

Review

Possible mechanisms of cannabinoid-induced antinociception
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Abstract

Anandamide is an endogenous ligand at both the inhibitory cannabinoid CB₁ receptor and the excitatory vanilloid receptor 1 (VR1). The CB₁ receptor and vanilloid VR1 receptor are expressed in about 50% and 40% of dorsal root ganglion neurons, respectively. While all vanilloid VR1 receptor-expressing cells belong to the calcitonin gene-related peptide-containing and isolectin B4-binding sub-populations of nociceptive primary sensory neurons, about 80% of the cannabinoid CB₁ receptor-expressing cells belong to those sub-populations. Furthermore, all vanilloid VR1 receptor-expressing cells co-express the cannabinoid CB₁ receptor. In agreement with these findings, neonatal capsaicin treatment that induces degeneration of capsaicin-sensitive, vanilloid VR1 receptor-expressing, thin, unmyelinated, nociceptive primary afferent fibres significantly reduced the cannabinoid CB₁ receptor immunostaining in the superficial spinal dorsal horn. Synthetic cannabinoid CB₁ receptor agonists, which do not have affinity at the vanilloid VR1 receptor, and low concentrations of anandamide both reduce the frequency of miniature excitatory postsynaptic currents and electrical stimulation-evoked or capsaicin-induced excitatory postsynaptic currents in substantia gelatinosa cells in the spinal cord without any effect on their amplitude. These effects are blocked by selective cannabinoid CB₁ receptor antagonists. Furthermore, the paired-pulse ratio is increased while the postsynaptic response of substantia gelatinosa neurons induced by α -amino-3-hydroxy-5-methylisoxasole-propionic acid (AMPA) in the presence of tetrodotoxin is unchanged following cannabinoid CB₁ receptor activation. These results strongly suggest that the cannabinoid CB₁ receptor is expressed presynaptically and that the activation of these receptors by synthetic cannabinoid CB₁ receptor agonists or low concentration of anandamide results in inhibition of transmitter release from nociceptive primary sensory neurons. High concentrations of anandamide, on the other hand, increase the frequency of miniature excitatory postsynaptic currents recorded from substantia gelatinosa neurons. This increase is blocked by ruthenium red, suggesting that this effect is mediated through the vanilloid VR1 receptor. Thus, anandamide at high concentrations can activate the VR1 and produce an opposite, excitatory effect to its inhibitory action produced at low concentrations through cannabinoid CB₁ receptor activation. This “dual”, concentration-dependent effect of anandamide could be an important presynaptic modulatory mechanism in the spinal nociceptive system. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Cannabinoid; Vanilloid VR1 receptor; Synaptic transmission; Spinal cord; Nociception; C-fibre

1. Introduction

Derivatives of cannabis sativa, endogenous ligands of the cannabinoid CB₁ receptor, such as anandamide and 2-arachidonoylglycerol, synthetic cannabinoid CB₁ receptor agonist molecules and inhibitors of the cannabinoid re-uptake system have been shown to produce antinociceptive or antihyperalgesic effects in various animal models of pain (Calignano et al., 1998; Herzberg et al., 1997; Mao et al., 2000; Martin et al., 1998, 1999; Mazzari et al., 1996;

Richardson et al., 1998a,b; Smith et al., 1998; Strangman et al., 1998; Vivian et al., 1998; Welch et al., 1998). In addition, there is anecdotal clinical evidence that marijuana-smoking attenuates pain in multiple sclerosis patients (Consroe et al., 1997).

Cannabinoids activate two known receptor subtypes: the cannabinoid CB₁ and CB₂ receptors. The cannabinoid CB₁ receptor is broadly found in the central and peripheral nervous systems, while the cannabinoid CB₂ receptor is expressed in non-neuronal cells. The antinociceptive effects of endogenous and exogenous cannabinoids are exerted primarily via the cannabinoid CB₁ receptor, which is coupled with G_i/G_o proteins (Matsuda et al., 1990; Munro et al., 1993). Activation of the cannabinoid CB₁ receptor leads to inhibition of adenylyl cyclase activity (Felder et

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al., 1995; Howlett and Fleming 1984). In addition, cannabinoid CB₁ receptor activation inhibits Ca²⁺ currents (Caulfield and Brown, 1992; Twitchell et al., 1997) and modulates various potassium currents (Deadwyler et al., 1995; Mackie et al., 1995; Poling et al., 1996) at different sites in the central nervous system (CNS).

In general, cannabinoids inhibit glutamatergic transmission in the brain (Levenes et al., 1998; Shen et al., 1996; Szabo et al., 2000) and participate in the control of neuronal excitability and firing (McAllister et al., 1999; Mu et al., 1999; Pan et al., 1998; Poling et al., 1996; Schweitzer, 2000; Shen et al., 1996).

Cannabinoid CB₁ receptors are expressed in supraspinal (Herkenham et al., 1991; Katona et al., 1999; Lichtman et al., 1996; Mailleux and Vanderhaeghen, 1995; Martin et al., 1996, 1998; Tsou et al., 1998), spinal (Farquhar-Smith et al., 2000; Herkenham et al., 1991; Tsou et al., 1998) and peripheral (Ahluwalia et al., 2000; Hohmann and Herkenham, 1999) centres associated with nociceptive processing. Strong immunostaining of cannabinoid CB₁ receptors in the dorsal horn and in the dorsal root ganglia suggests that one of the major sites of antinociceptive action is the spinal cord. This is supported by behavioural studies using intrathecal injection of cannabinoid receptor agonists (Litchman and Martin, 1991; Mao et al., 2000; Richardson et al., 1998a,b,c), electrophysiological extracellular recordings (Hohmann et al., 1995, 1998, 1999) and inhibition of C-fibre induced neurotransmitter release in the spinal cord (Drew et al., 2000).

2. Anatomical evidence for cannabinoid-induced presynaptic modulation of the spinal nociceptive synaptic transmission

Hohmann and Herkenham (1999) have shown that the spinal binding of cannabinoid CB₁ receptor ligands was decreased by about 50% after dorsal rhizotomy suggesting that cannabinoid CB₁ receptor are expressed both on primary sensory fibres and other structures in the spinal dorsal horn. However, neonatal capsaicin injection, which induces degeneration of the majority of nociceptive primary sensory neurons, produces only a moderate decrease in cannabinoid receptor binding in the spinal dorsal horn (Hohmann and Herkenham, 1998). In agreement with these findings, these authors have also found a sizeable subpopulation of primary sensory neurons expressing the cannabinoid CB₁ receptor mRNA, with minimal co-expression of the cannabinoid CB₁ receptor mRNA and mRNA of calcitonin gene-related peptide (CGRP), which is a marker of a sub-population of nociceptive primary sensory neurons (Hohmann and Herkenham, 1999). These findings suggest that only a small proportion of the cannabinoid CB₁ receptor expressing primary sensory neurons is nociceptive. Recently, however, based on immunohistochemical findings showing that dorsal rhizotomy had negligible effect on the expression of the cannabinoid CB₁ receptor protein in the superficial dorsal horn, an area where nociceptive primary sensory neurons terminate, Farquhar-Smith et al. (2000) have suggested that very few

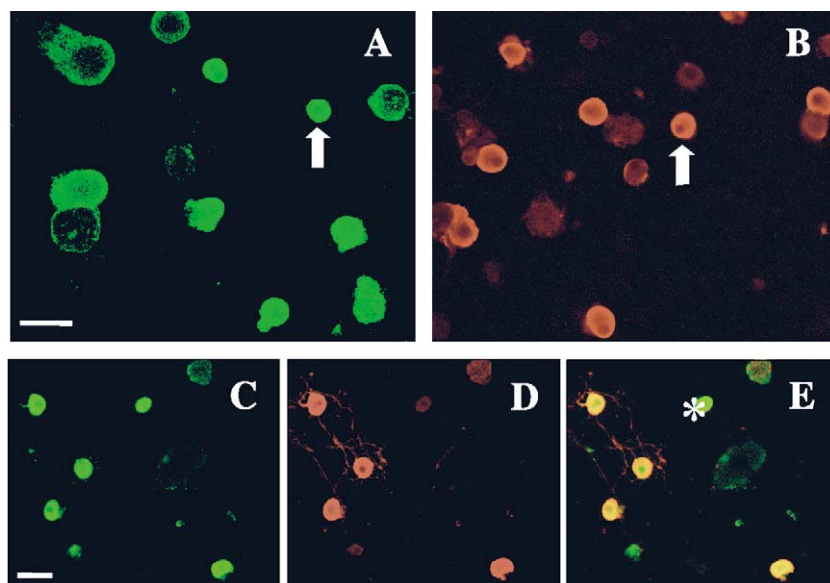


Fig. 1. Co-expression of cannabinoid CB₁ receptors with markers and vanilloid VR1 receptor in primary afferents. Confocal images of rat dorsal root ganglion neurons from three different cultures incubated in (A) anti-cannabinoid CB₁ receptor antiserum; (B) anti-vanilloid VR1 receptor antiserum; (C, D, E) anti-cannabinoid CB₁ receptor and anti-vanilloid VR1 receptor antisera, together. (A) Green fluorescence indicates cannabinoid CB₁ receptor immunostaining. Note that the majority of the immunoreactive neurons are small cells (solid arrow). (B) Red fluorescence shows vanilloid VR1 receptor-like immunopositive dorsal root ganglion cells. The vanilloid VR1 receptor-like immunopositive neurons belong to the small size sub-population of dorsal root ganglion cells (solid arrow). (C, D, E, images from the same visual field) Dorsal root ganglion cells immunolabelled for both cannabinoid CB₁ (C) and vanilloid VR1 (D) receptors. Double-labelled cells show up in yellow colour (E) after superimposing images (C) and (D). The visual field also contains a cannabinoid CB₁ receptor-like immunopositive neuron (asterisk). Bar = 25 μ m (from Ahluwalia et al., 2000).

primary sensory neurons, if any at all, express the cannabinoid CB₁ receptor. Interestingly, hemisection of the spinal cord did not reduce the cannabinoid CB₁ receptor immunostaining, either. Although based on these data, Farquhar-Smith et al. (2000) have concluded that the majority of the cannabinoid CB₁ receptors in the dorsal horn is likely to be expressed on spinal interneurons, they were unable to identify cannabinoid CB₁ receptor-positive perikarya.

Most recently, Ross et al. (2001) have found that a specific cannabinoid CB₁ receptor antibody produced labelling of cultured dorsal root ganglion neurons. The distribution, proportion, type and neurochemical properties of acutely dissociated primary sensory neurons expressing the cannabinoid CB₁ receptor was provided by Ahluwalia et al. (2000). It is generally accepted that about 2/3 of the total number of primary sensory neurons are nociceptive, and based on their responsiveness to different neurotrophic factors, nociceptive cells can be divided into two major sub-populations. About half of the nociceptive cells expresses the high affinity receptor for nerve growth factor (NGF), while the other half of the neurons expresses receptor for glial cell-derived neurotrophic factor (GDNF) (Averill et al., 1995; Bennett et al., 1998; Michael et al., 1997; Molliver et al., 1997). NGF- and GDNF-responsive nociceptive primary sensory neurons have distinct neurochemical properties (Lawson, 1996; Snider and McMahon, 1998). While NGF-responsive neurons produce different neuropeptides such as CGRP, GDNF-responsive neurons bind the isolectin IB4 (Bennet et al., 1998; Snider and McMahon, 1998). Primary sensory neurons responding neither to NGF nor GDNF are considered as non-nociceptive neurons.

Triple immunohistochemical labelling using IB4 and antibodies raised against the cannabinoid CB₁ receptor and CGRP have revealed that approximately half of the dorsal root ganglion neurons were positive to the cannabinoid CB₁ receptor while about 1/5 showed CGRP immunostaining and 1/3 bound IB4. About a third and about the half of the cannabinoid CB₁ receptor immunopositive neurons were positively stained also for CGRP and IB4, respectively. On the other hand, about 3/4 of the neurons showing CGRP immunopositivity and a similar proportion of the IB4-binding cells showed cannabinoid CB₁ receptor immunostaining. About 1/4 of the cannabinoid CB₁ receptor immunopositive neurons did not contain either CGRP or bind IB4 suggesting that these cells were non-nociceptive cells. These results clearly indicate that primary sensory neurons do express the cannabinoid CB₁ receptor protein and the majority of these neurons are nociceptive belonging to both the NGF- and GDNF-responsive sub-populations (Ahluwalia et al., in press).

It has been shown that the majority of both peptide-containing, NGF-responsive and IB4-binding, GDNF-responsive primary sensory neurons expresses the vanilloid VR1 receptor (Guo et al., 1999; Michael and Priestley, 1999;

Tominaga et al., 1998), a recently identified ligand-gated ion channel (Caterina et al., 1997). Thus, it was suggested that a sub-population of the CGRP-containing and IB4-binding cannabinoid CB₁ receptor-expressing cells are capsaicin-sensitive, vanilloid VR1 receptor expressing neurons. Double immunohistochemical labelling of acutely-dissociated primary sensory neurons using specific antibodies raised against the vanilloid VR1 and cannabinoid CB₁ receptors revealed that virtually all vanilloid VR1 receptor-expressing cells were also positive for the cannabinoid CB₁ receptor (Fig. 1; Ahluwalia et al., 2000). This co-expression of the cannabinoid CB₁ and vanilloid VR1 receptor in the spinal cord is also shown in the superficial dorsal horn of the spinal cord. Confocal microscopy on spinal cord sections double-immunostained with antibodies raised against the vanilloid VR1 and cannabinoid CB₁ receptors revealed that VR1 positive fibres were also positive for the cannabinoid CB₁ receptor. Furthermore, reduction of vanilloid VR1- and cannabinoid CB₁ receptor-immunostaining was parallel in the superficial dorsal horn after neonatal capsaicin treatment, which causes degeneration of unmyelinated nociceptive fibres (Fig. 2).

While data by Ahluwalia et al. (2000) and Hohmann and Herkenham (1998) are in agreement that a sub-popula-

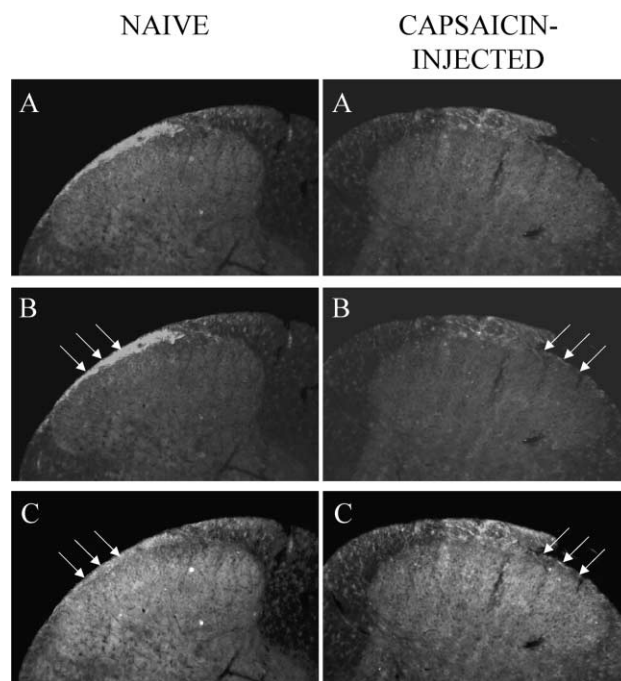


Fig. 2. The effects of neonatal capsaicin treatment on spinal expression of cannabinoid CB₁ and the vanilloid VR1 receptors. Neonatal treatment with capsaicin depleted the vanilloid VR1 receptor (B) and the majority of cannabinoid CB₁ receptor (C) immunostaining in the dorsal horn of the lumbar spinal cord. Fluorescent microscopic micrographs (A) show the overlap of cannabinoid CB₁ receptor (green) and vanilloid VR1 receptor (red) positive staining in the dorsal horn, and the loss of the stained profiles after neonatal capsaicin treatment.

tion of primary sensory neurons expresses the cannabinoid CB₁ receptor, there is disagreement on the proportion of the cannabinoid CB₁ receptor-expressing cells belonging to the nociceptive sub-population. Hohmann and Herkenham (1998) have found only a small reduction in the cannabinoid CB₁ receptor binding after neonatal capsaicin treatment. This discrepancy may originate from the use of various antibodies, recognising different epitopes which could be exposed by different conformation or subtypes of the cannabinoid CB₁ receptor. The great majority of the capsaicin-insensitive spinal cannabinoid CB₁ receptor binding sites found by Hohmann and Herkenham (1998) could represent receptors, which were identified by the cannabinoid CB₁ receptor antibody used by Farquhar-Smith et al. (2000).

In summary, the findings of these immunohistochemical, binding and in situ hybridisation experiments strongly suggest the presence of cannabinoid CB₁ receptors in the dorsal horn of the spinal cord. The larger proportion of this population is expressed presynaptically on the spinal terminals of nociceptive primary sensory neurons.

3. Functional significance of the cannabinoid CB₁ receptor expression on capsaicin-sensitive nociceptive primary sensory neurons

Further evidence for the putative location of cannabinoid CB₁ receptors in the spinal dorsal horn has been provided by Morisset and Urban (2001) by using the patch-clamp recording technique in substantia gelatinosa neurons. Synthetic cannabinoids, such as (*R*)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN55,212-2) reduced the frequency but not the amplitude of miniature excitatory postsynaptic currents (mEPSCs) and EPSCs evoked by primary afferent stimulation (Morisset and Urban, 2001). A significant increase of the paired-pulse ratio in the presence of the cannabinoid CB₁ receptor agonist was measured, further supporting the presynaptic modulation of the evoked-EPSCs in the substantia gelatinosa. It is therefore likely that cannabinoids have depressed the synaptic transmission by decreasing the release probability of glutamate from the primary afferent terminals. In the same preparation, there was little evidence for postsynaptic cannabinoid effect as the AMPA/kainate-mediated inward postsynaptic current was not altered in the presence of the cannabinoid agonist WIN55,212-2. While these data were obtained in substantia gelatinosa cells, one cannot exclude that neurons in other regions of the dorsal horn might not have some postsynaptic cannabinoid receptors as it has been suggested by Farquhar-Smith et al. (2000).

A strong presynaptic inhibition of the primary afferent C-fibres was also observed in experiments where capsaicin was used to selectively stimulate these fibres (Guo et al.,

1999; Oh et al., 1996). The frequency of capsaicin-induced EPSCs was reduced by WIN55,212.2. This finding is supported by further studies, which have described an

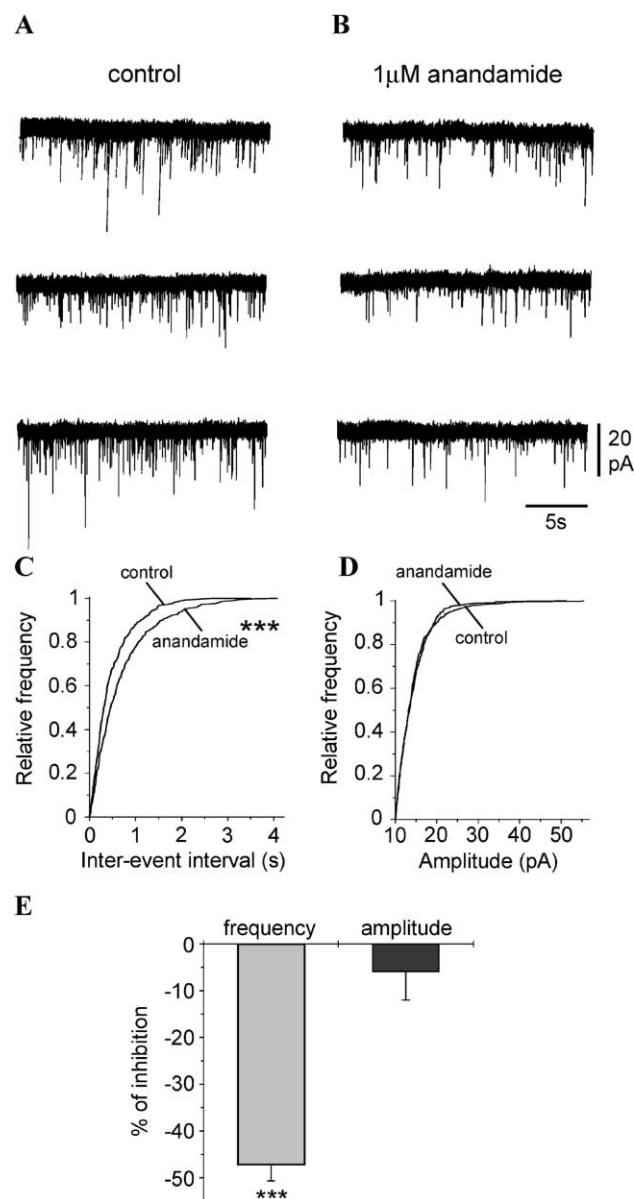


Fig. 3. Low concentration of anandamide inhibits mEPSCs in the spinal substantia gelatinosa cells. (A) Continuous recording of mEPSCs in the presence of TTX, bicuculline and strychnine. (B) Anandamide (1 μM) decreased the frequency of mEPSC in substantia gelatinosa neurons in the spinal cord (also see C) but not their amplitude (D). (E) The graphic representation of the mean percentage of inhibition of the mEPSC frequency and the lack of effect of anandamide on the amplitude of mEPSCs. The methods used here have been described in details previously (Morisset and Nagy, 1998; Morisset and Urban, 2001). Briefly, spinal cord transverse slices were superfused in a submerged-type recording chamber with an artificial cerebrospinal fluid equilibrated with 95% O₂–5% CO₂, at 30 °C (pH 7.4). Lamina II neurons were visually identified using infrared differential interference contrast microscopy and whole-cell patch-clamp recordings in voltage-clamp mode were performed. Selective activation of C-fibres was produced by superfusion of capsaicin. Data analysis and drug application were described previously (Morisset and Urban, 2001).

inhibition of capsaicin-induced CGRP release by cannabinoids in the spinal cord (Richardson et al., 1998c).

The inhibitory effect of cannabinoids on mEPSCs could be completely blocked by the selective cannabinoid CB₁ receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-

carboxamide (SR141716A). This, together with the fact that cannabinoid CB₂ receptors have not been found in the spinal cord (Chapman, 1999; Tsou et al., 1998), provide evidence for an inhibitory role of cannabinoid CB₁ receptors in the spinal dorsal horn. However, the presence of a tonic cannabinoid CB₁ receptor-mediated inhibition of ex-

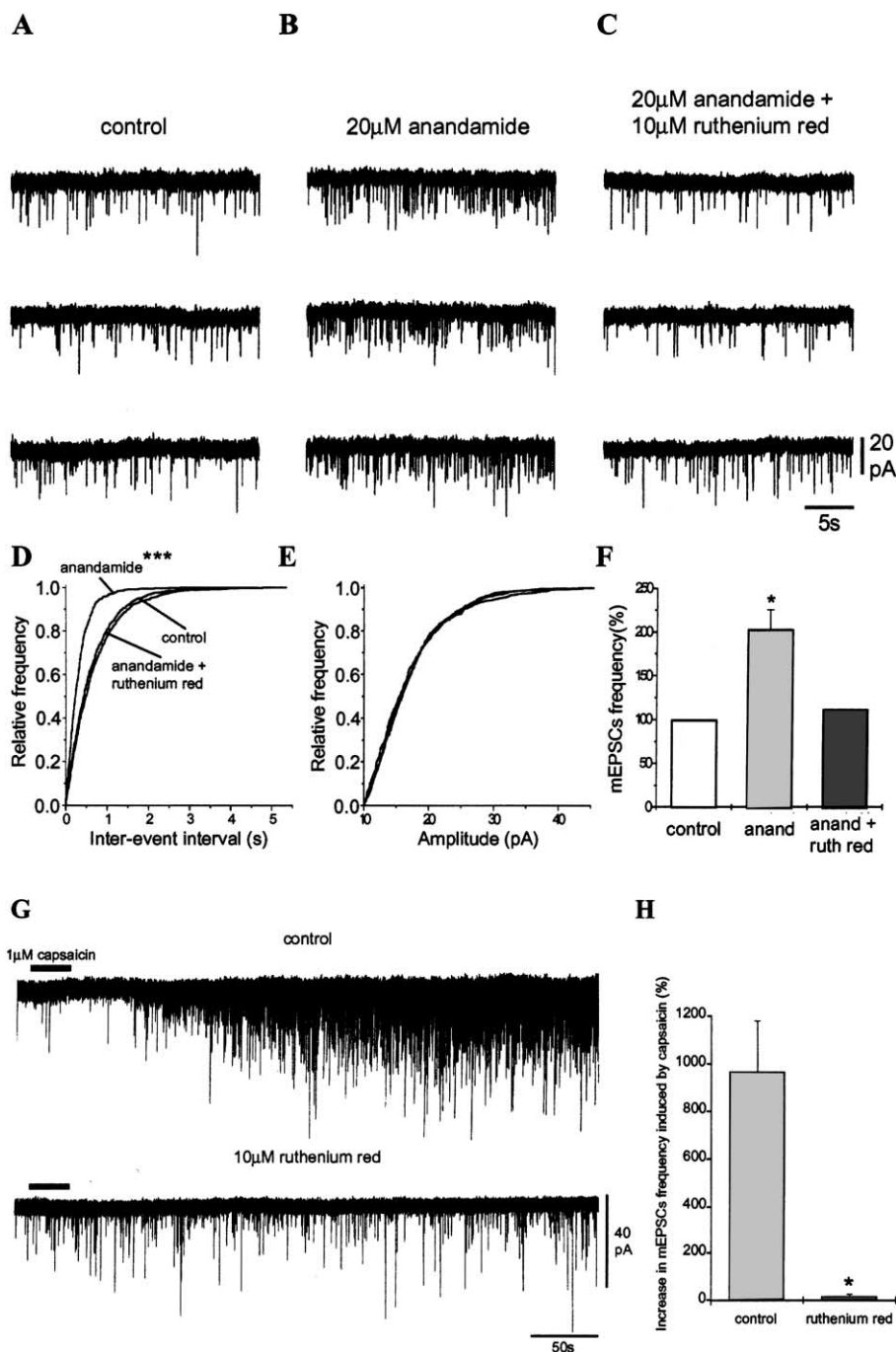


Fig. 4. High concentration of anandamide enhances mEPSCs in the spinal substantia gelatinosa cells. (A) Continuous trace of mEPSCs in control conditions (B). Effects of 20 μ M anandamide on mEPSCs. (C) The effect of anandamide was reversed by co-application with ruthenium red. While a ruthenium red sensitive increase of mEPSC frequency was recorded (D), no change in the distribution of amplitude of mEPSCs were observed (E). (F) Graphic presentation of the mean effect of anandamide and ruthenium red on the mEPSC frequency. (G) The effects of capsaicin on mEPSCs in a substantia gelatinosa cell. Ruthenium red completely abolished the capsaicin effect. (H) Graphic presentation of the mean effect of ruthenium red on the capsaicin-induced increase of the mEPSC frequency (for methodology, see legend for Fig. 3).

citatory transmission in the spinal cord is further debated (Chapman, 1999; Richardson et al., 1998a,b,c). In our study, using the spinal slice preparation, we could not find tonic inhibition on mEPSCs in the substantia gelatinosa cells. Some of the earlier observations describing tonic inhibition might have been due to the use of antagonists (SR141716A) with inverse agonist actions (Rinaldi-Carmona et al., 1994, 1998). However, no inverse agonist effects of 2 μ M SR141716 was seen in the substantia gelatinosa cells (Morisset and Urban, 2001). Alternatively, the tonic inhibition is produced by the activity of descending fibres, which are likely to be lost in the spinal slice preparation. Nevertheless, the patch-clamp data are in agreement with the morphological findings (Ahluwalia et al., 2000, in press) showing that the cannabinoid CB₁ receptor-mediated reduction in the synaptic input from nociceptive primary afferents to the spinal cord is produced by the activation of presynaptically expressed cannabinoid CB₁ receptors.

4. Concentration-related effects of anandamide: a dynamic presynaptic modulatory system in the dorsal horn?

An exciting aspect of cannabinoid CB₁ receptor and vanilloid VR1 receptor co-expression was raised after recent findings by Zygmund et al. (1999) suggesting that anandamide, the endogenous cannabinoid CB₁ receptor ligand, although at high concentrations (Tognetto et al., 2001), also activates the vanilloid VR1 receptor.

We investigated this dual effect of anandamide on nociceptive primary afferents by using patch-clamp recordings from substantia gelatinosa neurons. Anandamide at 1 μ M concentration inhibited mEPSCs similar to that of WIN55,212-2 (Fig. 3). However, anandamide at concentrations between 10 and 50 μ M increased the frequency of mEPSCs by almost 100% in substantia gelatinosa cells (Fig. 4). While the anandamide-induced reduction in the mEPSP frequency was blocked by SR141716A, a selective cannabinoid CB₁ receptor antagonist, this compound did not affect the high concentration-induced increase in the frequency. This latter effect of anandamide was completely blocked, however, by ruthenium red, a vanilloid VR1 receptor channel blocker (Fig. 4). Interestingly, the synthetic non-selective cannabinoid receptor agonist WIN55,212-5 did not produce this dual effect, indicating that it largely depends on the structure of the molecule. Nevertheless, these results, together with that of Tognetto et al. (2001) indicate that significantly larger concentration of anandamide is needed to activate the vanilloid VR1 receptor than the cannabinoid CB₁ receptor.

While it is generally accepted that the local concentration of anandamide both at the periphery and in the spinal cord can reach sufficient level to activate the cannabinoid CB₁ receptor, it is debated whether the local concentration

could rise to the level that can activate vanilloid VR1 receptor.

5. Summary

In summary, morphological and physiological evidences suggest a strong presynaptic cannabinoid CB₁ receptor-mediated modulation of the nociceptive input to substantia gelatinosa neurons in the spinal cord. The attenuation of the capsaicin-induced increase in excitability and depolarisation of the substantia gelatinosa cells suggests that the strong inhibitory effect of the cannabinoids is able to reduce the nociceptive input to the spinal dorsal horn. This strong inhibitory effect is likely to be one of the mechanisms of the antihyperalgesic and analgesic effects of cannabinoids in various animal models of acute and chronic pain (Calignano et al., 1998; Herzberg et al., 1997; Lichtman and Martin, 1991; Lichtman et al., 1996; Martin et al., 1993, 1999; Mazzari et al., 1996; Meng et al., 1998; Richardson et al., 1998a,b; Smith et al., 1998; Strangman et al., 1998; Tsou et al., 1996; Vivian et al., 1998; Welch et al., 1998).

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