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European Journal of Pharmacology 345 (1998) 145–153

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# Antihyperalgesic effects of spinal cannabinoids

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Received 15 December 1997; accepted 23 December 1997

**Abstract**

Cannabinoids have been widely reported to produce antinociception in models such as tail flick and hot plate. However, their role in modulating thermal hyperalgesia is unknown. The potency of some drugs, such as the opioids, increases during hyperalgesia. Thus, we evaluated whether there is a change in the effectiveness of intrathecal cannabinoids with hyperalgesia. Additionally, we evaluated whether cannabinoids could inhibit capsaicin-evoked neurosecretion from isolated rat spinal cord. Our results indicate that 1 fmol anandamide (i.t.) completely blocked carrageenan-induced thermal hyperalgesia. However, anandamide at doses as high as 100 pmol had no effect on thermal latencies in normal animals. Additionally, anandamide inhibited $K^+$- as well as capsaicin-evoked immunoreactive calcitonin gene-related peptide release. Finally, cannabinoid receptors were identified in sensory neurons. Collectively, these results indicate that there is an increased effectiveness of modulation of thermal nociceptive thresholds by spinal cannabinoids during hyperalgesia. This antihyperalgesic effect may be the result of cannabinoid-induced inhibition of neurosecretion from certain primary afferent fibers. © 1998 Elsevier Science B.V.

*Keywords:* Cannabinoid; Hyperalgesia; Spinal cord; Superfusion; Capsaicin; Trigeminal ganglion

## 1. Introduction

Although antinociceptive effects of cannabinoids have been widely reported, few studies have evaluated their antihyperalgesic effects. Antinociception is the ability to raise the nociceptive threshold in the absence of hyperalgesia. In contrast, antihyperalgesia is the ability to decrease the hyperalgesic response to a nociceptive stimulus. Some drugs, such as the opioids, demonstrate both antinociceptive and antihyperalgesic effects but demonstrate different potencies with the different conditions. Agonists at the $\mu$-opioid receptor subtype are more potent in their ability to increase response latencies in the hot plate test after carrageenan-induced hyperalgesia when compared with normal animals (Joris et al., 1990; Hylden et al., 1991). Additionally, $\mu$-opioid receptor agonists are more potent in inhibiting C fiber-evoked spinal responses in inflamed animals compared with normal animals (Stanfa et al., 1992). Cannabinoids share many properties with the opioids including interacting with $G_{i/o}$ proteins and producing spinal antinociception. However, it is not known if cannabinoids also share an increased potency during hyperalgesia.

Cannabinoid-induced antinociception is due, at least in part, to spinal mechanisms. Cannabinoid receptors have been measured in rat spinal cord (Herkenham et al., 1991). Additionally, cannabinoid-induced antinociception remains after spinalization of animals (Lichtman and Martin, 1991). Furthermore, cannabinoid agonists inhibit firing of spinal wide dynamic range neurons in response to noxious stimuli (Hohmann et al., 1995) and will prevent the expression of c-*fos* in the dorsal horn (Tsou et al., 1996). Our laboratory has recently found that spinal cannabinoid receptors are tonically active and that hypoactivity of these receptors results in a glutamate-dependent hyperalgesia (Richardson et al., 1997, 1998a). One potential mechanism by which cannabinoids may inhibit firing of wide dynamic range neurons in response to noxious stimuli and both glutamate-dependent and carrageenan-induced hyperalge-

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sia is via the inhibition of neurosecretion into the spinal cord from nociceptive primary afferent fibers, such as those sensitive to capsaicin.

In the present study, we used the mouse model of carrageenan hyperalgesia (Aanonsen et al., 1996) to test the hypothesis that intrathecal administration of cannabinoids produce antihyperalgesia. Additionally, we evaluated the hypothesis that cannabinoid receptors inhibit neurosecretion from the central terminals of capsaicin-sensitive primary afferent fibers by interacting with cannabinoid receptors located on these fibers.

## 2. Materials and methods

### 2.1. Animals

Male ND4 Swiss mice or male Sprague–Dawley rats (20–25 g, Harlan Laboratories, Indianapolis, IN) were maintained on a 12:12 light:dark cycle with free access to food and water. All procedures were approved by the University of Minnesota Animal Care and Use Committee.

### 2.2. Materials

Materials were obtained from the following companies: anandamide, RBI (Natick, MA); SR 141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxyamide], a gift from Sanofi Recherché (Montpellier, France); sterile saline, Baxter (McGaw Park, IL); [$^3$H]CP 55,940 [(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)-cyclohexanol)}, DuPont NEN (NET-1051, Boston, MA); capsaicin, Fluka (Ronkonkoma, NY); CGRP antisera (MI-2; kindly provided by Mike Iadorola); goat anti-rabbit antisera coupled to ferric beads, PerSeptive Diagnostics (Cambridge, MA); thiorphan, Bachem (Torrance, CA); rat trigeminal ganglion (Zivic–Miller, Zellenople, PA); UltimaGold scintillation cocktail, Packard (Meriden, CT); Bradford assay reagents, BioRad (Cambridge, MA). All other reagents were obtained from Sigma (St. Louis, MO).

### 2.3. Drug administration

A 6% carrageenan solution was made up in saline. Mice were restrained by holding at the pelvis. One hindleg was extended by the researcher restraining the animals while another researcher injected carrageenan into the hindpaw in a 10-$\mu$l volume using a 30 gauge needle (Aanonsen et al., 1996). The procedure was repeated with the other hindpaw. Immediately following carrageenan administration, saline or anandamide was administered intrathecally in a 5-$\mu$l volume (Hylden and Wilcox, 1980). In control animals, saline was injected bilaterally into the mouse hindpaws and then immediately into the intrathecal space. Normal (i.e., uninflamed) animals received only an intrathecal administration of saline or anandamide.

### 2.4. Hot plate latencies

Mice were placed on a Harvard Hot Plate Analgesia Meter (Edinbridge, KY) set at 54–55°C and immediately removed when a response to the thermal noxious stimulus, either licking of a hindpaw or jumping from the surface of the hot plate, was observed. A 40 s cut-off was used to prevent tissue damage. Hot plate latencies were recorded in triplicate for each animal with approximately 5 min separating each trial. The average baseline latency was $17.6 \pm 0.2$ s (mean ± S.E.M.; $n = 364$). After recording baseline latencies, drugs were administered as described above. Post-injection latencies were recorded 30 min after drug administration in the carrageenan studies (the time of peak hyperalgesia). Because time course studies indicate that antinociception induced by intrathecal administration of anandamide disappears by 20 min (Smith et al., 1994), these experiments were designed to determine whether anandamide inhibits the development of hyperalgesia. In addition, we evaluated whether anandamide was antinociceptive in uninflamed animals. In this latter experiment, animals had to be tested while the drug was still active, i.e., within 20 min of drug administration (Smith et al., 1994). Thus, post-injection latencies were recorded 5 min after drug administration in the uninflamed animals. Difference scores were determined for each mouse by subtracting its average baseline latency from its average post-injection latency. When analyzing blocked results across different days of testing, inter-day experimental variability was removed by subtracting the vehicle control from the experimental groups. Observers were blind to treatment allocation.

### 2.5. Tissue collection

Animals were sacrificed via decapitation and their spinal cords removed via hydraulic extrusion. Tissue was dissected and immediately processed for superfusion or frozen on dry ice for subsequent membrane preparation and radioreceptor binding assays.

### 2.6. $P_2$ membrane preparation

$P_2$ membranes were prepared using the method described by Devane et al. (1988). Tissue was homogenized in 30 ml of sucrose (320 mM), EDTA (2 mM), and $MgCl_2$ (5 mM) and then centrifuged at $2000 \times g$ for 10 min. The pellet was washed twice. The supernatants were then centrifuged at $39\,000 \times g$ for 15 min. The pellet was resuspended in 30 ml buffer A [Tris–HCl (50 mM, pH 7.0 at 30°C), EDTA (2 mM), and $MgCl_2$ (5 mM)] at 37°C for 10

min. This was centrifuged at 23 000 × *g* for 10 min. The pellet was resuspended in 30 ml buffer A at 30°C for 40 min. This was centrifuged at 11 000 × *g* for 15 min. The pellet was resuspended in 1 ml Tris–HCl (50 mM, pH 7.4 at 30°C), EDTA (1 mM), and $MgCl_2$ (3 mM) and stored at −80°C.

### 2.7. Radioreceptor binding

Homogenates (60 μg of protein for spinal cord and 100 μg of protein for trigeminal ganglion, determined by the Bradford assay) were incubated for 1 h at room temperature in assay buffer (Tris–HCl (50 mM, pH 7.4), $MgCl_2$ (3 mM), EDTA (1 mM), and fatty acid-free bovine serum albumin (1 mg/ml)) with various concentrations of the cannabinoid agonist [$^3$H]CP-55940 (101–158 Ci/mmol). Total volume per reaction tube was 200 μl. Nonspecific binding was determined with the cannabinoid receptor antagonist SR 141716A (1 μM). The reaction was terminated with rapid filtration over a Whatman GF/C filter (Hillsboro, OR) which had been soaked for 15 min in 0.5% polyethylenimine. Tissue was then washed 3 times with 4 ml of ice cold assay buffer. After washing, filters were transferred to a scintillation vial and UltimaGold scintillation cocktail was added. Disintegrations per minute were determined by counting the vials with a liquid scintillation counter for one minute each. Binding parameters were determined from three separate experiments each in triplicate using homogenates of tissue from 15–30 animals. Specific binding ranged from 20–80%.

### 2.8. Superfusion

The dorsal half of the lumbar enlargement of rat spinal cord was dissected and chopped into 200 μm cubes with a McIlwain tissue chopper (Mickle Laboratory Engineering, Gomshall UK). The tissue was placed in a 1 cc chamber and continually superfused with oxygenated Kreb's buffer (37°C, pH 7.4, 500 μl/min, Brandel Superfusion Pump; Brandel, Gaithersburg, MD). The modified Kreb's buffer consists of NaCl (135 mM), KCl (3.5 mM), MgCl (1 mM), $NaHCO_3$ (20 mM), $NaH_2PO_4$ (1 mM), $CaCl_2$ (2.5 mM), dextrose (3.3 mM), bovine serum albumin (fatty acid free; 0.1%), ascorbate (0.1 mM), and thiorphan (10 μM). The superfusate was collected by a fraction collector (Gilson FC203B; Gilson, Middleton, WI) into test tubes containing 1 M MES (2-[*N*-morpholino]ethanesulfonic acid) which maintains the pH of the superfusate at 6.1. One fraction represents 3 min of collection time. Following equilibration of the tissue (ca. 1 h), anandamide or vehicle was administered for 6 min. Capsaicin was then coadministered in the presence of the pretreated drug or vehicle for an additional 6 min. Superfusate was collected for an additional 52 min. In the experiment with the antagonist, SR 141716A or vehicle was administered for 3 min prior to anandamide or vehicle administration.

### 2.9. Radioimmunoassay

Superfusate from the dorsal half of the spinal cord was analyzed for immunoreactive calcitonin gene-related peptide (CGRP) using radioimmunoassay (RIA). CGRP was used as an indicator of neurosecretion from primary afferent fibers (Willis and Coggeshall, 1991). The CGRP antiserum binds near the C-terminal end of CGRP and does not cross-react with cholecystokinin, neuropeptide Y, or other peptides with similar C-terminal residues, such as the phenylalanine–methionine–arginine–phenylalanine amide peptides. Immediately following the experiment, samples were pre-incubated for 24 h (4°C) with 100 μl of the antibody and then received 100 μl of [$^{125}$I]CGRP-(28–37) (approximately 20,000–25,000 c.p.m.) and 50 μl of goat anti-rabbit antisera coupled to ferric beads. After another 24 h incubation, the [$^{125}$I]CGRP-(28–37) bound to the CGRP antibody was separated from free tracer via immunomagnetic separation (PerSeptive Diagnostics, Cambridge, MA). The liquid was aspirated from each test tube, and the immunoprecipitated reaction product counted on a gamma counter. Standard curves were generated with all drugs to exclude the possibility of non-specific effects in the RIA. Levels of immunoreactive CGRP were determined for each fraction using logit–log analysis. Under non-equilibrium conditions, the minimum detection limit for the assay is approximately 1–3 fmol/tube with 50% displacement at 10–30 fmol/tube. The intra- and inter-assay coefficients of variation are less than 5% and 12%, respectively.

### 2.10. Statistics

Data were analyzed with analysis of variance (ANOVA) followed by Duncan's multiple range test (parametric data). In Fig. 7 data were analyzed with Kruskal–Wallis followed by Dunn's multiple comparison test for nonparametric data because the data violated the homogeneity of variance requirement for parametric data. Receptor binding results were analyzed with GraphPad Prism software (San Diego, CA). Results were considered significant when the probability that they occurred due to chance alone was less than 5% (i.e., $P < 0.05$). $K_d$ and $B_{max}$ values were determined with nonlinear regression and one site and two site analyses were compared to determine the better fit. Data are reported as mean ± S.E.M.

## 3. Results

The carrageenan model of inflammation was used to evaluate intrathecal cannabinoid effects on the induction of thermal hyperalgesia. Thirty minutes after bilateral intraplantar carrageenan and intrathecal saline administration (carrageenan/saline), animals demonstrated significant hyperalgesia ($-7.5 \pm 1.1$ vs. $-3.4 \pm 1.4$ s; $P < 0.05$;

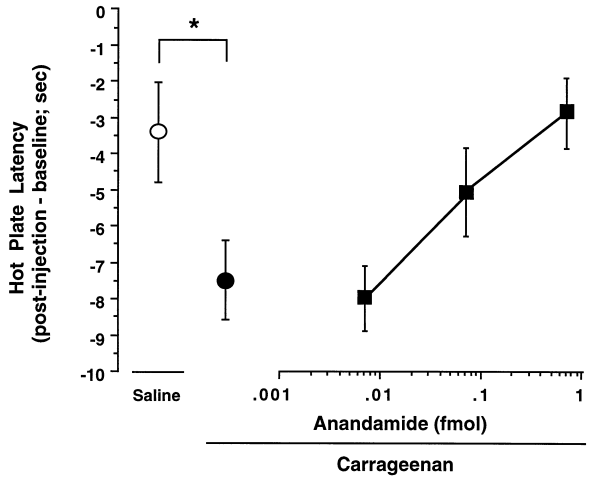


Fig. 1. Effect of intrathecal anandamide on carrageenan-induced hyperalgesia. Animals received a bilateral intraplantar injection of either saline or carrageenan. Animals receiving an intraplantar injection of saline immediately received an intrathecal injection of saline ($n = 8$; open circle). Animals receiving an intraplantar injection of carrageenan immediately received an intrathecal injection of saline ($n = 9$; closed circle) or 0.7 fmol ($n = 9$), 0.07 fmol ($n = 10$), or 0.0007 ($n = 8$) anandamide (closed squares). Baseline hot plate latencies were recorded prior to injections. Post-injection latencies were recorded 30 min after drug administration. Error bars are S.E.M. $* P < 0.05$ compared with saline.

ANOVA ($F(4,39) = 4.1$)) when compared with animals receiving the saline/saline combination (Fig. 1). Animals receiving the carrageenan/anandamide combination demonstrated a dose-dependent reversal of the carrageenan-induced hyperalgesia ($P < 0.005$; ANOVA/Linear Regression ($F(1,25) = 11.3$)). Hot plate latencies of animals receiving the 0.7 fmol dose of anandamide did not differ from those of animals receiving the saline/saline combination ($-2.9 \pm 0.9$ vs. $-3.4 \pm 1.4$ s). These results indicate that intrathecal administration of anandamide is capable of preventing the development of carrageenan-induced hyperalgesia.

The next question addressed was whether there is a change in the ability of cannabinoids to increase hot plate response latencies in inflamed as compared to uninflamed animals. Fig. 2 reveals that intrathecal doses of anandamide from 0.07 fmol to 70 pmol were incapable of producing antinociception in normal animals ($P =$ ns; ANOVA ($F(4,50) = 0.54$)). These results indicate that anandamide evokes antihyperalgesic effects at dosages which are devoid of antinociceptive activity.

Available evidence suggests that intrathecal administration of drugs results in a mostly spinal action (Hylden and Wilcox, 1980). Thus, we evaluated the presence of cannabinoid receptors in rat and mouse lumbar spinal cord. Cannabinoid receptors have previously been reported in cervical spinal cord (Herkenham et al., 1991). Binding of [$^3$H]CP 55,940 to the dorsal half of the rat lumbar enlargement is presented in Fig. 3. Analysis revealed a $K_d$ of $0.83 \pm 0.1$ nM and a $B_{max}$ of $0.82 \pm 0.03$ pmol/mg protein bound. The data fit a one site model better than a two site model. Consistent with a single site was the Hill coefficient of $1.08 \pm 0.07$. Similar binding parameters were determined in mouse lumbar spinal cord, with $K_d$ of $1.9 \pm 0.3$ nM and a $B_{max}$ of $1.2 \pm 0.1$ pmol/mg protein bound.

Having determined that cannabinoid receptor binding sites are present in the lumbar enlargement of rat and mouse spinal cord, we next evaluated whether the cannabinoid receptors were capable of modulating neurosecretion from capsaicin-sensitive fibers. We used the rat spinal cord superfusion model to test this hypothesis (Fig. 4). Anandamide administration had no effect on basal immunoreactive CGRP release from isolated rat spinal cord. However, coadministration of anandamide with 50 mM $K^+$ decreased $K^+$-evoked immunoreactive CGRP release from isolated rat spinal cord ($P < 0.01$; ANOVA ($F(6,48) = 6.99$)). Because CGRP located in the dorsal half of the spinal cord is primarily synthesized in dorsal root ganglion cells (Willis and Coggeshall, 1991), the inhibition of $K^+$-evoked immunoreactive CGRP release suggests that cannabinoids are capable of inhibiting neuropeptide release from the terminals of primary afferent fibers.

To test the hypothesis that anandamide inhibits neurosecretion from nociceptive primary afferent terminals, experiments were conducted with capsaicin. Capsaicin induces neurosecretion primarily from terminals of small diameter sensory neurons (Holzer, 1991).

We initially conducted a capsaicin concentration response curve in the spinal cord superfusion model (Fig. 5). Capsaicin evoked release of immunoreactive CGRP in a concentration-dependent manner with an $EC_{50}$ of 0.5 $\mu$M.

We then evaluated the ability of anandamide to inhibit capsaicin-evoked release of immunoreactive CGRP from rat spinal cord (Fig. 6). There was no effect of anandamide

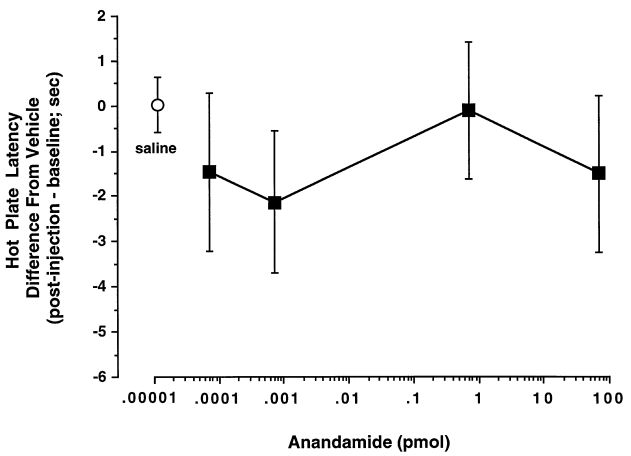


Fig. 2. Effect of intrathecal anandamide on hot plate latencies in normal animals. After baseline latencies were recorded, animals received an intrathecal injection of saline ($n = 19$, open circle) or 0.07 nmol ($n = 10$), 0.7 pmol ($n = 9$), 0.7 fmol ($n = 8$), or 0.07 fmol ($n = 9$) anandamide (closed squares). Hot plate latencies were again recorded 5 min after injection. Error bars are S.E.M.

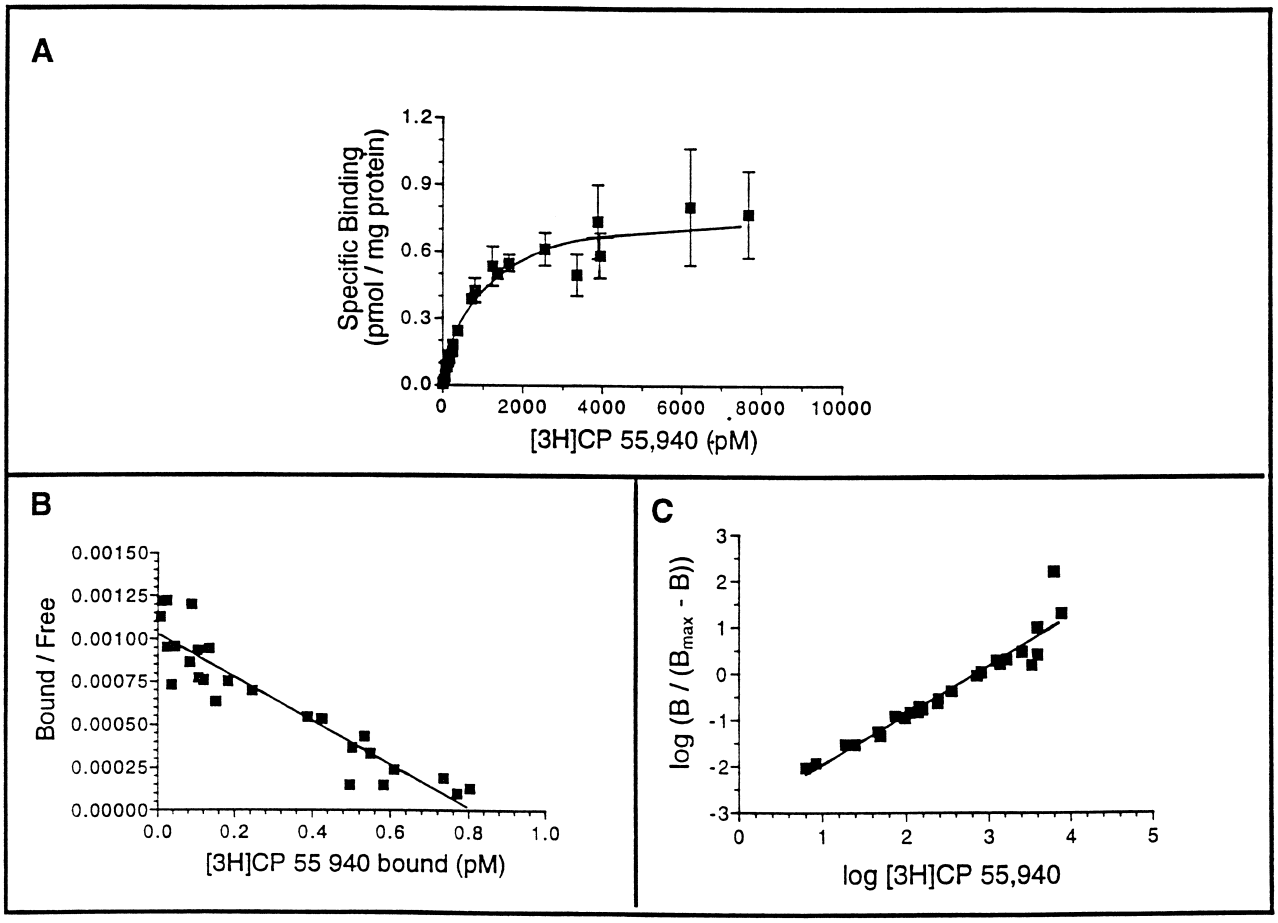


Fig. 3. Binding of [$^3$H]CP 55,940 in rat dorsal lumbar spinal cord. Tissue was collected, dissected, and immediately frozen on dry ice. Membranes were prepared and radioreceptor binding assay conducted as described in Section 2. (A) Saturation isotherm. Error bars are S.E.M. (B) Rosenthal (Scatchard) plot. (C) Hill plot.

on basal release of immunoreactive CGRP. There was, however, a significant time and drug interaction of anandamide after capsaicin administration ($P < 0.01$; ANOVA ($F(12,78) = 2.3$)). This indicates that 1 nM anandamide is capable of inhibiting immunoreactive CGRP release from the central terminals of capsaicin-sensitive primary affer-

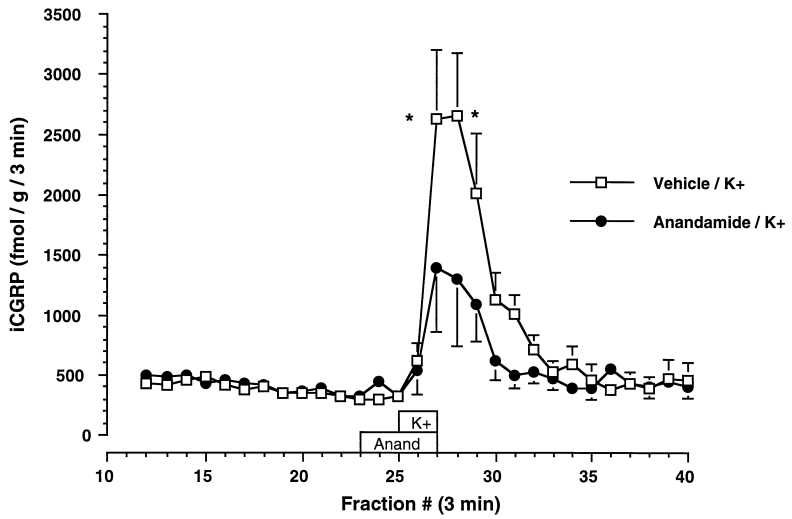


Fig. 4. Effect of anandamide on 50 mM $K^+$-evoked immunoreactive CGRP release from the dorsal half of lumbar spinal cord. Tissue was collected and superfused as described in Section 2. After equilibration, the tissue was treated with either vehicle ($n = 5$) or 100 nM anandamide ($n = 5$) for 6 min followed by 50 mM $K^+$ in the presence of the pretreated drug or vehicle for 6 min. Superfusate was assayed for immunoreactive CGRP using radioimmunoassay. Data represent mean ± S.E.M. $* P < 0.05$.

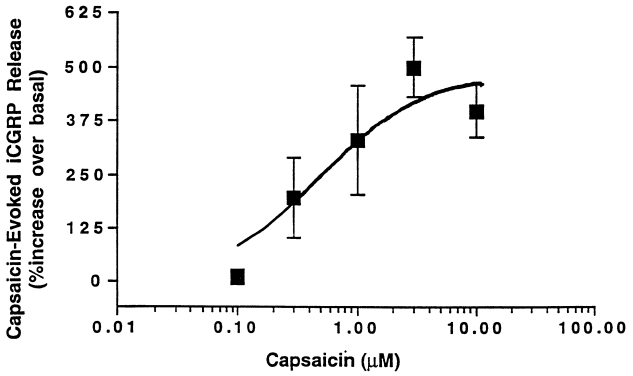


Fig. 5. Effect of capsaicin on immunoreactive CGRP release from the dorsal half of lumbar spinal cord. Tissue was collected and superfused as described in Section 2. After equilibration, tissue was treated with various concentrations of capsaicin for 6 min ($n = 4–8$/group). Data is represented as the percentage increase in immunoreactive CGRP release over basal release. Error bars are S.E.M.

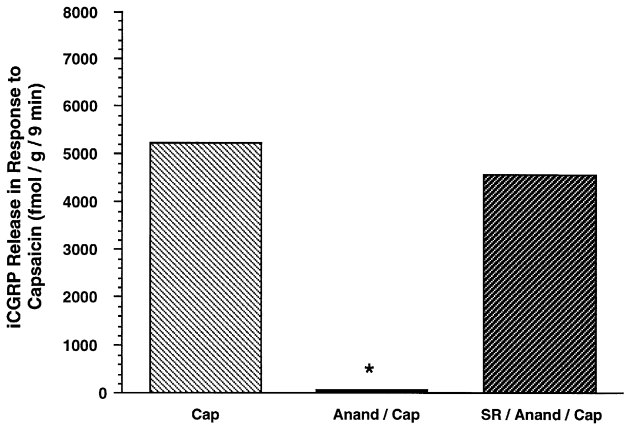


Fig. 7. Effect of anandamide ± the cannabinoid $CB_1$ receptor antagonist SR 141716A on capsaicin-evoked immunoreactive CGRP release from the dorsal half of rat lumbar spinal cord. Tissue was collected and superfused as described in Section 2. After equilibration, the tissue was treated with vehicle or 10 nM SR 141716A ('SR') for fraction 22 ($n = 3$). Tissue was then treated with vehicle, 10 nM SR 141716A/1 nM anandamide, or 1 nM anandamide ('Anand', $n = 4$) for 6 min (fraction 23 and 24). Tissue was stimulated with 300 nM capsaicin ('Cap') in the presence of the pretreated drug or vehicle ($n = 6$) for fractions 25 and 26. Superfusate was assayed using radioimmunoassay for immunoreactive CGRP. Data are represented as mean ± S.E.M. $* P < 0.05$ vs. Cap.

ent fibers. We next evaluated whether the inhibition by anandamide was reversible with the selective cannabinoid $CB_1$ receptor antagonist SR 141716A (Fig. 7). Pretreatment of the tissue with SR 141716A resulted in a reversal of anandamide's inhibition of capsaicin-evoked release ($P < 0.05$; Kruskal–Wallis).

The ability of anandamide to inhibit $K^+$- and capsaicin-evoked immunoreactive CGRP release supports the hypothesis that anandamide is interacting with receptors on the terminals of primary afferent fibers to modulate neurotransmitter release. An alternative explanation is that anandamide is indirectly modulating primary afferent release via interactions with receptors on fibers from cells located in the spinal cord or supraspinally. If cannabinoid receptors are located on the terminals of primary afferent fibers, we would expect them to be synthesized in the sensory neuron. Thus, we evaluated cannabinoid receptor binding in sensory neurons using the rat trigeminal ganglion as a model system. Analysis revealed concentration-dependent, saturable binding with a $K_d$ of $1.2 \pm 0.3$ nM and a $B_{max}$ of $0.22 \pm 0.02$ pmol/mg protein bound (Fig.

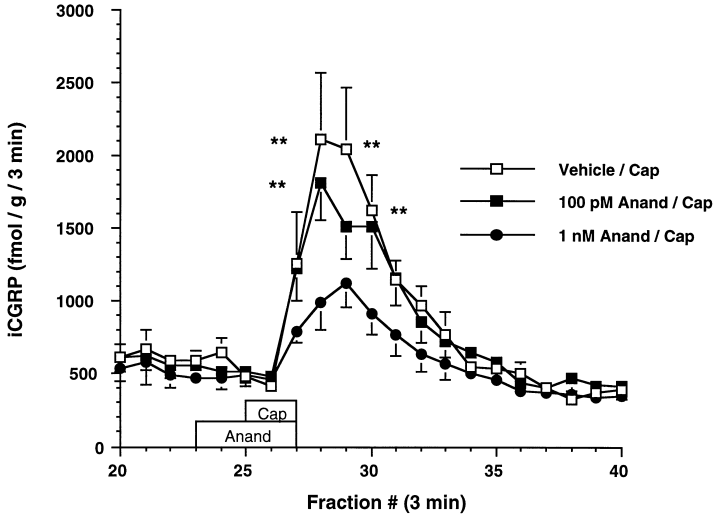


Fig. 6. Effect of 100 pM or 1 nM anandamide on capsaicin-evoked immunoreactive CGRP release from the dorsal half of rat lumbar spinal cord. Tissue was collected and superfused as described in Section 2. After equilibration, the tissue was treated with vehicle ($n = 4$) or 100 pM ($n = 6$) or 1 nM ($n = 6$) anandamide ('anand') for 6 min. Tissue was then treated with 3 μM capsaicin ('cap') in the presence of the pretreated drug or vehicle for 6 min. Superfusate was assayed for immunoreactive CGRP using radioimmunoassay. Data are represented as mean ± S.E.M. $** P < 0.01$ vs. 1 nM anandamide/capsaicin.

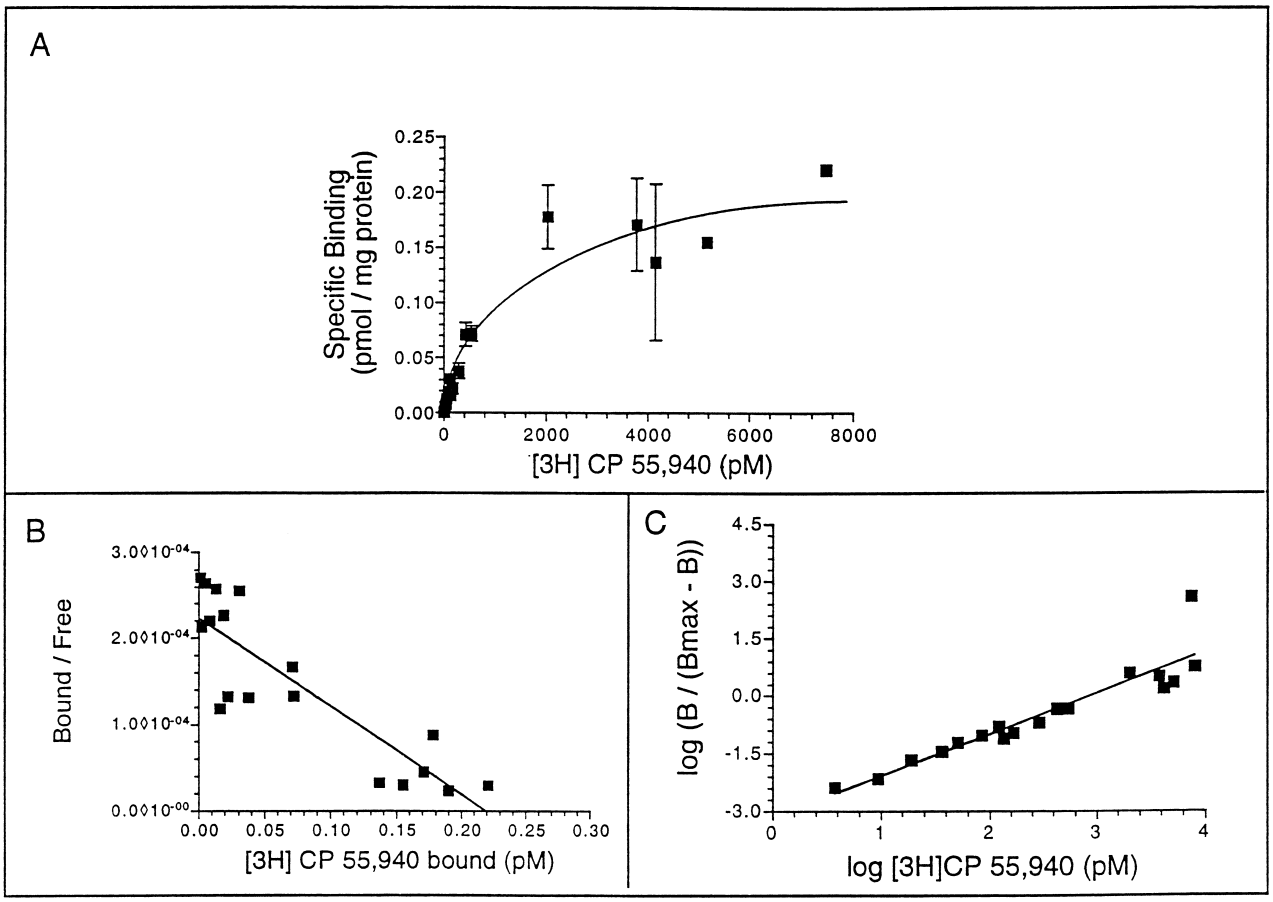


Fig. 8. Binding of [$^3$H]CP 55,940 to trigeminal ganglion. Membrane homogenates were prepared and radioreceptor binding assay was performed as described in Section 2. (A) Saturation isotherm. Error bars are S.E.M. (B) Rosenthal (Scatchard) plot. (C) Hill plot.

8). The Hill coefficient was $1.08 \pm 0.11$ consistent with a single site.

## 4. Discussion

The present study demonstrates that intrathecal anandamide is capable of preventing carrageenan-induced hyperalgesia in a dose-dependent manner when administered at the same time as carrageenan. To better understand the effects of cannabinoids on thermal nociceptive thresholds, we attempted to compare the potency of anandamide in normal and inflamed animals. However, we were unable to measure antinociception with anandamide in normal animals using concentrations comparable to the carrageenan studies (0.7 fmol) as well as concentrations 100 000 fold higher (70 pmol). The time course for evaluating the effects of anandamide on hot plate latencies was different in normal and inflamed animals. In the inflamed animals, we administered carrageenan immediately followed by anandamide. We tested hot plate latencies 30 min later. Because the effects of intrathecal anandamide peak at 5 min and are gone within 20 min (Smith et al., 1994), this design enabled us to determine that intrathecal anandamide can prevent the development of hyperalgesia. The ability of anandamide to inhibit the induction of hyperalgesia has also been observed after peripheral administration of anandamide (Richardson et al., 1998b). Anandamide's short timecourse after intrathecal administration, however, precluded us from using the same design in normal animals. Thus, in normal animals antinociception was evaluated 5 min after injection which is the time of peak effect on nociceptive thresholds after intrathecal administration (Smith et al., 1994). The lack of effect at these concentrations in normal animals is consistent with findings from other studies in which antinociception was observed after intrathecal administration of anandamide but only at concentrations much greater than those used in the present study (Smith et al., 1994). These results indicate that there is a greater effectiveness of spinal cannabinoids during hyperalgesia. Such increases have been reported for other ligands such as $\mu$-opioid agonists (Hylden et al., 1991; Stanfa et al., 1992). There are a number of explanations which could account for the increased effectiveness of the cannabinoids during inflammation. These include a decrease in the activity of a functional antagonistic system, synergism with other ligands that are active only during inflammation, an increase in efficacy of a cannabinoid receptor subtype with inflammation, and an increase in

cannabinoid receptor affinity. Since these hypotheses are not mutually exclusive, all or some combination may contribute to the observed increase in anandamide effectiveness.

Because carrageenan produces hyperalgesia by both peripheral and central mechanisms, anandamide's antihyperalgesic effect could theoretically be at either site. However, we have administered anandamide intrathecally to preferentially deliver it to the lumbar spinal cord. Hylden and Wilcox (1980) have demonstrated that 10 min after intrathecal administration, methylene blue is concentrated near the injection site and ''never extended rostral of thoracic segments.'' Additionally, 10 min after intrathecal administration, more than 95% of the injected dose of [$^3$H]morphine was restricted to the spinal cord. These results suggest that drugs administered intrathecally are likely to be concentrated in the spinal cord at least over short periods of time. The identification of cannabinoid binding sites in the lumbar enlargement of the spinal cord is consistent with a spinal site of action.

There are at least three possible mechanisms by which anandamide may act spinally to prevent carrageenan-induced hyperalgesia. First, it may prevent the afferent barrage by acting presynaptically to inhibit neurotransmitter release. The presynaptic inhibition of neurosecretion could be accomplished via either closing of $Ca^{2+}$ channels to prevent exocytosis or stabilization of the membrane potential at subthreshold levels to prevent depolarization and subsequent neurosecretion. Activation of the cannabinoid receptor could result in both of these via the closing of $Ca^{2+}$ channels (Mackie and Hille, 1992; Caulfield and Brown, 1992), inhibition of adenylyl cyclase activity (Howlett, 1984), and opening of $K^+$ channels (Deadwyler et al., 1993; Henry and Chavkin, 1995). Second, cannabinoids may act postsynaptically to stabilize membrane potentials at subthreshold levels via the enhancement of $K^+$ currents and thus, prevent the transduction of the nociceptive message. Finally, cannabinoids may lead to disinhibition of an inhibitory circuit, i.e., inhibition of terminal A removes the inhibition of terminal B resulting in the release of an antihyperalgesic substance. All three mechanisms have been proposed to mediate actions of opioids (Mudge et al., 1979; Dickenson, 1994; Stein, 1994).

If the cannabinoids are producing antihyperalgesia by acting presynaptically to inhibit neurosecretion, then we may be able to detect such an effect in the superfusion model of isolated spinal cord. We chose to evaluate the activity of anandamide on immunoreactive CGRP release because the majority of CGRP in the dorsal half of the spinal cord is located in terminals of primary afferent fibers (Willis and Coggeshall, 1991). Administration of anandamide suppressed $K^+$-evoked immunoreactive CGRP release from isolated rat spinal cord, suggesting that anandamide inhibits release of immunoreactive CGRP from central terminals of primary afferent fibers. To evaluate whether anandamide is inhibiting immunoreactive CGRP release from nociceptive fibers, we stimulated neurosecretion with capsaicin, which will activate a subset of mostly C- but also some A$\delta$-fibers (Holzer, 1991). Pretreatment of anandamide suppressed capsaicin-evoked immunoreactive CGRP release from isolated spinal cord in an SR 141716A-sensitive manner. These results suggest that cannabinoid $CB_1$ receptor activation inhibits neurosecretion from capsaicin-sensitive primary afferent fibers.

As mentioned above, anandamide may be acting directly at receptors located on the central terminals of primary afferent fibers to inhibit synaptic transmission. This has been demonstrated with opioids (Gamse et al., 1979; Aimone and Yaksh, 1989) which share many features with the cannabinoids such as signal transduction pathways. If cannabinoid receptors are located on these terminals, then one would expect the receptor to be synthesized in the sensory neuron. The sensory neuron sending projections to the lumbar enlargement is the dorsal root ganglion cells. However, for technical reasons relating to tissue availability, we chose the trigeminal ganglion as the prototype tissue to evaluate cannabinoid receptor binding on sensory neurons. Radioreceptor assays indicate that [$^3$H]CP 55,940 bound to a single site in the trigeminal ganglion in a saturable manner. This finding indicates that cannabinoid receptors are expressed in primary afferent fibers, possibly including the capsaicin-sensitive class of fibers.

The ability of cannabinoids to inhibit neurosecretion from certain nociceptive fibers may explain the increased effectiveness of cannabinoids during hyperalgesia. Both C polymodal and A mechanoheat fibers are mostly quiescent under normal conditions but are activated under conditions of hyperalgesia such as those produced by carrageenan (Perl, 1992; Meyer et al., 1994). Thus, activation of cannabinoid receptors on these terminals would have little effect under normal conditions. However, when the nociceptive fibers were activated by carrageenan, activation of cannabinoid receptors could now have a detectable effect. This hypothesis is consistent with the results of our superfusion experiments in that anandamide had little effect on basal release but detectable effect on capsaicin-stimulated release.

In conclusion, we have demonstrated that spinal administration of cannabinoids increases thermal nociceptive thresholds more effectively in hyperalgesic than in normal animals. One potential mechanism for the antihyperalgesia is the presynaptic inhibition of neurosecretion from the central terminals of capsaicin-sensitive fibers. The ability of cannabinoids to inhibit neurosecretion from capsaicin-sensitive primary afferent fibers may prove to be clinically beneficial and provide a novel therapeutic treatment for disorders characterized by primary afferent barrage. Furthermore, the increase in potency of cannabinoids during hyperalgesia may mean that there are dosages of cannabinoids that would be effective as antihyperalgesic agents but subthreshold for the untoward psychomimetic effects.

## Acknowledgements

SR 141716A was a gift from Sanofi Recherché (Montpellier, France). This work was supported by a predoctoral grant from the Howard Hughes Medical Institute (JDR) and by NIH DE9860 (KMH). The authors wish to thank Catherine Harding-Rose and Mary Ann Sabino for technical assistance.

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