Cannabinoid-Induced Presynaptic Inhibition of Glutamatergic EPSCs in Substantia Gelatinosa Neurons of the Rat Spinal Cord

VALERIE MORISSET AND LASZLO URBAN
Novartis, London WC1E 6BN, United Kingdom

Received 16 January 2001; accepted in final form 27 March 2001

Morisset, Valerie and Laszlo Urban. Cannabinoid-induced presynaptic inhibition of glutamatergic EPSCs in substantia gelatinosa neurons of the rat spinal cord. J Neurophysiol 86: 40–48, 2001. The effect of cannabinoids on excitatory transmission in the substantia gelatinosa was investigated using intracellular recording from visually identified neurons in a transverse slice preparation of the juvenile rat spinal cord. In the presence of strychnine and bicuculline, perfusion of the cannabinoid receptor agonist WIN55,212-2 reduced the frequency and the amplitude of spontaneous excitatory postsynaptic currents (sEPSCs). Furthermore, the frequency of miniature EPSCs (mEPSCs) was also decreased by WIN55,212-2, whereas their amplitude was not affected. Similar effects were reproduced using the endogenous cannabinoid ligand anandamide. The effects of both agonists were blocked by the selective CB1 receptor antagonist SR141716A. Electrical stimulation of high-threshold fibers in the dorsal root evoked a monosynaptic EPSC in lamina II neurons. In the presence of WIN55,212-2, the amplitude of the evoked EPSC (eEPSCs) was reduced, and the paired-pulse ratio was increased. The reduction of the eEPSC following CB1 receptor activation was unlikely to have a postsynaptic origin because the response to AMPA, in the presence of 1 µM TTX, was unchanged. To investigate the specificity of this synaptic inhibition, we selectively activated the nociceptive C fibers with capsaicin, which induced a strong increase in the frequency of EPSCs. In the presence of WIN55,212-2, the response to capsaicin was diminished. In conclusion, these results strongly suggest a presynaptic location for CB1 receptors whose activation results in inhibition of glutamate release in the spinal dorsal horn. The strong inhibitory effect of cannabinoids on C fibers may thereby contribute to the modulation of the spinal excitatory transmission, thus producing analgesia at the spinal level.

INTRODUCTION

Derivatives of Cannabis sativa, endogenous ligands, such as anandamide and 2-arachidonoylglycerol, synthetic receptor agonist molecules, and inhibitors of the endogenous cannabinoid re-uptake system have all been shown to exert antinociceptive or antihyperalgesic activity in various animal models of acute pain (Lichtman and Martin 1991; Martin et al. 1998; Smith et al. 1998; Strangman et al. 1998; Vivian et al. 1998; Welch et al. 1998) as well as inflammatory (Calignano et al. 1998; Martin et al. 1999; Mazzari et al. 1996; Richardson et al. 1998a–c; Smith et al. 1998) and neuropathic pain models (Herzberg et al. 1997; Mao et al. 2000). In addition, there is reasonable clinical evidence that marijuana smoking attenuates pain in multiple sclerosis patients (Consroe et al. 1997).

Cannabinoids activate two receptors, CB1 and CB2. The CB2 receptor is expressed exclusively in nonneuronal cells at the periphery, while the CB1 receptor is broadly found in the central and peripheral nervous systems. The antinociceptive effects of endogenous and exogenous cannabinoids are exerted primarily via the CB1 receptor. This is a Gi/Go-coupled receptor (Matsuda et al. 1990; Munro et al. 1993) whose activation leads to inhibition of adenyl cyclase activity (Felder et al. 1995; Howlett and Fleming 1984). In addition, CB1 receptor activation inhibits calcium 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).

CB1 receptors are expressed in neurons and are widely distributed in the CNS (Herkenham et al. 1991). In general, cannabinoids inhibit glutamatergic transmission in the cerebellum (Levenes et al. 1998), hippocampus (Shen et al. 1996), and the substantia nigra (Szabo et al. 2000). Cannabinoids have also been shown to participate in the control of neuronal excitability and firing (McAllister et al. 1999; Mu et al. 1999; Pan et al. 1998; Polling et al. 1996; Schweitzer 2000; Shen et al. 1996).

CB1 receptors are expressed in supraspinal (Herkenham et al. 1991; Katona et al. 1999; Lichtman et al. 1996; Maileux and Vanderhaegen 1992; 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 (Ahluwashia et al. 2000; Hofmann and Herkenham 1999) structures that are associated with nociceptive processing. Strong immunostaining of CB1 receptors in the dorsal horn and in the DRG suggests that one of the major sites of antinociceptive action is the dorsal horn of the spinal cord. In addition, lots of behavioral studies show antinociception after intrathecal injection (Litchman and Martin 1991; Mao et al. 2000; Richardson et al. 1998a). Using electrophysiological extracellular recordings, Hofmann and colleagues (1995, 1998, 1999) found that WIN55,212-2 depressed responses of wide dynamic range neurons evoked by noxious stimuli in the lumbar dorsal horn. In agreement with these findings, C-fiber-induced neurotransmitter release was inhibited in the spinal cord (Drew et al. 2000). However, there is no intracellular electrophysiological evidence for direct spinal action of cannabinoids in the spinal cord, and no informa-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
tion is available at cellular level on whether the spinal effect of cannabinoids is pre- or/and postsynaptic.

Considering the projection of the nociceptive Aδ and C fibers to the superficial dorsal horn, we have investigated the possible role of the endogenous cannabinoid agonist, anandamide, and the synthetic agonist, WIN55,212-2, in the modulation of the excitatory synaptic transmission of lamina II neurons in the spinal cord slice preparation, using whole cell patch-clamp recording.

METHODS

Slice preparation

The methods used to obtain spinal cord slices has been described previously (Morisset and Nagy 1998). Briefly, male Wistar-Han rats aged from 15 to 25 days were deeply anesthetized by inhalation of 2-chloro-1,1,2-trifluoroethyl difluoromethyl ether and decapitated. Following a dorsal laminectomy, the spinal cord was isolated, and 400-μm transverse slices were obtained from the L2–L6 lumbar region, using a vibratome (DSK Microslicer). Slices were then transferred to a submerged-type recording chamber where they were superfused at 2–3 ml/min with an artificial cerebrospinal fluid (ACSF) equilibrated with 95% O2-5% CO2, at a temperature of 30°C (pH 7.4). Silicon tubing was used to minimize adhesion of lipophylic drugs to the perfusion system. The ACSF contained (in mM) 124.0 NaCl, 2.4 KCl, 2.5 CaCl2, 1.2 MgCl2, 1.2 KH2PO4, 26.0 NaHCO3, and 10.0 glucose. Slices were allowed to recover for 1.5 h before the start of recordings.

Electrophysiology

Lamina II neurons were visually identified using infrared differential interference contrast microscopy (Olympus). Patch-clamp recordings in whole cell configuration were performed with pipettes (8–12 MΩ) containing (in mM) 120.0 K-Gluconate, 20.0 KCl, 0.1 CaCl2, 1.3 MgCl2, 1.0 EGTA, 10.0 HEPES, 0.1 GTP, 0.2 cAMP, 0.1 leupeptin, and 3.0 Na2-ATP (pH 7.3). Membrane currents were filtered at 2 kHz and recorded using an Axopatch 200B amplifier (Axon Instruments). Signals were digitized at 5–10 kHz using a Digidata

FIG. 1. The cannabinoid agonist, WIN55, 212-2 inhibits spontaneous excitatory postsynaptic currents (EPSCs) in substantia gelatinosa neurons. A, left: 78 s of continuous recording of spontaneous EPSCs in control conditions; right: after bath application of 10 μM WIN55,212-2. B: cumulative plots of the inter-event interval distribution before and after superfusion of WIN55,212-2 to the slice. C: cumulative plots of amplitude distributions. D: mean percentage of inhibition of the EPSC frequency and amplitude in the presence of WIN55,212-2 (n = 8 neurons).
1200 interface (Axon Instruments) with the pClamp 8 software (Axon Instruments). All neurons were held at −70 mV in voltage-clamp mode.

Synaptic responses were evoked using a concentric bipolar tungsten electrode (Rhodes SNEX-100) placed on the dorsal root. The stimulus intensity used to activate Aδ and C fibers and the conduction velocity of the primary afferents were determined previously by recording a compound action potential from an isolated dorsal root. The stimulus intensity and duration of electrical pulses used to activate Aδ and C fibers was >500 μA and 500 μs (Morisset and Nagy 1998). In this set of experiments, we did not differentiate between A- and C-fiber-evoked components of the postsynaptic potential. Selective activation of C fibers was produced by superfusion of capsaicin (1.0 μM).

Analysis

Data were analyzed off-line using the Mini Analysis Program (Synaptosoft) and pClamp 8. Data were compared using the nonparametric Kolmogorov-Smirnov test or the ANOVA test. Statistical significance was assessed at $P < 0.05$. All data are expressed as means ± SE; $n$ refers to the number of neurons studied.

Drugs

All drugs were bath applied. Stock solutions were diluted with ACSF just before application. Strychnine was purchased from Sigma. Tetrodotoxin (TTX), bicuculline, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), anandamide, WIN55,212-2, (S)-AMPA, and capsaicin were purchased from Tocris. SR141716A was purchased from SRI International.

RESULTS

Cannabinoid agonist WIN55,212-2 inhibits spontaneous EPSCs

In this study, excitatory postsynaptic currents (EPSCs) were isolated in the presence of 20 μM bicuculline and 50 μM strychnine to block GABA-A and glycinergic receptor-mediated inhibitory postsynaptic currents, respectively. Under control conditions, the average frequency of spontaneous EPSCs, calculated for 36 lamina II neurons held at −70 mV, was 1.17 ± 0.68 Hz (ranging from 0.38 to 3.02 Hz) and the mean amplitude was 21.7 ± 1.4 pA (8–105 pA). Bath application of the cannabinoid receptor agonist WIN55,212-2 (1–10 μM) caused a significant reduction in the spontaneous excitatory synaptic activity (Fig. 1A). In the presence of WIN55,212-2, the inter-event interval (Fig. 1B) and amplitude (Fig. 1C) distributions were shifted toward the right and the left, respectively, showing a decrease in the EPSC frequency ($P < 0.001$).
and amplitude ($P < 0.05$). The effect of WIN55,212-2 had a slow onset (10–15 min) and could not be washed out (see DISCUSSION). Similar findings were observed using WIN55,212-2, and were also reported elsewhere in the literature (Szabo et al. 2000; Takahashi and Linden 2000). On average ($n = 8$ neurons), WIN55,212-2 produced a significant decrease of $54.6 \pm 7.2\%$ (34–86%) in EPSCs frequency ($P < 0.001$) and a $20.5 \pm 4.4\%$ decrease in the EPSCs amplitude ($P < 0.01$; Fig. 1D). Moreover, WIN55,212-2 did not significantly change the holding current.

**WIN55,212-2 inhibits the frequency but not the amplitude of miniature EPSCs**

To investigate the possible location of the activated cannabinoid receptors, we analyzed the action of WIN55,212-2 on the miniature EPSCs (mEPSCs). These spontaneous unitary events were recorded in the presence of $1 \mu M$ TTX to block the action potential-mediated synaptic activity (Iyadomi et al. 2000). The mean frequency of mEPSCs was $1.20 \pm 0.21$ Hz (0.15–4.16 Hz), and the mean amplitude was $21.3 \pm 1.8$ pA (8–71 pA) under control conditions ($n = 22$ neurons).

WIN55,212-2 (1–5 $\mu M$) significantly reduced the mEPSCs transmission (Fig. 2A). There was no significant difference in the amplitude distributions of mEPSCs obtained under control conditions and in the presence of WIN55,212-2 (Fig. 2B). Conversely, WIN55,212-2 produced a significant shift toward the right of the inter-event interval distribution (Fig. 2C; $P < 0.001$), indicating a decrease of mEPSCs frequency. In five of five neurons tested, WIN55,212-2 decreased the frequency of mEPSCs on average by $36.3 \pm 7.3\%$ (Fig. 2D, $P < 0.01$), without affecting the mean amplitude ($-0.14 \pm 2.9\%$).

The mean membrane input resistance, calculated with a 20-mV hyperpolarizing voltage step for 16 neurons, remained the same under control condition ($693 \pm 66$ M$\Omega$) and after perfusion with WIN55,212-2 ($699 \pm 74$ M$\Omega$). These findings suggest that cannabinoid receptor activation results in inhibition of transmitter release in the superficial laminae of the dorsal horn.

**Anandamide, the endogenous cannabinoid agonist, also inhibits the mEPSCs**

In another set of experiments, we investigated whether we could reproduce the modulatory effect of WIN55,212-2 on the excitatory transmission, this time using the endogenous ligand, anandamide. Bath application of anandamide (0.5–1 $\mu M$) greatly depressed the occurrence of mEPSCs (Fig. 3A). The onset of the effect of anandamide was slow, reaching a plateau after perfusion of the slice for 15 min (Fig. 3B). In six of six neurons tested, anandamide reduced the mEPSCs frequency on average by $49.9 \pm 2.5\%$ ($P < 0.001$) without changing the mean amplitude ($5.8 \pm 6.3\%$ inhibition; see Fig. 3C).

**FIG. 3.** Anandamide, the endogenous cannabinoid agonist, inhibits the miniature EPSCs (mEPSCs). A: continuous recording of mEPSCs for 25 s in control condition (left) and after bath application of 1 $\mu M$ anandamide (right). B: mean time course of the inhibition of the mEPSC frequency in the presence of anandamide. C: mean percentage of inhibition of the mEPSC frequency and amplitude in the presence of anandamide calculated from 6 neurons.
Effect of WIN55,212-2 is mediated by CB1 receptors

In the presence of the CB1 receptor-specific antagonist SR141716A (1–2 μM), WIN55,212-2 failed to reduce the mEPSC transmission (Fig. 4A). The antagonist blocked the action of WIN55,212-2 on the mEPSC frequency (n = 4, Fig. 4B) and the mEPSC amplitude remained unchanged (n = 4, Fig. 4C). Therefore we assume that the modulation of the excitatory transmission was specifically mediated by CB1 receptor activation.

Superfusion of SR14176A alone affected neither the frequency of the mEPSCs (n = 6, Fig. 4B) nor their amplitude (n = 6, Fig. 4C). This indicates the lack of tonic modulation of the excitatory transmission by cannabinoids in the slice preparation.

WIN55,212-2 inhibits evoked synaptic responses

In the presence of 20 μM bicuculline and 50 μM strychnine, stimulation of the dorsal root attached to the slice evoked EPSCs (eEPSCs) in neurons held at −70 mV. Electrically evoked EPSPs were completely abolished by 20 μM CNQX (not shown), indicating that they were mediated by AMPA receptors. The eEPSCs in lamina II neurons are likely to be monosynaptic since no failure during 10-Hz dorsal root stimulation was found (not shown). The stimulus strength used was typically ranging between 50 and 500 μs and 200 and 500 μA. These parameters were determined from previous measurements of fiber conduction velocities (see METHODS) to activate high-threshold putative nociceptive fibers (Aδ and/or C fibers).

As illustrated in Fig. 5A, I and 2, perfusion of WIN55,212-2 (1–5 μM) significantly inhibited the eEPSCs in all neurons tested (n = 8). On average, the peak amplitude of the eEPSCs was decreased by 39.6 ± 4.1% (P < 0.001). As for the other sets of experiments, the effect of WIN55,212-2 had a slow onset (Fig. 5A2), and could only be washed out once after 40 min (not shown).

WIN55,212-2 increases the paired-pulse ratio

To further investigate the location of the CB1 receptors involved in the modulation of the eEPSCs, we tested the effects of WIN55,212-2 on paired pulses evoked at 50-ms interval. The paired-pulse ratio (PPR) was calculated as the amplitude of the second eEPSC divided by the amplitude of the first eEPSC. In control conditions, the second eEPSC was typically smaller than the first one (Fig. 5B1) even at a stimulus strength just above threshold. The PPR was on average 0.49 ± 0.13 (n = 3), indicating a paired-pulse depression. In the presence

![Fig. 4](http://jn.physiology.org/)

A: continuous recording of mEPSCs (200 s) in control conditions (left) and in the presence of 2 μM of the selective CB1 receptor antagonist SR141716A (middle) and after addition of 1 μM WIN55,212-2 (right). Relative frequency (B) and amplitude (C) of mEPSCs, in artificial cerebrospinal fluid only, in the presence of SR141716A, and after combined perfusion of SR141716A and WIN55,212-2. Data were pooled from 4 and 6 neurons, respectively.
of WIN55,212-2 (1 \( \mu \)M), the first eEPSC was reduced, whereas the second one was not significantly affected (Fig. 5B1), resulting in an average PPR of 0.70 ± 0.15 (n = 3). Activation of CB1 receptors increased the PPR on average by 43 ± 13% (P, 0.05, Fig. 5B2), further suggesting that the cannabinoid receptors involved were located presynaptically.

**WIN55,212-2 does not affect AMPA-induced postsynaptic responses**

To determine if the reduction of the eEPSCs in the presence of WIN55,212-2 (1 \( \mu \)M) was also partially due to direct modulation of the sensitivity of the postsynaptic glutamatergic receptor, we investigated whether the cannabinoid receptor agonist would affect the response to an exogenous application of AMPA. In the presence of 1 \( \mu \)M TTX, 10-s bath perfusion of 1–5 \( \mu \)M AMPA elicited an inward current that was unaffected following CB1 receptor activation (100 ± 5.5%, n = 3 neurons; Fig. 5C). It is therefore unlikely that a negative modulation of AMPA receptors could account for the reduction of the eEPSCs in the presence of WIN55,212-2.

**Effects of WIN55,212-2 on capsaicin-induced, C-fiber-mediated EPSCs**

To investigate whether cannabinoids have a specific effect on the nociceptive input to the spinal cord, we selectively activated primary afferent C fibers with capsaicin. Perfusion of the slice preparation with capsaicin (1 \( \mu \)M) for 30 s resulted in a large increase in EPSC frequency (1190 ± 167% of control; P < 0.001; n = 4 neurons) together with the gradual development of an inward current (7–12 pA; Fig. 6, A and B). The average amplitude of the EPSCs was not significantly changed (113 ± 8 pA; Fig. 6C). In the presence of 1 \( \mu \)M WIN55,212-2, similar application of capsaicin produced a 549 ± 107% (P < 0.01) increase in the EPSC frequency, and the inward current was almost completely suppressed (Fig. 6, A and B). This 55 ± 3% (P < 0.001) inhibition of the capsaicin response in the presence of WIN55,212-2 (n = 4 neurons) was not due to desensitization of the VR1 receptors since repeated application of capsaicin (after a 30-min washout period) in the presence of the cannabinoid receptor agonist produced a similar response to the first one (not shown).

**DISCUSSION**

Activation of cannabinoid receptors by the nonselective CB1/CB2 agonist, WIN55,212-2, produced a strong inhibitory effect on glutamatergic spontaneous, miniature, and dorsal-root-evoked synaptic events in the superficial dorsal horn of the spinal cord. A similar inhibition of the miniature EPSCs was produced by anandamide. Our results show that these effects are mainly presynaptic in origin. As we will discuss, they may
We found that cannabinoid agonists decreased the frequency, and to a lesser extent, the amplitude of the spontaneous synaptic activity. However, only the frequency but not the amplitude of the spontaneous events was decreased by 54.6%, whereas the frequency of the miniature was only reduced by 36.3%. The inhibitory effects of cannabinoids on the glutamatergic transmission are likely to affect calcium-dependent mechanisms. Moreover, although we did not find any change in the input resistance or holding current following the activation of cannabinoid receptors in the presence of TTX, we cannot exclude the possibility of some postsynaptic effects of cannabinoids. Indeed, WIN55,212-2 has been reported to modulate some voltage-dependent potassium or calcium channels (Deadwyler et al. 1995; Twitchell et al. 1997).

The cannabinoid-mediated presynaptic inhibition of the excitatory transmission in the substantia gelatinosa shown by our results suggests that the CB1 receptor is expressed on axons of intrinsic spinal neurons (Farquhar-Smith et al. 2000). In addition to intrinsic spinal neurons, other sites of presynaptic location for CB1 receptors, namely descending systems and sensory afferents could be considered. Descending systems innervating spinal neurons could originate from various sources in the brain expressing CB1 receptors (Tsou et al. 1998); however, there is no direct evidence for CB1-positive fibers of descending sources.

To further investigate the putative location of CB1 receptors, we studied the effect of cannabinoids on EPSCs evoked by primary afferent stimulation and showed that WIN55,212-2 decreased the amplitude of electrically evoked EPSCs. To determine whether this effect could have a presynaptic origin, we used the paired-pulse paradigm (Betz 1970) Modification of the paired-pulse ratio was found specific for presynaptic inhibition in various preparations of the brain (Sullivan 1999; Szabo et al. 2000; Takahashi and Linden 2000). In the present study, the significant increase of the paired-pulse ratio in the presence of the cannabinoid agonist further supports 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 probability of release of glutamate from the primary afferent terminals. Similar findings showed that the possible mechanism of this phenomenon was due to the inhibition of voltage-gated calcium channels by cannabinoid agonists (Caulfield and Brown 1992; Twitchell et al. 1997).

Another possible mechanism for the inhibition of the primary afferent-evoked response following CB1 receptor activation is the modulation of the postsynaptic receptors by cannabinoids. In agreement with previous data (Yoshimura and Nishi 1993), we found that the EPSCs evoked by dorsal root stimulation were blocked by 10 μM CNQX (not shown), suggesting AMPA/kainate receptor activation. The inward postsynaptic current evoked by perfusion of AMPA was not altered in the presence of the cannabinoid agonist WIN55,212-2. Thus we can exclude a postsynaptic effect on AMPA receptors and postulate that the inhibition of the dorsal root-evoked EPSCs by WIN55,212-2 occurred at a presynaptic site. Our data strongly suggest that, in substantia gelatinosa, the cannabinoid-mediated inhibition of the primary afferent response rely on presynaptic mechanisms. There is also accumulating evidence showing that in the CNS, a major general role for CB1 recep-

![Figure 6](http://jn.physiology.org/)

**FIG. 6.** WIN55,212-2 decreased the response to C fibers selectively activated by capsaicin. *A*: increase of EPSC frequency in response to 30-s bath application of 1 μM capsaicin in control conditions (top) and in the presence of 1 μM WIN55,212-2 (bottom). *B* and *C*: the relative change in frequency and amplitude of EPSCs. Data were pooled from 4 neurons.

involve calcium-dependent as well as calcium-independent mechanisms.

Anandamide, the endogenous ligand for cannabinoid receptors, was used at 1 μM concentration, which inhibited mEPSCs similar to that of WIN55,212-2. Enhancement of synaptic activity has not been observed, therefore it is unlikely that activation occurred (Smart et al. 2000; Zygmunt et al. 1999). However, it is possible that higher doses of anandamide may produce, like capsaicin, an increase in mEPSCs frequency (Yang et al. 1998).

This latter finding strongly suggests that activation of cannabinoid receptors reduce synaptic activity by acting at a presynaptic site in the substantia gelatinosa. Since miniature events occur independently from action potentials, it is unlikely that a reduction of calcium entering into the axon terminals could account for the decrease of mEPSCs frequency mediated by cannabinoids. Another possible explanation could be that cannabinoids act directly on the release machinery and perturbate the reaction cascade triggering the exocytosis of glutamate vesicles. However, since the frequency of the spontaneous events was decreased by 54.6%, whereas the frequency of the miniature was only reduced by 36.3%, the inhibitory effects of cannabinoids on the glutamatergic transmission are likely to affect calcium-dependent mechanisms. Moreover, although we did not find any change in the input resistance or holding current following the activation of cannabinoid receptors in the presence of TTX, we cannot exclude the possibility of some postsynaptic effects of cannabinoids. Indeed, WIN55,212-2 has been reported to modulate some voltage-dependent potassium or calcium channels (Deadwyler et al. 1995; Twitchell et al. 1997).

The cannabinoid-mediated presynaptic inhibition of the excitatory transmission in the substantia gelatinosa shown by our results supports immunohistochemical investigations, which suggest that the CB1 receptor is expressed on axons of intrinsic spinal neurons (Farquhar-Smith et al. 2000). In addition to intrinsic spinal neurons, other sites of presynaptic location for CB1 receptors, namely descending systems and sensory afferents could be considered. Descending systems innervating spinal neurons could originate from various sources in the brain expressing CB1 receptors (Tsou et al. 1998); however, there is no direct evidence for CB1-positive fibers of descending sources.

To further investigate the putative location of CB1 receptors, we studied the effect of cannabinoids on EPSCs evoked by primary afferent stimulation and showed that WIN55,212-2 decreased the amplitude of electrically evoked EPSCs. To determine whether this effect could have a presynaptic origin, we used the paired-pulse paradigm (Betz 1970) Modification of the paired-pulse ratio was found specific for presynaptic inhibition in various preparations of the brain (Sullivan 1999; Szabo et al. 2000; Takahashi and Linden 2000). In the present study, the significant increase of the paired-pulse ratio in the presence of the cannabinoid agonist further supports 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 probability of release of glutamate from the primary afferent terminals. Similar findings showed that the possible mechanism of this phenomenon was due to the inhibition of voltage-gated calcium channels by cannabinoid agonists (Caulfield and Brown 1992; Twitchell et al. 1997).

Another possible mechanism for the inhibition of the primary afferent-evoked response following CB1 receptor activation is the modulation of the postsynaptic receptors by cannabinoids. In agreement with previous data (Yoshimura and Nishi 1993), we found that the EPSCs evoked by dorsal root stimulation were blocked by 10 μM CNQX (not shown), suggesting AMPA/kainate receptor activation. The inward postsynaptic current evoked by perfusion of AMPA was not altered in the presence of the cannabinoid agonist WIN55,212-2. Thus we can exclude a postsynaptic effect on AMPA receptors and postulate that the inhibition of the dorsal root-evoked EPSCs by WIN55,212-2 occurred at a presynaptic site. Our data strongly suggest that, in substantia gelatinosa, the cannabinoid-mediated inhibition of the primary afferent response rely on presynaptic mechanisms. There is also accumulating evidence showing that in the CNS, a major general role for CB1 recep-
tors is the presynaptic inhibition of glutamate release (Szabo et al. 2000).

To address the specificity of the inhibition of the primary afferent-evoked synaptic response, we selectively activated C fibers with capsaicin (Guo et al. 1999; Oh et al. 1996). WIN 55,212-2 clearly decreased the frequency of the capsaicin-induced EPSCs in substantia gelatinosa neurons, suggesting a presynaptic site for cannabinoid receptors on nociceptive primary afferents. This finding is supported by previous reports, which have described an inhibition of capsaicin-induced CGRP release by cannabinoids in the spinal cord (Richardson et al. 1998), and a 50% reduction in CB1 binding sites in the dorsal horn after dorsal rhizotomy (Hohmann et al. 1999) or neonatal capsaicin treatment (Hohmann and Herkenham 1998).

The inhibitory effect of WIN55,212-2 on mEPSCs was completely blocked by the selective CB1 receptor antagonist SR141716A. This, together with the fact that CB2 receptors have not been found in CNS neurons and more specifically in the spinal cord (Chapman 1999; Tsou et al. 1998), provide strong evidence for an inhibitory role of CB1 receptors in substantia gelatinosa.

It has been suggested that endogenous cannabinoids maintain a sustained, tonic inhibition in the spinal cord of the naïve rat (Chapman 1999; Richardson et al. 1998). However, this is debated (Ward 2000) based on the inverse agonist actions of the “antagonist,” SR 141716A (Rinaldi-Carmona et al. 1994, 1998). In our experiments, SR 141716 (2 μM), the CB1 antagonist did not have any effect on the mEPSCs. Therefore from our results, it seems that there is no tonic CB1-related inhibition of the excitatory transmission in the spinal slice of a naïve rat.

In summary, our results suggest a strong presynaptic inhibition of the nociceptive input to substantia gelatinosa neurons in the spinal slice. The attenuation of the capsaicin-induced increase in excitability and depolarization of the substantia gelatinosa cells suggest that the strong inhibitory effect of the cannabinoids is able to reduce nociceptive input to the spinal dorsal horn.

Our study gives for the first time clear evidence that cannabinoids mediate a presynaptic inhibition of the excitatory synaptic activity in the substantia gelatinosa together with a strong inhibition of the nociceptive input to the superficial dorsal horn. We believe that 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; Martin et al. 1993, 1998; 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).

We are very grateful to Dr. A. Fox, F. Nagy, and I. Nagy for careful reading of and critical comments on the manuscript.

REFERENCES


Pugh GJR, Smith PB, Dombrowski DS, and Welch SP. The role of endogenous opioids in enhancing the antinoiception produced by the combination of delta9-tetrahydrocannabinol and morphine in the spinal cord. J Pharmacol Exp Ther 279: 608–616, 1996.