



## Cannabinoids reduce hyperalgesia and inflammation via interaction with peripheral CB<sub>1</sub> receptors

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### Abstract

Central antinociceptive effects of cannabinoids have been well documented. However, relatively little is known about the peripheral effects of the cannabinoids on inflammation. In the present study, we evaluated the effects of peripherally administered cannabinoids on three indices of inflammation: carrageenan-induced thermal hyperalgesia, carrageenan-induced edema, and capsaicin-induced plasma extravasation. In addition, we determined the effect of cannabinoids on capsaicin-evoked neuropeptide release from isolated rat hindpaw skin. Our results indicate that cannabinoids produce antihyperalgesia via interaction with a peripheral CB<sub>1</sub> receptor. Peripheral, but not systemic, administration of 0.01 ng anandamide inhibited the induction of hyperalgesia. Peripheral administration of anandamide also attenuated hyperalgesia after its development via interaction with the CB<sub>1</sub> cannabinoid receptor subtype as indicated by its reversal with the CB<sub>1</sub> receptor antagonist SR 141716A. Additionally, peripheral, but not systemic, administration of 0.01 ng anandamide inhibited edema. Peripherally administered cannabinoids also interacted with CB<sub>1</sub> receptors to inhibit capsaicin-evoked plasma extravasation into the hindpaw. One potential mechanism for the anti-inflammatory actions of the cannabinoids is the inhibition of neurosecretion from the peripheral terminals of nociceptive primary afferent fibers. This hypothesis is supported by the finding that anandamide inhibited capsaicin-evoked release of calcitonin gene-related peptide from isolated hindpaw skin. Collectively, these results indicate that cannabinoids reduce inflammation via interaction with a peripheral CB<sub>1</sub> receptor. A potential mechanism for this effect is the inhibition of neurosecretion from capsaicin-sensitive primary afferent fibers. © 1998 International Association for the Study of Pain. Published by Elsevier Science B.V.

**Keywords:** Cannabinoid; Peripheral; Hyperalgesia; Capsaicin; Neurotransmitter release; Carrageenan

### 1. Introduction

Reports of marijuana-induced analgesia date back millennia (Hall et al., 1994). However, studying the antinociceptive and antihyperalgesic roles of cannabinoids has proved difficult because of the lack of identified receptors and selective agonists and antagonists. Recently, the CB<sub>1</sub> and CB<sub>2</sub> receptor subtypes were cloned and determined to be members of the G protein-coupled receptor family (Matsuda

et al., 1990; Munro et al., 1993). Additionally, in the last few decades selective synthetic cannabinoids have been developed and endogenous cannabinoids discovered (Lemberger and Rowe, 1975; Johnson et al., 1981; D'Ambra et al., 1992; Devane et al., 1992; Hanus et al., 1993; Mechoulam et al., 1995; Sugiura et al., 1995). Such developments have resulted in the determination that activation of central cannabinoid receptors produces antinociception (Yaksh, 1981; Herkenham et al., 1991; Lichtman and Martin, 1991; Martin et al., 1993, 1995, 1996; Smith et al., 1994; Hohmann et al., 1995; Lichtman et al., 1996; Hohmann and Herkenham, 1997; Richardson et al., 1998a,b).

While the role of peripheral cannabinoid receptors in nociceptive modulation is not as well understood as that of the central cannabinoid receptors, peripheral CB<sub>2</sub> receptors have been reported to modulate inflammation. Palmitoylethanolamide, a selective CB<sub>2</sub> cannabinoid receptor

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agonist, can attenuate mechanical hyperalgesia induced by carrageenan (Mazzari et al., 1996). One potential mechanism for the antihyperalgesic effect of palmitoylethanolamide is the inhibition of mast cell degranulation (Facci et al., 1995; Mazzari et al., 1996) which would modulate the release of histamine and serotonin. Both of these compounds are capable of producing vasodilatation, increased vascular permeability (plasma extravasation), and activation of nociceptors. These contribute to the observed effects of hyperthermia, edema, and hyperalgesia which are characteristic of carrageenan-induced hyperalgesia (Vinegar et al., 1976; Hargreaves et al., 1988; Rang et al., 1994). While the role of peripheral CB<sub>2</sub> receptors in inflammation has been evaluated, the potential role of peripheral CB<sub>1</sub> receptors is not known.

The CB<sub>1</sub> receptor has been found throughout the central nervous system. Although binding studies have found peripheral tissues to be negative for cannabinoid binding sites (Jansen et al., 1992; Lynn and Herkenham, 1994; Rinaldi-Carmona et al., 1996), CB<sub>1</sub> cannabinoid receptor mRNA is detectable in a number of peripheral tissues (Galiègue et al., 1995; Shire et al., 1995). We have previously demonstrated that activation of spinal CB<sub>1</sub> receptors can result in both antihyperalgesia and inhibition of neuropeptide release from central terminals of capsaicin-sensitive primary afferent nociceptive fibers (Richardson et al., 1998b). Additionally, we have demonstrated that cannabinoid binding sites can be measured in sensory neurons (Richardson et al., 1998b). Hohmann and Herkenham (1997) have reported that CB<sub>1</sub> receptor mRNA is expressed in substance P containing neurons in the dorsal root ganglia. Thus, we have hypothesized that CB<sub>1</sub> cannabinoid receptors are synthesized in sensory neurons and transported bidirectionally to both the peripheral and central terminals of primary afferent nociceptive fibers. Furthermore, activation of these CB<sub>1</sub> receptors could serve to inhibit neuropeptide release which, in the periphery, could result in both anti-inflammatory and antihyperalgesic effects. These hypotheses were tested in the present study.

## 2. Methods

### 2.1. Animals

Male Sprague–Dawley rats (Harlan, 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. Reagents

Reagents were obtained from the following companies: anandamide and methanandamide, Cayman Chemical (Ann Arbor, MI); SR 141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-

carboxamide), a gift from Sanofi Recherche (Montpellier, France); capsaicin, Fluka (Ronkonkoma, NY); CGRP antisera (MI-2), kindly provided by Mike Iadorola; goat anti-rabbit antisera coupled to ferric beads, PerSeptive Diagnostics (Boston, MA); thiorphan, Bachem (Torrance, CA); all other reagents, Sigma (St. Louis, MO).

### 2.3. Thermal hyperalgesia

Paw withdrawal latencies were recorded using the radiant heat method (Hargreaves et al., 1988). Briefly, animals were placed in a clear acrylic box on a glass platform (Plantar Test, Ugo Basile, Italy). A beam of radiant heat was applied through the platform to the plantar surface of the hindpaw. A photocell detected paw withdrawal and the latency was recorded. After recording baseline paw withdrawal latencies in duplicate, animals received an intraplantar (i.pl.) injection of 3 mg carrageenan in a 100  $\mu$ l volume. This was immediately followed by either a 50  $\mu$ l i.pl. or intraperitoneal (i.p.) injection of the endogenous cannabinoid anandamide (0.01 ng) with vehicle (1% ethanol) injection in the opposite site to maintain a double-dummy experimental design. Paw withdrawal latencies were recorded in duplicate 1 h after drug administration. In another set of studies, paw withdrawal latencies were recorded 2 h after carrageenan administration. This was followed by a 50  $\mu$ l i.pl. injection of anandamide (10 ng), 100 ng of the selective CB<sub>1</sub> receptor antagonist SR 141716A (Rinaldi-Carmona et al., 1994; Felder et al., 1995; Rinaldi-Carmona et al., 1995), anandamide (10 ng)/SR 141716A (100 ng), or vehicle (1% ethanol). The final paw withdrawal latency was recorded in duplicate at 2.5 h post-carrageenan. Treatments were randomly allocated to animals and observers were blind to treatment allocations.

### 2.4. Edema

Edema was measured using a plethysmometer (Stoelting, Chicago, IL). After baseline paw volumes were measured, animals received a bilateral i.pl. injection of 3 mg carrageenan. This was followed by either a unilateral 50  $\mu$ l i.pl. injection of 0.01 ng anandamide and an i.p. injection of the 1% ethanol/saline vehicle or a unilateral i.pl. injection of the vehicle and an i.p. injection of 0.01 ng anandamide. Paw volumes were measured again 3 h after drug administration.

### 2.5. Plasma extravasation

Plasma extravasation was determined as described by Joris et al. (1990) with slight modification. Animals were anesthetized with sodium pentobarbital (50 mg/kg) and injected intravenously (i.v.) with 75 mg/kg Evans blue dye. After 5 min they received a 50  $\mu$ l i.pl. injection of either the vehicle (0.1% ethanol/saline), methanandamide (175 ng), SR 141716A (250 ng), or methanandamide (175 ng)/

SR 141716S (250 ng). After another 5 min they received a 20  $\mu\text{g}$  i.pl. injection of capsaicin (50  $\mu\text{l}$ ). Twenty minutes after capsaicin, 6 mm punch biopsies were collected from the hind paw plantar skin and extracted with 1 ml formamide for 24 h and then read on a spectrophotometer at a wavelength of 568 nm to determine optical density. These values were compared to a standard curve in order to determine the amount of Evans blue dye in each sample.

## 2.6. Superfusion

A model of rat skin superfusion has recently been developed in our laboratory (Kilo et al., 1997). In this model, immunoreactive calcitonin gene-related peptide (iCGRP) release can be evoked from an in vitro preparation of rat hindpaw skin in a concentration-dependent manner by capsaicin. The  $\text{EC}_{50}$  for this effect is approximately 30  $\mu\text{M}$  with maximal release occurring at 100  $\mu\text{M}$ . Capsaicin-evoked release can be inhibited by the elimination of calcium from the buffer, addition of the capsaicin receptor antagonist capsazepine, or neonatal treatment of the animals with capsaicin to destroy capsaicin-sensitive fibers. In the present study, we have used the rat skin superfusion model to evaluate the ability of anandamide to inhibit neurosecretion from the peripheral terminals of capsaicin-sensitive fibers.

The dorsal hindpaw skin was dissected from proximal digits to the ankle ( $209 \pm 8$  mg). The biopsy was placed in a 2 cc chamber with the corium side exposed to a continual flow of oxygenated synthetic interstitial fluid (SIF) buffer ( $34^\circ\text{C}$ ,  $\text{pH} = 7.4$ , ca. 250  $\mu\text{l}/\text{min}$ ; Brandel Superfusion Pump, Brandel, Gaithersburg, MD). SIF buffer consists of NaCl (108 mM), KCl (3.5 mM),  $\text{MgSO}_4$  (0.7 mM),  $\text{NaHCO}_3$  (26 mM),  $\text{NaH}_2\text{PO}_4$  (1.67 mM), Na gluconate (9.6 mM),  $\text{CaCl}_2$  (1.5 mM), glucose (5.5 mM), sucrose (7.6 mM), ascorbate (0.09 mM), and thiorphan (10  $\mu\text{M}$ ). A similar buffer has been used in electrophysiology experiments in rat hindpaw skin (Reeh, 1986). The superfusate was collected into test tubes by a fraction collector (Gilson, Middleton, WI). Each fraction represents 10 min of collection time. Following equilibration of the tissue and baseline collection (ca. 90 min), anandamide or vehicle was administered for 10 min. Capsaicin was then co-administered in the presence of the pretreated drug or vehicle for 7 min. Superfusate was collected for an additional eight fractions.

## 2.7. Radioimmunoassay

Superfusate was analyzed for immunoreactive calcitonin gene-related peptide (iCGRP) using a previously validated radioimmunoassay (RIA). 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 FMRF-amide peptides. Immediately following the experiment, samples were

pre-incubated for 48 h ( $4^\circ\text{C}$ ) with 100  $\mu\text{l}$  of the antibody and then received 100  $\mu\text{l}$  of [ $^{125}\text{I}$ ]CGRP $_{28-37}$  (approximately 20 000–25 000 cpm) and 50  $\mu\text{l}$  of goat anti-rabbit antisera coupled to ferric beads. After another 48 h incubation, the [ $^{125}\text{I}$ ]CGRP $_{28-37}$  bound to the CGRP antibody was separated from free via immunomagnetic separation (PerSeptive Diagnostics, Boston, MA). The liquid was aspirated from each test tube, and the immunoprecipitated reaction product was counted on a gamma counter. Standard curves were generated with all drugs to exclude the possibility of non-specific effects in the RIA. iCGRP levels were determined for each fraction using logit-log analysis. Under non-equilibrium conditions, the minimum detection limit for the assay is approximately 2–3 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.

## 2.8. Statistics

Data were analyzed with contrast, *t*-test with Welch's correction, ANOVA or ANCOVA followed by a multiple comparison test as appropriate. Results were considered significant when the probability that they occurred due to chance alone was less than 5% (i.e.,  $P < 0.05$ ). Data are reported as mean  $\pm$  SEM.

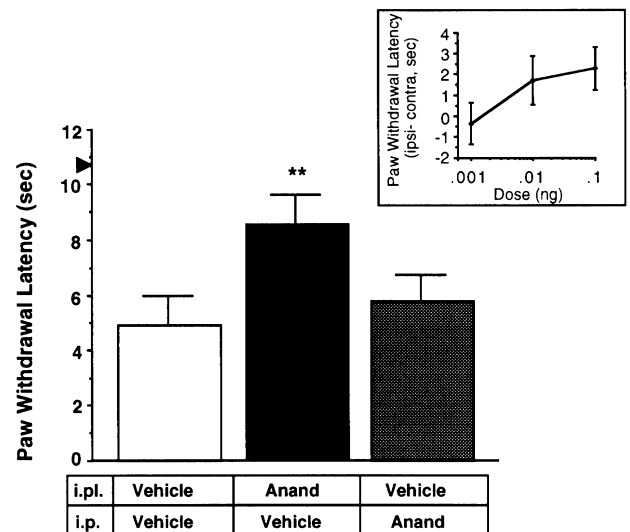


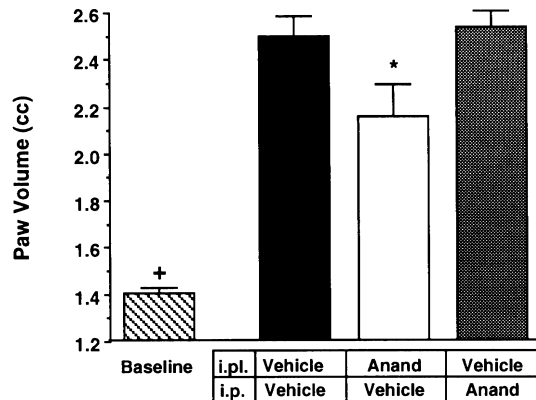
Fig. 1. Effect of systemic and peripheral anandamide administration on carrageenan-induced hyperalgesia. Animals received an intraplantar (i.pl.) injection of carrageenan followed by an intraperitoneal (i.p.) injection of either 0.01 ng anandamide or vehicle. Animals then received an i.pl. injection of the opposite drug. Paw withdrawal latencies were measured prior to carrageenan and 1 h after carrageenan. Arrowhead depicts mean baseline paw withdrawal values. Data are mean  $\pm$  SEM;  $n = 8$  for all groups.  $**P < 0.05$  compared with both vehicle and i.p. anandamide groups. The inset represents results from animals receiving a bilateral i.pl. injection of carrageenan followed by a unilateral i.pl. administration of 0.001, 0.01, or 0.1 ng anandamide. Results are represented as difference between the ipsilateral ('ipsi') paw withdrawal latency and the contralateral ('contra') paw withdrawal latency 1 h after carrageenan. Data are mean  $\pm$  SEM;  $n = 8$  for all groups.

### 3. Results

We first evaluated whether peripheral administration of the endogenous cannabinoid anandamide was capable of inhibiting the induction of carrageenan-induced hyperalgesia. There were no differences in baseline paw withdrawal latencies for any of the groups (ANOVA ( $F_{(2,21)} = 1.3$ );  $P = \text{NS}$ ). Animals were hyperalgesic 1 h after intraplantar (i.pl.) administration of carrageenan ( $11.1 \pm 0.37$  vs.  $4.94 \pm 1.2$  s;  $P < 0.001$ ). Intraplantar, but not intraperitoneal (i.p.), administration of anandamide inhibited carrageenan-induced hyperalgesia ( $P < 0.05$ ;  $F_{(1,35)} = 4.8$ ; Fig. 1). Additionally, this effect is dose related (Fig. 1 inset;  $P < 0.05$ ;  $F_{(4,67)} = 2.91$ ). These results suggest that cannabinoids are capable of blocking the induction of carrageenan-induced hyperalgesia via a local site of action in the periphery rather than by diffusing into the blood stream and being distributed to a distant site of action.

Fig. 2 presents the effect of peripheral versus systemic

#### A. Ipsilateral Hindpaws



#### B. Contralateral Hindpaws

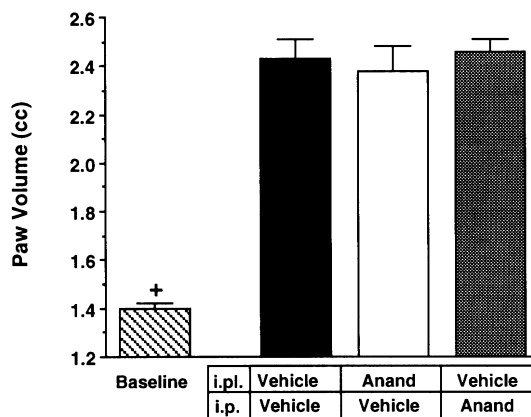


Fig. 2. Effect of systemic versus peripheral anandamide on carrageenan-induced edema. Animals received an i.pl. injection of carrageenan followed by a unilateral i.pl. injection of either 0.01 ng anandamide or vehicle. Animals then received an i.p. injection of the opposite drug. Paw volumes were measured prior to carrageenan ('Baseline') and 3 h after carrageenan in the ipsilateral (A) and contralateral (B) paw. Data are means  $\pm$  SEM;  $n = 8$  for all groups.  $^+P < 0.05$  vs. all other groups;  $*P < 0.05$  compared with both vehicle and i.p. anandamide.

anandamide on the induction of edema by carrageenan. There were no differences in baseline paw volumes between groups for either the right (ANOVA ( $F_{(2,21)} = 0.09$ );  $P = \text{NS}$ ) or left (ANOVA ( $F_{(2,21)} = 0.07$ );  $P = \text{NS}$ ) paw. Three hours after carrageenan administration there was an increase in paw volume in both paws ( $1.40 \pm 0.02$  vs.  $2.49 \pm 0.9$  ml;  $P < 0.05$ ; ANOVA ( $F_{(3,44)} = 89.3$ ) and  $1.42 \pm 0.02$  vs.  $2.43 \pm 0.08$  ml;  $P < 0.05$ ; ANOVA ( $F_{(3,44)} = 138.8$ )). The increased paw volume was reduced by the concurrent unilateral i.pl. administration of anandamide but not by i.p. anandamide administration ( $2.15 \pm 0.14$  vs.  $2.53 \pm 0.07$  ml). Additionally, there was no effect of either the unilateral i.pl. or i.p. administration of anandamide on the contralateral paw ( $2.38 \pm 0.10$  and  $2.46 \pm 0.05$  ml). These results suggest that anandamide is capable of inhibiting carrageenan-induced edema via a local site of action in the periphery rather than by diffusing into the blood stream and being distributed to a distant site of action.

In Fig. 1 we demonstrated that anandamide could inhibit the induction of hyperalgesia; we also evaluated whether anandamide could attenuate hyperalgesia once it had been induced (Fig. 3). There were no differences in baseline latencies between the groups (ANOVA ( $F_{(3,50)} = 1.68$ );  $P = \text{NS}$ ). Two hours post-carrageenan, paw withdrawal latencies were measured to assess the induction of hyperalgesia. There was no difference among the groups in paw withdrawal latencies at the 2 h timepoint; however, there was an overall decrease in paw withdrawal latencies when compared to baseline latencies indicating the induction of hyperalgesia ( $8.97 \pm 0.22$  vs.  $3.24 \pm 0.13$  s; Fig. 3;  $P < 0.001$ ; ANOVA ( $F_{(5,156)} = 91.1$ )). Immediately following the measurement of paw withdrawal latencies at the 2 h timepoint, animals received an i.pl. injection of anandamide (10 ng), the selective CB<sub>1</sub> receptor antagonist SR 141716A (100 ng), anandamide (10 ng)/SR 141716A (100 ng), or the vehicle. Thermal hyperalgesia was still present 30 min after vehicle administration ( $4.70 \pm 0.48$  s). It was, however, attenuated by the i.pl. administration of anandamide ( $6.87 \pm 0.48$  s). SR 141716A administration blocked development of anandamide-induced antihyperalgesia ( $5.12 \pm 0.45$  s) but had no effect on its own ( $5.13 \pm 0.35$ ). These results suggest that anandamide is capable of inhibiting the maintenance of carrageenan-induced hyperalgesia via interaction with a peripheral CB<sub>1</sub> cannabinoid receptor.

Carrageenan-induced edema has been reported to have a neurogenic component (Nagahisa et al., 1992) so we evaluated the ability of the anandamide congener methanandamide, which is reported to be more potent and more stable than anandamide (Abadji et al., 1994), to inhibit plasma extravasation evoked by capsaicin, which selectively induces a neurogenic inflammation (Holzer, 1991). In the absence of capsaicin,  $7.3 \pm 2.1$   $\mu\text{g}$  Evans blue dye was detected in the biopsy (Fig. 4). Evans blue dye was increased with capsaicin administration to  $14.0 \pm 2.3$   $\mu\text{g}$  ( $P < 0.05$ ;  $F_{(1,25)} = 4.0$ ). Intraplantar administration of 175 ng methanandamide blocked the increase in plasma

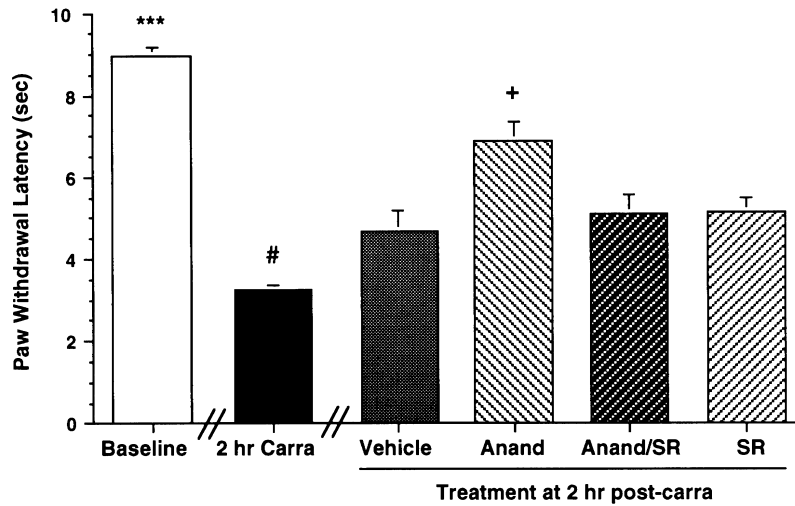


Fig. 3. Effect of peripheral anandamide and SR 141716A administration on paw withdrawal latencies after the induction of hyperalgesia. Animals received an i.pl. injection of carrageenan (3 mg). Paw withdrawal latencies were recorded prior to injection ('Baseline',  $n = 54$ ) and 2 h after injection ('2 h Carra',  $n = 54$ ). Animals then received an i.pl. injection of either the vehicle (1% ethanol,  $n = 12$ ), 10 ng anandamide ('Anand',  $n = 15$ ), 10 ng anandamide/100 ng SR 141716A ('Anand/SR',  $n = 14$ ), or 100 ng SR 141716A ('SR',  $n = 13$ ). Paw withdrawal latencies were measured 30 min later. \*\*\* $P < 0.001$  vs. all groups; # $P < 0.05$  vs. Vehicle, Anand, Anand/SR, and SR; + $P < 0.05$  vs. Vehicle, Anand/SR, and SR.

extravasation ( $8.2 \pm 2.5 \mu\text{g}$ ) in an SR 141716A-sensitive manner ( $13.4 \pm 1.3 \mu\text{g}$ ). These results indicate that peripheral administration of cannabinoids can inhibit neurogenic inflammation as measured by plasma extravasation of Evans blue dye.

One mechanism by which capsaicin induces neurogenic inflammation is via neurosecretion from peripheral terminals of primary afferent nociceptive fibers. The results from the previous experiment, i.e., the ability of cannabinoids to inhibit capsaicin-induced neurogenic inflammation, may be due to the inhibition of capsaicin-evoked neurosecretion. This hypothesis was tested in the next set of experiments and the results are presented in Fig. 5. Anandamide (1 nM) has no effect on basal release but has a simple main effect on inhibiting capsaicin-evoked iCGRP release ( $P < 0.05$ ; ANOVA ( $F_{(1,14)} = 4.54$ ); Fig. 5A). Fig. 5B represents the effects of three different concentrations of anandamide on the ability of capsaicin to evoke iCGRP release from isolated rat hindpaw skin. There is a significant increase in iCGRP release with capsaicin or 0.1 nM anandamide/capsaicin compared to baseline. Both 1 nM anandamide/capsaicin and 1000 nM anandamide/capsaicin significantly inhibit capsaicin-evoked iCGRP release ( $P < 0.001$ ; ANOVA ( $F_{(4,47)} = 17.1$ )). These results are consistent with the hypothesis that cannabinoids modulate neurogenic inflammation via inhibition of neurosecretion from the peripheral terminals of capsaicin-sensitive fibers.

#### 4. Discussion

In this study we demonstrate that intraplantar (i.pl.) administration of the endogenous cannabinoid anandamide inhibits carrageenan-induced thermal hyperalgesia. We have demonstrated that both co-administration of ananda-

midamide with carrageenan and administration of anandamide after the induction of hyperalgesia are effective in decreasing hyperalgesia. These results indicate that anandamide can inhibit the induction as well as the maintenance of hyperalgesia.

The demonstration that anandamide is capable of inhibiting carrageenan-induced hyperalgesia with intraplantar administration is, in itself, insufficient to conclude that cannabinoids act peripherally to produce antihyperalgesia. It is well supported in the literature that cannabinoids can act at a number of sites in the central nervous system to raise nociceptive thresholds (Yaksh, 1981; Herkenham et al., 1991; Lichtman and Martin, 1991; Martin et al., 1993; Richardson et al., 1998a,b; Smith et al., 1994; Hohmann et al., 1995;

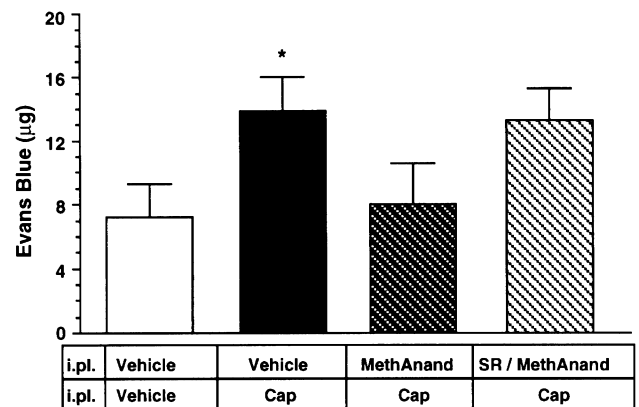


Fig. 4. Effect of the cannabinoid agonist methanandamide on capsaicin-evoked neurogenic inflammation. Rats received an i.v. injection of Evans blue dye followed by an i.pl. injection of the vehicle, 175 ng methanandamide ('MethAnand'), or 175 ng methanandamide + 250 ng SR 141716A ('SR/MethAnand'). This was immediately followed by an i.pl. injection of vehicle or capsaicin. Extravasation was determined as described in Section 2. Error bars are SEM;  $n = 6-19$ /group. \* $P < 0.05$  vs. Vehicle/Vehicle and MethAnand/Cap.

Martin et al., 1995; Lichtman et al., 1996; Martin et al., 1996; Hohmann and Herkenham, 1997; Richardson et al., 1998a,b). Thus, one possible interpretation of the ability of i.p. administered anandamide to produce antihyperalgesia is that it is diffusing into the blood stream and acting at distant, potentially central, sites. However, the inability of the peripherally-effective dose of anandamide to produce antihyper-algesia and to inhibit edema when administered systemically (i.e., i.p.) supports a local site of action in the inflamed hindpaw. This is also supported by the lack of contralateral effects following intraplantar anandamide. Additionally, a low dose of the selective CB<sub>1</sub> receptor antagonist SR 141716A was capable of inhibiting anandamide-induced antihyperalgesia. Considerably larger systemic doses of SR 141716A are required for centrally-mediated cannabinoid effects. For example, i.p. administration of 250 ng SR 141716A is ineffective in antagonizing  $\Delta^9$ -THC-induced antinociception in the tail flick assay (Compton et al., 1996; Reche et al., 1996). In the present study, 100 ng SR 141716A (i.pl.) completely reversed

anandamide-induced antihyperalgesia. These results are consistent with a peripheral cannabinoid receptor mediating the observed thermal antihyperalgesia.

Previous studies have suggested that CB<sub>2</sub> receptors located on mast cells have an antihyperalgesic role (Mazzari et al., 1996). Our results support both an antihyperalgesic and an anti-inflammatory role for peripheral CB<sub>1</sub> receptors. We demonstrated that anandamide has both antihyperalgesic and anti-edemic effects. Most studies have found that anandamide does not activate the CB<sub>2</sub> receptor (Facci et al., 1995; Bayewitch et al., 1995) except at very high concentrations (greater than 75  $\mu$ M; Lee et al., 1995) supporting the hypothesis that anandamide's effects in the present study are mediated by the CB<sub>1</sub> receptor. Moreover, anandamide-induced antihyperalgesia was inhibited by the coadministration of the selective CB<sub>1</sub> receptor antagonist SR 141716A. Thus, the reported inability of anandamide to interact with the CB<sub>2</sub> receptor, the ability of SR 141716A to inhibit anandamide's effects, and the lack of activity of anandamide after systemic administration suggest that the effects of ana-

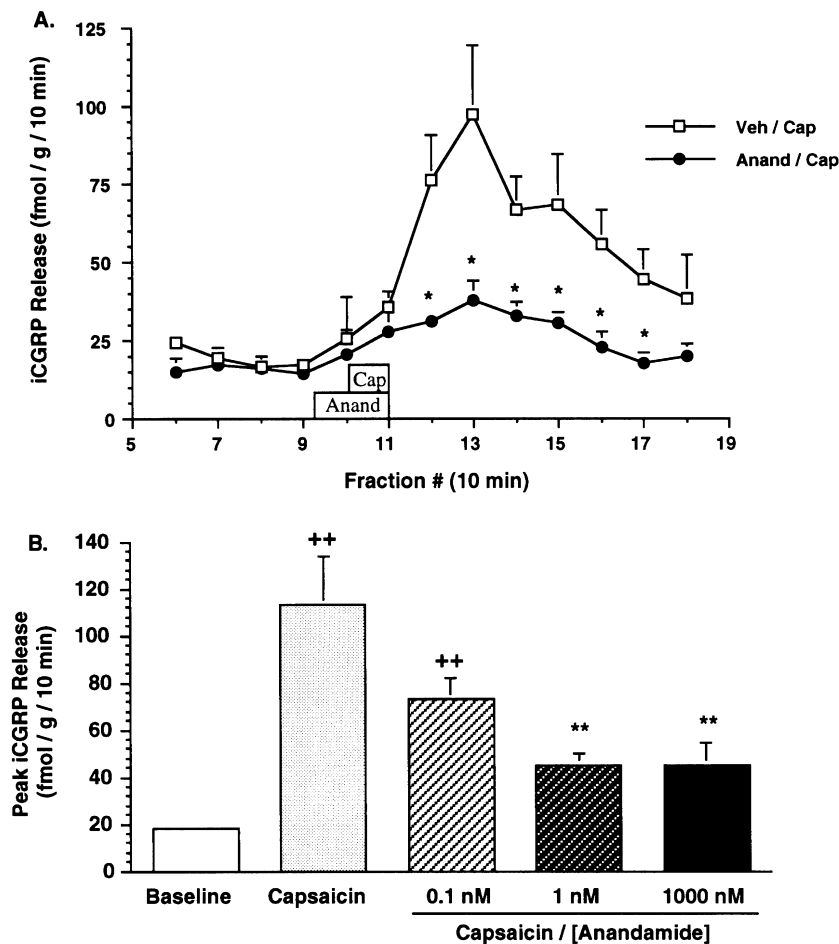


Fig. 5. Effect of anandamide on capsaicin-evoked iCGRP release from rat hindpaw skin. After equilibration, the dorsal hindpaw skin was treated with the vehicle or anandamide ('Anand') for 10 min followed by capsaicin ('Cap') in the presence of the pretreated drug or vehicle for 7 min. Data are represented as means  $\pm$  SEM. Error bars not visible are contained within the symbol. (A) Release of iCGRP under basal conditions and in response to 1 nM anandamide/100  $\mu$ M capsaicin ( $n = 6$ ) and vehicle/100  $\mu$ M capsaicin ( $n = 10$ ). \* $P < 0.05$ . (B) Comparison of peak iCGRP release in response to no drug (baseline,  $n = 26$ ), 100  $\mu$ M capsaicin ( $n = 10$ ), 0.1 nM anandamide/capsaicin ( $n = 5$ ), 1 nM anandamide/capsaicin ( $n = 6$ ), or 1000 nM anandamide/capsaicin ( $n = 5$ ). ++ $P < 0.01$  vs. baselines; \*\* $P < 0.01$  vs. capsaicin.

ndamide in this study are mediated via a peripheral CB<sub>1</sub> receptor.

Cannabinoids have previously been reported to either reduce carrageenan-induced edema (Sofia et al., 1973) or to have no effect (Kosersky et al., 1973). The anti-edemic effect was attributed to the activation of the pituitary-adrenal axis as demonstrated by the elimination of anti-edema by bilateral adrenalectomy and hypophysectomy. In the present study, we demonstrate that anandamide is effective in inhibiting carrageenan-induced edema via a peripheral site of action as determined both by the lack of effect of i.p. anandamide and by the inability of ipsilateral i.pl. administration of anandamide to inhibit edema in the contralateral hindpaw. The inhibition of edema observed in the present study was not as great as that reported by Sofia et al. (1973). There are at least two potential explanations for the difference. First, we have not conducted a full dose-response curve and thus the dose applied may not be a maximally effective dose. Interpretations from a full dose-response curve with local administration are confounded if the agonist also has systemic effects, such as stimulation of the pituitary-adrenal axis. Thus, it is difficult to determine the efficacy using local drug injection protocols. Secondly, we have not conducted a time-course for the anti-edemic effect of anandamide. Co-administration of anandamide and carrageenan may not produce the greatest effect on edema. Taken together this suggests that cannabinoids are capable of inhibiting edema by both a central and peripheral mechanism.

Edema is a result of plasma extravasation, i.e., the entering of materials from the vascular compartment into the injured tissue due to increased permeability of the microvasculature. Plasma extravasation can be evaluated using Evans blue dye, which binds to circulating albumin and remains mostly in the vascular compartment under control conditions. However, during plasma extravasation, the dye-albumin complex extravasates into the tissue. Capsaicin-induced release of neuropeptides is capable of producing plasma extravasation (Nagahisa et al., 1992; Brain, 1996). In the present study, we evaluated the ability of methanandamide, a stable congener of anandamide, to inhibit capsaicin-induced plasma extravasation. Methanandamide inhibited capsaicin-induced plasma extravasation in an SR 141716A-reversible manner. This suggests that cannabinoids inhibit plasma extravasation via interaction with a peripheral CB<sub>1</sub> receptor.

There are at least two mechanisms by which activation of the cannabinoid receptor could produce the effects observed in the present study. Activation of the CB<sub>1</sub> receptor has been reported to inhibit adenylyl cyclase activity (Howlett, 1984), close certain calcium channels (Caulfield and Brown, 1992; Mackie and Hille, 1992), and enhance certain potassium currents (Deadwyler et al., 1993; Henry and Chavkin, 1995). All three of these could lead to decreased neurotransmitter release. Indeed, in the present study, we demonstrate that anandamide can inhibit capsaicin-induced iCGRP release from isolated hindpaw skin. The

inhibition of neurosecretion from primary afferent fibers could result in the reduction of carrageenan-induced hyperalgesia and edema as well as capsaicin-induced plasma extravasation. A second mechanism for the effects of anandamide is the activation of potassium currents and subsequent decreased excitability of the peripheral terminal. Together these mechanisms would result in both the inhibition of neurogenic inflammation and the inhibition of nociceptive transmission to the central nervous system.

The results of this study demonstrate that cannabinoids are capable of inhibiting carrageenan-induced hyperalgesia and edema via interaction with a peripheral CB<sub>1</sub> receptor. In addition, cannabinoids inhibit capsaicin-evoked plasma extravasation in vivo and neurosecretion from isolated hindpaw skin. Taken together, these indicate that cannabinoids have a peripheral anti-inflammatory effect mediated by the CB<sub>1</sub> receptor.

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