities of both of these TRP channels [4,19,20], ultimately resulting in peripherally mediated antihyperalgesia [4,19]. The simultaneous desensitization of multiple TRP channels might lead to a broader inhibition of nociceptor responsiveness than that observed by inhibition of only one channel and, thus, represents a potentially novel approach for the generation of analgesic compounds. Studies conducted in cell expression systems indicate that TRPV1-selective cannabinoids can only desensitize TRPA1 when TRPV1 is co-expressed. Similarly, TRPA1-selective cannabinoids desensitize TRPV1 only under conditions in which TRPA1 is present [4]. Thus, the cross-desensitization produced by these TRP-selective cannabinoids requires the co-expression of both TRPV1 and TRPA1.

To understand how activation of ICRs leads to inhibition of nociceptors, molecular mechanisms of desensitization of TRP channels by ICR-activating cannabinoids were

investigated. The results indicate that cross-desensitization between the TRPA1 and TRPV1 channels in sensory neurons seems to involve multiple separate mechanisms.

First, TRPV1 is desensitized by TRPV1- and TRPA1-activating cannabinoids via Ca^{2+}-dependent calcineurin (phosphatase 2B)-induced dephosphorylation of the channel [19,20] (Figure 1). A similar mechanism is employed in the desensitization of TRPV1 by the TRPA1-specific agonist mustard oil (MO) [33,44]. Interestingly, Ca^{2+}-induced PtdIns(4,5)P_2 biosynthesis, a mechanism involved in tachyphylaxis of capsaicin responses in heterologous expression systems [34,38], does not play a part in desensitization of TRPA1 by activation of TRPV1 or in cross-desensitization of TRPV1 by activation of the TRPA1 channel in sensory neurons [33]. Furthermore, Ca^{2+}-dependent activation of kinases can be responsible for recovery from desensitization [37]. Overall, Ca^{2+}-dependent desensitization prevails over sensitization because calcineurin is more effectively induced by low concentrations of Ca^{2+} than kinases [45]. Thus, calcineurin-mediated dephosphorylation is an important mechanism for cannabinoid desensitization of TRPV1 and compounds that block calcineurin produce a significant inhibition of peripheral cannabinoid antihyperalgesia [18] (Figure 1).

A second potential mechanism for TRPA1 desensitization is via a Ca^{2+}-dependent depletion of PtdIns(4,5)P_2 [33]. However, unlike capsaicin [33,34], tested cannabinoids cannot induce Ca^{2+}-dependent depletion of PtdIns(4,5)P_2, possibly owing to their slow or reduced accumulation of intracellular Ca^{2+} levels [20]. Thus, this mechanism does not seem to be utilized for cannabinoid desensitization of TRP channels.

A third mechanism for desensitization of TRPA1 is via activation of a Ca^{2+}-independent pathway [33,44]. This pathway is evident for TRPA1 desensitization by low concentrations of MO [46] and possibly by a partial agonist for the TRPA1 channel, notably the cannabinoid WIN55212 [4,33]. Altogether, certain cannabinoids are able to inhibit nociception by activating ICRs – TRP channels.

Roles of ICRs in peripheral antihyperalgesia

There is broad agreement that cannabinoids can produce peripherally mediated antihyperalgesic and antinociceptive effects by any of several proposed mechanisms [4,19, 47–49]. Experimental findings support at least four distinct hypotheses (Figure 2). The first hypothesis is that cannabinoids mediate their actions via different metabotropic or ionotropic receptors. Thus, peripherally restricted doses of WIN55212 significantly inhibit capsaicin-induced nocifensive behavior in wild-type animals, and this effect is lost in either TRPV1 or CB_1-gene knockouts but not in mice with genetic deletion of the CB_2 receptor [4]. Thus, cannabinoids might activate multiple receptor mechanisms to produce peripherally mediated antihyperalgesia. The second hypothesis is based upon the observation that cannabinoid receptor systems are differentially activated in various pain models (e.g. inflammatory versus neuropathic versus basal pain thresholds) or by distinct experimental approaches (e.g. local versus systemic versus intrathecal injection). For example, certain immune cells expressing CB_2 might have greater contributions to nociceptor activation in inflammatory pain models compared with neuropathic pain models. In addition, inflammation has been reported to trigger upregulation of the CB_1 receptor in sensory neurons [50]. The third hypothesis is based upon the finding that cannabinoids can modulate sensory neurons in addition to non-neuronal peripheral cells. Thus, the overall cannabinoid effect might be an integration of activity across several peripheral cell types (e.g. nociceptor terminals or keratinocytes [32]). Fourth, it is possible that ICRs and MCRs can functionally cooperate under certain conditions [5,51] because ionotropic and metabotropic receptors are co-expressed in many cells [52].

How important are ionotropic cannabinoid mechanisms in such a diversity of analgesic cannabinoid hypotheses? Interestingly, evidence from knockout animals indicates that both

pathways are equally important in at least some pain models because knockout of either ICR or MCR genes reduces peripherally mediating antihyperalgesic effects of cannabinoids [4]. Several hypotheses could explain these findings. First, ICRs and MCRs operate independently and mediate the actions of cannabinoids on different types of cells. For example, CB_2-mediated antinociception occurs via activation of CB_2 on keratinocytes [53], whereas TRPA1-mediated effects of cannabinoids take place on nociceptors [4]. Thus, the relative role of specific cell types in various pain models could alter the contribution of distinct cannabinoid receptor systems. Second, ICRs and MCRs effectively mediate antihyperalgesia or antinociception in distinct pain models. Third, MCRs could cooperate with ionotropic receptors on cells where both are co-expressed. This could result in either direct inhibition of nociceptors or promotion of Ca^{2+}-dependent release of nociception inhibitory factors (i.e. opioids, endocannabinoids etc) by non-neuronal peripheral cells. When co-expressed, ICRs and MCRs can cooperate in a variety of ways. For example, the activation of CB_1 on mesencephalic dopaminergic neurons can produce intracellular 12(S)-hydroxyeicosatetraenoic acid, which is a TRPV1 agonist [5]. Cannabinoids can also be coupled to $G_{q/11}$ proteins [54,55], and this could lead to activation of the phospholipase C pathway which, in turn, could result in the activation of several TRP channels including TRPA1 [56]. In addition, fatty acid amine hydrolase (FAAH) is involved in anandamide metabolism and is co-localized with TRPV1 in several areas, indicating an important regulatory interaction [57]. Indeed, inhibition of FAAH rapidly leads to accumulation of fatty acid amides, some of which are efficacious TRP agonists [58]. Thus, URB597, a potent and systemically active inhibitor of FAAH, activates TRPA1 channels in addition to peroxisome proliferator-activated receptor-α [58,59].

Conclusion and future perspectives

Despite the wealth of information on cannabinoid-induced peripheral antihyperalgesic and antinociceptive actions in many pain models, the molecular mechanism(s) for these effects remains unknown. Recent investigation in these mechanisms has yielded the hypothesis that activation of ICRs can result in desensitization of the TRPA1 and TRPV1 channel activities, inhibition of nociceptors and antihyperalgesia and antinociception in certain pain models. One important conclusion is that, although cannabinoids differ in their activations of various receptors, they could exert inhibitory effects by acting through ICRs. In addition, the control of intracellular activities by cannabinoids could occur via two very distinct pathways (i.e. metabotropic versus ionotropic), providing multiple mechanisms for triggering antihyperalgesia and antinociception. Thus, it is conceivable that partial TRP-channel-specific agonists could constitute a novel class of peripherally selective analgesics without the typical side effects of conventional cannabinoids.

Nevertheless, many challenges still remain. Among these is the detailed characterization of molecular mechanisms responsible for desensitization of nociceptor-specific TRP channels by ICR-activating cannabinoids, the investigation of the function of ICR in peripheral cells (such as blood and skin cells) that might have roles in the process of nociception, and the evaluation of possible co-operation between MCRs and ICRs in nociceptive neurons in addition to other peripheral cells contributing to nociception.

Acknowledgments

Research was supported by grants DE014928 (A.N.A.), DA19585 and UL1 RR025767 (K.M.H.) and DE016500 (N.A.J.).

Glossary

Activation*	increased functional activity of neurons (e.g. action potential or exocytosis).
Allodynia	occurrence of nociception in response to a non-noxious stimulus.
Antiallodynia	reduction of allodynia.
Antihyperalgesia	reduction of hyperalgesia.
Antinociception	increase in nociceptive thresholds above basal nociceptive levels.
Basal nociceptive thresholds	stimulus intensity capable of evoking an escape response under basal (un-injured) conditions. Usually a thermal or mechanical stimulus.
Hyperalgesia	increased magnitude of nociception in response to a noxious stimulus.
Inflammatory pain models	animal models in which nociception occurs owing to injury of a peripheral tissue.
Inverse agonist	a compound that binds to the same site as an agonist but reverses the constitutive activity of the receptor.
Neuropathic pain models	animal models in which nociception occurs owing to injury to a peripheral nerve.
Nociception	the neural encoding and modulation of noxious stimuli.
Pain	an unpleasant sensory and emotional experience associated with actual or potential tissue damage.
Peripheral nociceptive neurons	specialized class of peripheral afferent neurons that detects and encodes stimuli that induce tissue injury or chemical factors released during tissue injury.
Sensitization	increased responsiveness of neurons.

References

1. Matsuda LA, et al. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 1990;346:561–564. [PubMed: 2165569]
2. Matsuda LA. Molecular aspects of cannabinoid receptors. Crit. Rev. Neurobiol 1997;11:143–166. [PubMed: 9209828]
3. Zimmer A, et al. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB_1 receptor knockout mice. Proc. Natl. Acad. Sci. U. S. A 1999;96:5780–5785. [PubMed: 10318961]
4. Akopian AN, et al. Cannabinoids desensitize capsaicin and mustard oil responses in sensory neurons via TRPA1 activation. J. Neurosci 2008;28:1064–1075. [PubMed: 18234885]
5. Kim SR, et al. Roles of transient receptor potential vanilloid subtype 1 and cannabinoid type 1 receptors in the brain: neuroprotection versus neurotoxicity. Mol. Neurobiol 2007;35:245–254. [PubMed: 17917113]
6. Pegorini S, et al. Vanilloid VR_1 receptor is involved in rimonabant-induced neuroprotection. Br. J. Pharmacol 2006;147:552–559. [PubMed: 16444289]
7. Ryberg E, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. Br. J. Pharmacol 2007;152:1092–1101. [PubMed: 17876302]

*Some terms are modified from Ref. [60].

8. D'Mello R, Dickenson AH. Spinal cord mechanisms of pain. Br. J. Anaesth 2008;101:8–16. [PubMed: 18417503]
9. Hucho T, Levine JD. Signaling pathways in sensitization: toward a nociceptor cell biology. Neuron 2007;55:365–376. [PubMed: 17678851]
10. Hagenacker T, et al. Feedback mechanisms in the regulation of intracellular calcium ($[Ca2+]_i$) in the peripheral nociceptive system: role of TRPV-1 and pain related receptors. Cell Calcium 2008;43:215–227. [PubMed: 17673288]
11. Richardson JD, et al. Cannabinoids reduce hyperalgesia and inflammation via interaction with peripheral CB_1 receptors. Pain 1998;75:111–119. [PubMed: 9539680]
12. Sun Y, Bennett A. Cannabinoids: a new group of agonists of PPARs. PPAR Res 2007;2007:23513. [PubMed: 18288264]
13. Mackie K. Understanding cannabinoid psychoactivity with mouse genetic models. PLoS Biol 2007;5:e280. [PubMed: 17927451]
14. Mackie K. Signaling via CNS cannabinoid receptors. Mol. Cell. Endocrinol 2008;286:S60–S65. [PubMed: 18336996]
15. Hiley CR, Kaup SS. GPR55 and the vascular receptors for cannabinoids. Br. J. Pharmacol 2007;152:559–561. [PubMed: 17704825]
16. Pertwee RG. Pharmacological actions of cannabinoids. Handb. Exp. Pharmacol 2005;168:1–51. [PubMed: 16596770]
17. Brown AJ. Novel cannabinoid receptors. Br. J. Pharmacol 2007;152:567–575. [PubMed: 17906678]
18. Evans RM, et al. Modulation of sensory neuron potassium conductances by anandamide indicates roles for metabolites. Br. J. Pharmacol 2008;154:480–492. [PubMed: 18376419]
19. Patwardhan AM, et al. The cannabinoid WIN 55,212-2 inhibits transient receptor potential vanilloid 1 (TRPV1) and evokes peripheral antihyperalgesia via calcineurin. Proc. Natl. Acad. Sci. U. S. A 2006;103:11393–11398. [PubMed: 16849427]
20. Jeske NA, et al. Cannabinoid WIN 55,212-2 regulates TRPV1 phosphorylation in sensory neurons. J. Biol. Chem 2006;281:32879–32890. [PubMed: 16954222]
21. Zygmunt PM, et al. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 1999;400:452–457. [PubMed: 10440374]
22. Tominaga M, Caterina MJ. Thermosensation and pain. J. Neurobiol 2004;61:3–12. [PubMed: 15362149]
23. Price TJ, et al. Modulation of trigeminal sensory neuron activity by the dual cannabinoid-vanilloid agonists anandamide, *N*-arachidonoyl-dopamine and arachidonyl-2-chloroethylamide. Br. J. Pharmacol 2004;141:1118–1130. [PubMed: 15006899]
24. Fischbach T, et al. Effects of anandamide and noxious heat on intracellular calcium concentration in nociceptive drg neurons of rats. J. Neurophysiol 2007;98:929–938. [PubMed: 17581853]
25. Lee MG, et al. Effect of olvanil and anandamide on vagal C-fiber subtypes in guinea pig lung. Br. J. Pharmacol 2005;146:596–603. [PubMed: 16056239]
26. Price TJ, et al. Cannabinoid receptor-independent actions of the aminoalkylindole WIN 55,212-2 on trigeminal sensory neurons. Br. J. Pharmacol 2004;142:257–266. [PubMed: 15155534]
27. McCarberg BH, Barkin RL. The future of cannabinoids as analgesic agents: a pharmacologic, pharmacokinetic, and pharmacodynamic overview. Am. J. Ther 2007;14:475–483. [PubMed: 17890938]
28. Hohmann AG, Suplita RL 2nd. Endocannabinoid mechanisms of pain modulation. AAPS J 2006;8:E693–E708. [PubMed: 17233533]
29. Mbvundula EC, et al. Cannabinoids in pain and inflammation. Inflammopharmacology 2004;12:99–114. [PubMed: 15265314]
30. Jordt SE, et al. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 2004;427:260–265. [PubMed: 14712238]
31. Liu L, et al. The responses of rat trigeminal ganglion neurons to capsaicin and two nonpungent vanilloid receptor agonists, olvanil and glyceryl nonamide. J. Neurosci 1997;17:4101–4111. [PubMed: 9151727]

32. Mohapatra DP, Nau C. Regulation of Ca^{2+}-dependent desensitization in the vanilloid receptor TRPV1 by calcineurin and cAMP-dependent protein kinase. J. Biol. Chem 2005;280:13424–13432. [PubMed: 15691846]
33. Akopian AN, et al. Transient receptor potential TRPA1 channel desensitization in sensory neurons is agonist dependent and regulated by TRPV1-directed internalization. J. Physiol 2007;583:175–193. [PubMed: 17584831]
34. Lukacs V, et al. Dual regulation of TRPV1 by phosphoinositides. J. Neurosci 2007;27:7070–7080. [PubMed: 17596456]
35. Docherty RJ, et al. Inhibition of calcineurin inhibits the desensitization of capsaicin-evoked currents in cultured dorsal root ganglion neurones from adult rats. Pflugers Arch 1996;431:828–837. [PubMed: 8927498]
36. Koplas PA, et al. The role of calcium in the desensitization of capsaicin responses in rat dorsal root ganglion neurons. J. Neurosci 1997;17:3525–3537. [PubMed: 9133377]
37. Bhave G, et al. cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. Neuron 2002;35:721–731. [PubMed: 12194871]
38. Liu B, et al. Functional recovery from desensitization of vanilloid receptor TRPV1 requires resynthesis of phosphatidylinositol 4,5-bisphosphate. J. Neurosci 2005;25:4835–4843. [PubMed: 15888659]
39. Karashima Y, et al. Modulation of the transient receptor potential channel TRPA1 by phosphatidylinositol 4,5-biphosphate manipulators. Pflugers Arch 2008;457:77–89. [PubMed: 18461353]
40. Vennekens R, et al. Vanilloid transient receptor potential cation channels: an overview. Curr. Pharm. Des 2008;14:18–31. [PubMed: 18220815]
41. Bautista DM, et al. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 2006;124:1269–1282. [PubMed: 16564016]
42. Caterina MJ, et al. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 2000;288:306–313. [PubMed: 10764638]
43. Kwan KY, et al. TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron 2006;50:277–289. [PubMed: 16630838]
44. Ruparel NB, et al. Homologous and heterologous desensitization of capsaicin and mustard oil responses utilize different cellular pathways in nociceptors. Pain 2008;135:271–279. [PubMed: 17590514]
45. Jung J, et al. Phosphorylation of vanilloid receptor 1 by Ca^{2+}/calmodulin-dependent kinase II regulates its vanilloid binding. J. Biol. Chem 2004;279:7048–7054. [PubMed: 14630912]
46. De Petrocellis L, et al. Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. J. Pharmacol. Exp. Ther 2008;325:1007–1015. [PubMed: 18354058]
47. Rice AS, et al. Endocannabinoids and pain: spinal and peripheral analgesia in inflammation and neuropathy. Prostaglandins Leukot. Essent. Fatty Acids 2002;66:243–256. [PubMed: 12052040]
48. Malan TP Jr, et al. CB_2 cannabinoid receptor agonists: pain relief without psychoactive effects? Curr. Opin. Pharmacol 2003;3:62–67. [PubMed: 12550743]
49. Walker JM, Hohmann AG. Cannabinoid mechanisms of pain suppression. Handb. Exp. Pharmacol 2005;168:509–554. [PubMed: 16596786]
50. Amaya F, et al. Induction of CB_1 cannabinoid receptor by inflammation in primary afferent neurons facilitates antihyperalgesic effect of peripheral CB_1 agonist. Pain 2006;124:175–183. [PubMed: 16709443]
51. Evans RM, et al. Chronic exposure of sensory neurones to increased levels of nerve growth factor modulates CB_1/TRPV1 receptor crosstalk. Br. J. Pharmacol 2007;152:404–413. [PubMed: 17700720]
52. Cristino L, et al. Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. Neuroscience 2006;139:1405–1415. [PubMed: 16603318]

53. Ibrahim MM, et al. CB_2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. Proc. Natl. Acad. Sci. U. S. A 2005;102:3093–3098. [PubMed: 15705714]
54. De Petrocellis L, et al. Mechanisms for the coupling of cannabinoid receptors to intracellular calcium mobilization in rat insulinoma beta-cells. Exp. Cell Res 2007;313:2993–3004. [PubMed: 17585904]
55. McIntosh BT, et al. Agonist-dependent cannabinoid receptor signalling in human trabecular meshwork cells. Br. J. Pharmacol 2007;152:1111–1120. [PubMed: 17922024]
56. Bandell M, et al. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 2004;41:849–857. [PubMed: 15046718]
57. Zimov S, Yazulla S. Vanilloid receptor 1 (TRPV1/VR1) co-localizes with fatty acid amide hydrolase (FAAH) in retinal amacrine cells. Vis. Neurosci 2007;24:581–591. [PubMed: 17686199]
58. Niforatos W, et al. Activation of TRPA1 channels by the fatty acid amide hydrolase inhibitor 3′-carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597). Mol. Pharmacol 2007;71:1209–1216. [PubMed: 17314320]
59. Sagar DR, et al. Inhibition of fatty acid amide hydrolase produces PPAR-α-mediated analgesia in a rat model of inflammatory pain. Br. J. Pharmacol. (in press).
60. Loeser JD, Treede RD. The Kyoto protocol of IASP Basic Pain Terminology. Pain 2008;137:473–477. [PubMed: 18583048]
61. Huang SM, et al. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc. Natl. Acad. Sci. U. S. A 2002;99:8400–8405. [PubMed: 12060783]
62. Watanabe H, et al. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. Nature 2003;424:434–438. [PubMed: 12879072]
63. Qin N, et al. TRPV2 is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons. J. Neurosci 2008;28:6231–6238. [PubMed: 18550765]
64. Zygmunt PM, et al. The anandamide transport inhibitor AM404 activates vanilloid receptors. Eur. J. Pharmacol 2000;396:39–42. [PubMed: 10822052]
65. Roberts LA, et al. Anandamide is a partial agonist at native vanilloid receptors in acutely isolated mouse trigeminal sensory neurons. Br. J. Pharmacol 2002;137:421–428. [PubMed: 12359623]

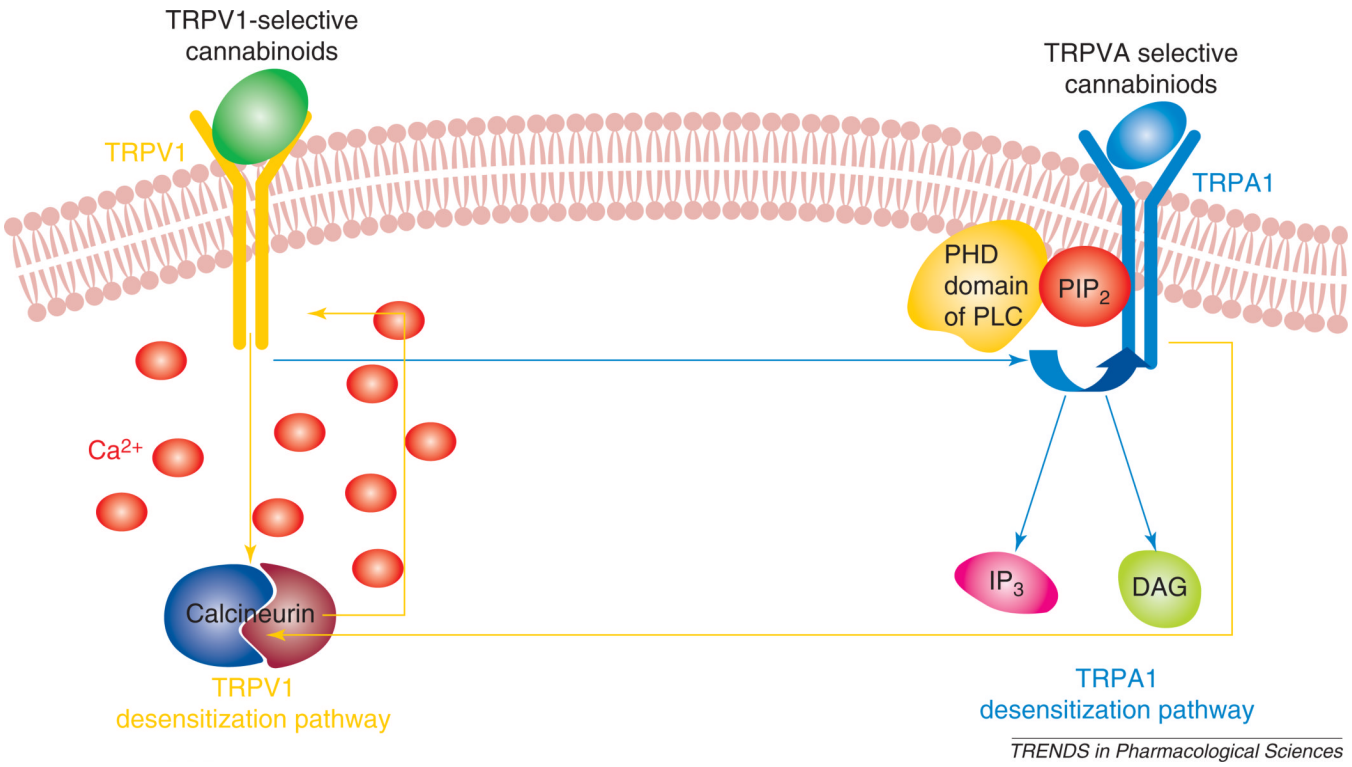

Figure 1.

Mechanisms for cannabinoid cross-desensitization of TRPV1 and TRPA1. Cannabinoids desensitize TRPV1 via activation of calcineurin and dephosphorylation of the ion channel. Homologous desensitization of TRPV1 can occur by application of TRPV1-selective cannabinoids (e.g. ACEA), and heterologous desensitization of TRPV1 can occur by administration of TRPA1-selective cannabinoids (e.g. WIN55212). Cannabinoids desensitize TRPA1 via activation of a calcium-independent pathway. Abbreviations: DAG, diacylglycerol; IP_3, inositol (1,4,5)-trisphosphate; PHD, pleckstrin homology domain; PIP_2, phosphatidylinositol (4,5)-bisphosphate; PLC, phospholipase C.

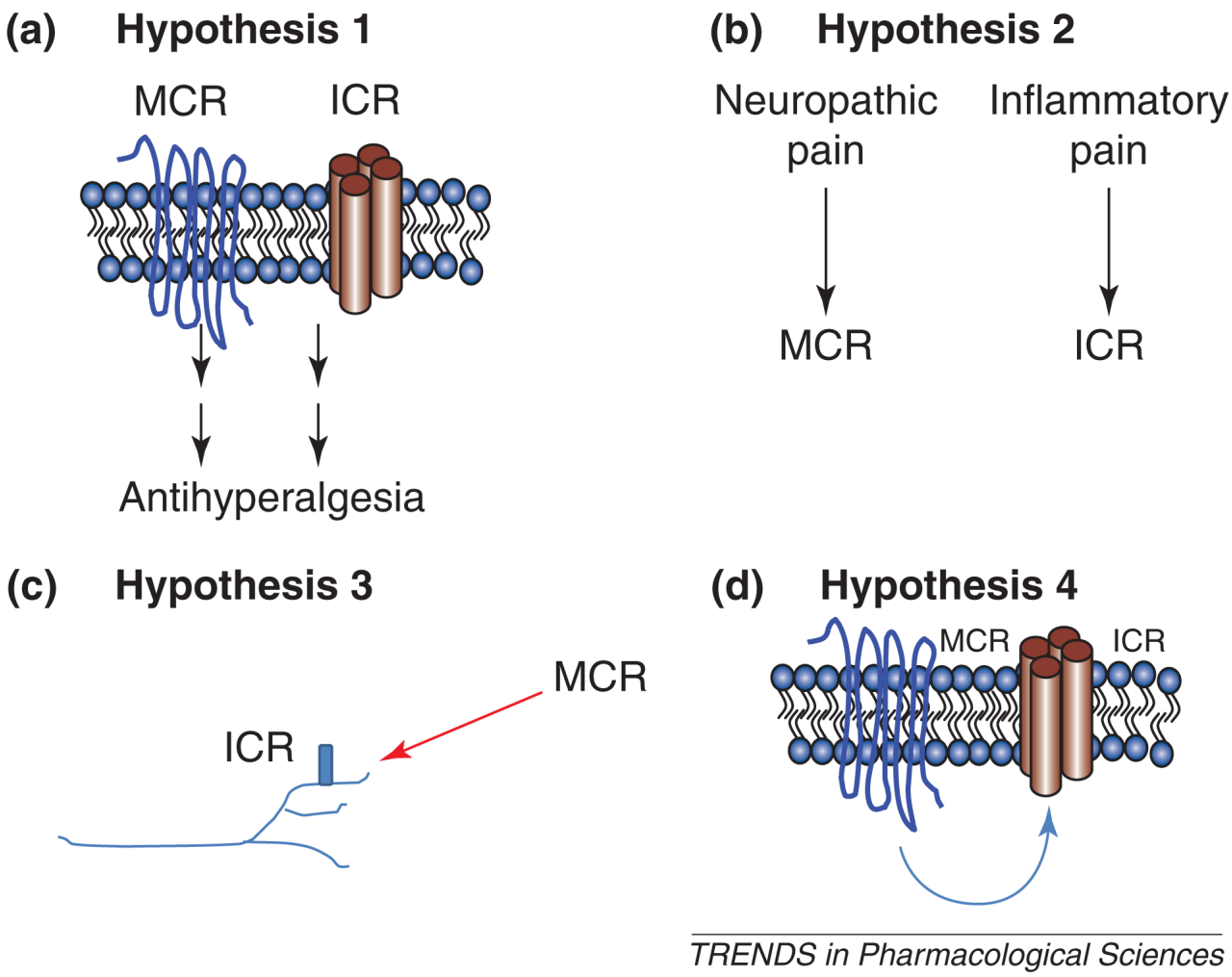

Figure 2.

Proposed mechanisms for the peripherally mediated antihyperalgesic effects of cannabinoids. (a) Hypothesis 1 proposes that cannabinoids produce peripheral antihyperalgesia by activation of both MCRs and ICRs. (b) Hypothesis 2 proposes that different pain models (or routes of drug injection) selectively activate either MCR or ICR pathways. (c) Hypothesis 3 proposes that the net effect of cannabinoids on modulating nociception is due to activation of MCRs and/or ICRs located on multiple cell types that are capable of interacting. (d) Hypothesis 4 proposes that MCRs and ICRs interact in the same cell leading to desensitization of activity. Note that the selective involvement of MCR and/or ICR in each hypothesis is for illustrative purposes only.

Table 1

Action of cannabinoids on ionotropic cannabinoid receptors[a]

Cannabinoid	Type	Action on ICR	MCR	Current[b]
Anandamide	Endogenous	TRPV1 [21]	CB_1; CB_2	200–500 pA
		>0.3 μM[c]	>10 nM	
NADA	Endogenous	TRPV1 [61]	CB_1; CB_2	300–700 pA
		>10 nM	>100 nM	
5′,6'-EA	Endogenous	TRPV4 [62]	NA[d]	20–50 pA
		>1 μM		
ACEA	Synthetic	TRPV1 [4,23]	CB_1	300–700 pA
		>5 μM	>1 nM	
Δ^9-THC	Plant	TRPV2 [63]; TRPA1 [30]	CB_1; CB_2	100–200 pA
		>10 μM	>10 nM	
Cannabinol	Plant	TRPV2 [63]; TRPA1 [30]	NA[d]	50–100 pA
		>10 μM		
Cannabidiol	Plant	TRPV2 [63]; TRPA1 [30]	NA[d]	50–100 pA
		>10 μM		
Cannabigerol	Plant	TRPM8 [46]	NA[d]	NA
		NA		
AM404	Synthetic	TRPV1 [64,65]	AEA-trans	200–500 pA
		>1 μM	NA	
WIN55212	Synthetic	TRPA1 [4]	CB_1; CB_2	200–300 pA
		>5 μM	>10 nM	
AM1241	Synthetic	TRPA1-TRPV1[e]	CB_2	100–200 pA
		>25 μM	>1 nM	

[a] Abbreviations: Δ9-THC, delta(9)-tetrahydrocannabinol; 5',6'-EA, 5',6'-epoxyeicosatrienoic acid; AEA-trans, antagonist for putative anandamide transporter; ICR, ionotropic cannabinoid receptor; MCR, metabotropic cannabinoid receptor; NA, non-applicable; NADA , *N*-arachidonoyl-dopamine.

[b] Approximate value of current magnitudes in sensory neurons.

[c] Approximate threshold concentrations of cannabinoids to activate receptors.

[d] Cannabinoid-like compounds do not activate CB_1 and CB_2.

[e] Activation of TRPA1 and TRPV1 co-expressing cells.