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Cannabinoid CB_1 receptor inhibition of mechanically evoked responses of spinal neurones in control rats, but not in rats with hindpaw inflammation

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Abstract

Spinally administered cannabinoid receptor agonists are anti-nociceptive in a variety of models of acute and persistent pain. The present study investigated the effects of activation of spinal cannabinoid CB_1 receptors on mechanically evoked responses of spinal neurones in acute and inflammatory pain states. In vivo electrophysiology studies were carried out in anaesthetised rats. Effects of spinal administration of a selective cannabinoid CB_1 receptor agonist, arachidonyl-2-chloroethylamide (ACEA), on mechanically evoked responses of dorsal horn neurones in control rats and rats with peripheral hindpaw carrageenan-induced inflammation were compared. ACEA (0.27 nM–27 μM) significantly inhibited innocuous and noxious mechanically evoked responses of dorsal horn neurones in control rats. Pre-administration of the CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1-*H*-pyrazole-3-carboxyamide, SR141716A, (0.43 μM) attenuated the inhibitory effects of ACEA (27 μM). ACEA did not alter mechanically evoked responses of dorsal horn neurones in rats with hindpaw carrageenan-induced inflammation. Following peripheral inflammation, there is a loss of spinal CB_1 receptor-mediated inhibition of mechanically evoked responses, which is suggestive of a functional down-regulation of CB_1 receptors under these conditions.

Keywords: Spinal cord; Cannabinoid CB_1 receptor; Carrageenan inflammation; von Frey monofilament; Dorsal horn neurone; Cannabinoid CB_1 receptor agonist, selective

1. Introduction

Cannabinoid receptor agonists have potentially important therapeutic effects, including analgesic actions, as well as a wide range of other undesirable sedative and psychoactive effects (for review, see Kumar et al., 2001; Pertwee, 2001). Understanding of the sites and mechanisms of action of cannabinoids is important for the effective use of cannabinoids as analgesics and the development of novel cannabinoid-based drugs.

The cannabinoid CB_1 receptor, cloned in 1990, and cannabinoid CB_2 receptor, cloned in 1993, mediate many of the effects of the active constituents of herbal cannabis, endogenous cannabinoids and synthetic cannabinoid receptor ligands (for review, see Howlett et al., 2002). Both receptors are negatively coupled to adenylyl cyclase through $G_{i/o}$ proteins and positively coupled to mitogen-activated protein kinases.

CB_1 receptors, which are present mainly on neurones, are able to regulate ion channels (for review, see Howlett et al., 2002). These receptors are positively coupled to inwardly rectifying potassium (K^+) channels and A-type K^+ channels and negatively coupled to N-type and P/Q type calcium (Ca^{2+}) channels through $G_{i/o}$ proteins. Modulation of K^+ and Ca^{2+} channels by CB_1 receptors has a broad range of physiological effects and contributes to both the therapeutic and undesirable effects of this class of drugs.

CB_1 receptors are located at many of the sites associated with peripheral and central processing of nociceptive messages. Studies of rat dorsal root ganglia neurones have demonstrated the presence of CB_1 receptors on cell bodies (Ross et al., 2001), and high co-expression with vanilloid VR1 receptors and other markers of nociceptive primary sensory neurones has been reported (Ahluwalia et al., 2000, see, however, Hohmann and Herkenham, 1999). There is also evidence for the presence of CB_1 receptors on non-nociceptive neurones (Ahluwalia et al., 2002; Hohmann and Herkenham, 1999). Recent work has shown that CB_1-immunoreactivity is co-localized with a marker for myelination, and that neurones expressing cannabinoid CB_1

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receptor-immunoreactivity are not responsive to capsaicin, suggesting that they are low- or high-threshold mechanoreceptors (Khasabova et al., 2002). Thus, there are discrepancies between these studies, one explanation for these differences is the existence of different CB_1 receptor subtypes in dorsal root ganglia neurones (for further discussion, see Ahluwalia et al., 2002).

The spinal cord plays an important role in the integration and modulation of nociceptive inputs and CB_1 receptors are localized to the superficial laminae of the dorsal horn of the spinal cord (Farquahr-Smith et al., 2000). In this study, there was, however, no evidence for fibre co-localization of CB_1-immunoreactivity and markers for primary afferent nociceptors. By contrast, immunocytochemical studies performed with antibodies raised against the N-terminal of the CB_1 receptor have described the presence of CB_1-immunoreactivity in dorsal root ganglia neurones and Lissauer's tract, providing strong evidence for a pre-synaptic localization of spinal cannabinoid receptors on primary afferent fibres (Salio et al., 2002). In addition, post-synaptic localization of CB_1 receptors, in particular on γ-aminobutyric acid (GABAergic) interneurones in laminae IIo, have been described (Salio et al., 2002). It has been proposed that the differences in the reported CB_1 receptor localization may arise due to the presence of a CB_1 receptor variant (CB_{1A}) with a truncated N-terminal tail in the dorsal horn of the spinal cord (see Salio et al., 2002 for further discussion).

The majority of previous studies of the functional role of spinal cannabinoid receptors have used nonselective cannabinoid drugs. Spinal administration of cannabinoids has been shown to be anti-nociceptive in models of acute (Drew et al., 2000; Hohmann et al., 1998; Smith and Martin, 1992; Welch and Stevens, 1992) and inflammatory (Drew et al., 2000; Harris et al., 2000; Martin et al., 1999b; Richardson et al., 1998) pain. The CB_1 receptor antagonist, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1-*H*-pyrazole-3-carboxyamide (SR141716A), attenuated, at least in part, the anti-nociceptive effects of cannabinoid agonists in these studies. Immunocytochemical and in situ hybridization studies discussed above, however, suggest the possibility of multiple CB_1 receptor subtypes at the level of the spinal cord, which may have a differential location and/or function.

The aim of the present study was to investigate the role of spinal CB_1 receptors with a selective CB_1 receptor agonist, arachidonyl-2-chloroethylamide (ACEA), which is a synthetic derivative of anandamide and has greater than 2000-fold selectivity for the CB_1 receptor over the CB_2 receptor (Hillard et al., 1999). Evidence suggests that CB_1 receptors are present on nociceptive and non-nociceptive primary afferent fibres. In the present study, we compared the effects of spinal administration of ACEA on innocuous and noxious mechanically evoked responses of spinal neurones in control rats. Since models of acute nociception are not representative of persistent pain states, the second aim of this study was to investigate the effects of spinal ACEA in rats with peripheral hindpaw carrageenan-induced inflammation.

2. Materials and methods

All experiments were carried out on male Sprague–Dawley rats (Charles River, UK) weighing 250–300 g. Experimental procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986 and IASP guidelines.

2.1. Surgical procedures

Anaesthesia was induced with 2–3% halothane in 66% N_2O/33% O_2 and a tracheal cannula was inserted (Drew et al., 2000). Rats were placed in a stereotaxic frame to ensure stability during electrophysiological recordings. A laminectomy was performed, lumbar vertebrae L1–L3 were located and segments L4–L5 of the spinal cord were exposed. The exposed segment of spinal cord was held rigid by the use of clamps rostral and caudal to the exposed area and bathed in saline. Upon completion of surgery, the level of halothane was reduced to 1–1.5%, which maintained a state of complete areflexia. Core body temperature was monitored and maintained (36–37 °C) by means of a heating blanket connected to a rectal probe via an automatic feedback control unit.

2.2. In vivo electrophysiology

Extracellular recordings of single wide dynamic range dorsal horn neurones were made using glass-coated tungsten electrodes (Merrill and Ainsworth, 1972), which were advanced through the cord by a SCAT microdrive. Data were captured and analysed by a CED 1401 interface (Cambridge Electronic Design, Cambridge, UK) coupled to a Pentium computer with Spike 2 software. Receptive fields of neurones covering one or two toes were identified with brush and pinch stimuli. The depth of the neurone from the surface of the dorsal horn of the spinal cord was recorded. All neurones selected had a clear short latency Aβ-fibre-evoked response followed by Aδ-fibre-evoked response and a longer latency C-fibre-evoked response. Stimulating electrodes were inserted into the centre of the receptive field (one or two toes). Responses were elicited by a train of 16 electrical stimuli (2-ms pulse width) at three times the threshold for C-fibre activation, at a frequency of 0.5 Hz. Aβ-fibre-evoked responses were taken as the number of action potentials recorded 0–20-ms post-stimulus, Aδ-fibre-evoked responses were taken as the number of action potentials recorded 20–90-ms post-stimulus. C-fibre-evoked responses were taken as the number of action potentials recorded 90–300-ms post-stimulus.

Following the characterisation of neuronal responses to transcutaneous electrical stimulation and confirmation of the contribution of A- and C-fibres to evoked responses, responses of neurones to mechanical punctate stimulation with von Frey hairs (Semmes-Weinstein monofilaments, North Coast Medical, USA) of differing bending forces

(8, 12, 21, 45 and 80 g) were characterised. The range of von Frey hairs selected included non-noxious and noxious mechanical punctate stimuli (noxious withdrawal threshold in awake animals is 15 g, Chaplan et al., 1994). von Frey hairs were applied to the centre of the receptive field in ascending weight order, for 10 s. Frequencies (Hz) of mechanically evoked firing over the 10-s stimulation period were quantified. Mechanically evoked responses of dorsal horn neurones were followed for 40–60 min, the last three stable evoked responses served as pre-drug control values.

2.3. Drug administration

Drugs were applied topically to the surface of the exposed L4/5 segments of the spinal cord. Effects of spinal administration of ACEA (0.27 nM–27 μM; top dose 0.2% ethanol in distilled H_2O, 50 μl) ($n=6$ neurones in six rats/each concentration), or vehicle (0.2% ethanol in distilled H_2O, 50 μl) ($n=5$ neurones in five rats) on mechanically evoked (8–80 g von Frey hairs) responses of spinal neurones were followed for 60 min, at 10-min intervals, in non-inflamed rats. Following the 60-min period of drug application, any remaining fluid was removed from the spinal cord and the next ascending concentration of ACEA was applied to the spinal cord. Due to the long time course of studying the effects of six concentrations of drug on mechanically evoked responses, data sets were broken up and effects of no more than four concentrations of ACEA on mechanical evoked responses were studied on any one neurone.

In a second set of experiments, 50 μl of spinal SR141716A (0.43 μM, in distilled H_2O and 0.04% ethanol) was administered alone ($n=6$ neurones in 6 rats) or 40 min before the spinal application of ACEA (27 μM, 50 μl) ($n=7$ neurones in 7 rats) in non-inflamed rats. Drug effects on mechanically evoked responses of spinal neurones were followed for 60 min at 10-min intervals.

In a separate group of rats ($n=6$), 100 μl of 2% carrageenan (in saline) was injected into the plantar surface of a hindpaw to generate peripheral inflammation. At 3 h following carrageenan injection, corresponding to peak behavioural hyperalgesia (Hargreaves et al., 1988), effects of spinal administration of ACEA (27 nM–27 μM, 50 μl) on peripheral mechanically evoked responses of spinal neurones ($n=6$ neurones in 6 rats) were studied in rats with hindpaw carrageenan-induced inflammation (as described for control rats). Effects of each concentration of ACEA were monitored for 60 min at 10-min intervals.

2.4. Drugs and chemicals

Arachidonyl-2-choroethylamide (ACEA) was obtained from Tocris Cookson (UK). *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1-*H*-pyrazole-3-carboxyamide (SR141716A) was provided by Research Biochemicals International as part of the chemical synthesis program of the National Institute of Mental Health, contract N01MH30003. Carrageenan was obtained from Sigma (UK).

2.5. Statistical analysis

Data are presented as mean maximal % of pre-drug control responses, statistical analysis was performed with repeated measures analysis of variance (ANOVA) and Dunnett's post hoc test, paired *t*-test and Mann–Whitney test where appropriate.

3. Results

3.1. Control-evoked neuronal responses

Depths, C-fibre threshold and latency of response, and control electrical-evoked A-fibre and C-fibre responses of dorsal horn neurones were similar in non-inflamed rats and rats with hindpaw carrageenan-induced inflammation (Table 1). Application of mechanical punctate stimuli (calibrated von Frey hairs 8–80 g) to the centre of the receptive field of dorsal horn neurones evoked spinal neuronal firing. Frequencies of mechanically evoked firing of dorsal horn neurones in non-inflamed rats and rats with hindpaw carrageenan-induced inflammation were not significantly different ($P>0.05$, Mann–Whitney test) (Fig. 1).

3.2. Effects of spinal ACEA on peripheral mechanically evoked neuronal responses in non-inflamed rats and rats with peripheral carrageenan inflammation

Spinally administration of ACEA (0.27 nM–27 μM) significantly inhibited innocuous 8-g-evoked responses of dorsal horn neurones in non-inflamed rats (minimum effective dose 0.27 nM, $P<0.05$, $n=6$, Fig. 2A), but not in rats with hindpaw carrageenan-induced inflammation ($n=6$, Fig. 3A). ACEA significantly inhibited innocuous 12-g-evoked responses of dorsal horn neurones in non-inflamed rats (minimum effective dose 0.27 nM, $P<0.05$, $n=6$, Fig. 2A), but not in rats with hindpaw carrageenan-induced inflammation ($n=6$, Fig. 3A). Similarly, spinally administered ACEA significantly inhibited noxious 21-g (minimum

Table 1
A comparison of control C-fibre threshold, latency, and magnitude of evoked responses of dorsal horn neurons of non-inflamed rats ($n=6$) and rats with peripheral carrageenan inflammation ($n=6$) (Aβ-fibre: 0–20 ms; Aδ-fibre: 20–90 ms; C-fibre: 90–300 ms post-stimulus)

	C-fibre threshold (mA)	C-fibre latency (ms)	Evoked responses (number of action potentials)		
			Aβ-fibre	Aδ-fibre	C-fibre
Non-inflamed	1.8 ± 0.2	183 ± 20	77 ± 17	89 ± 11	239 ± 41
Carrageenan inflamed	2.0 ± 0.3	159 ± 21	77 ± 11	67 ± 19	186 ± 34

Values are means ± S.E.M.

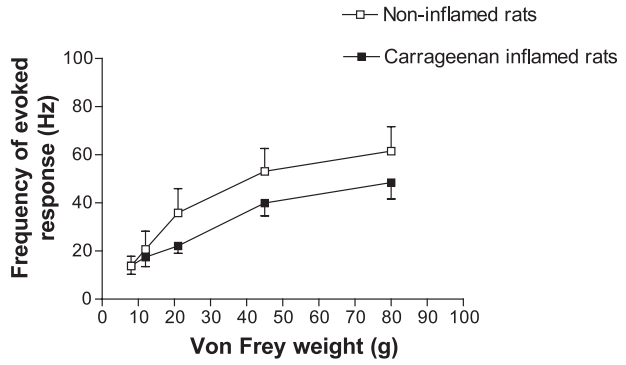

Fig. 1. Mechanically evoked responses of frequency of firing of dorsal horn neurones in non-inflamed rats ($n=6$). Application of increasing von Frey filament weights evoked a graded increase in frequency of firing in non-inflamed rats ($n=6$) and rats with peripheral carrageenan inflammation ($n=6$). Data expressed as mean control-evoked response ± S.E.M.

effective dose 0.27 nM $P<0.001$)-, 45-g (minimum effective dose 0.27 nM $P<0.001$)- and 80-g (minimum effective dose 0.27 nM $P<0.001$)-evoked responses of dorsal horn

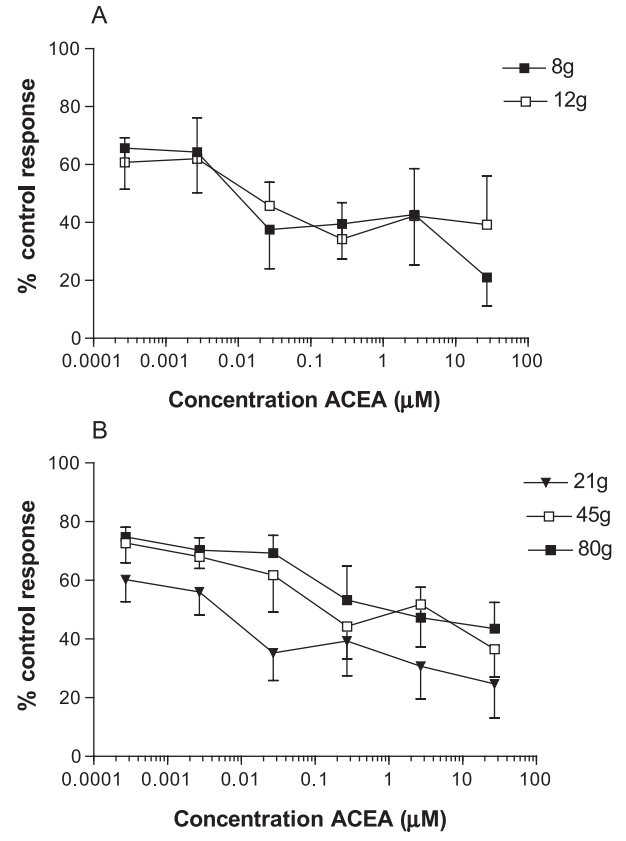

Fig. 2. Effects of spinal administration of ACEA (0.27 nM–27 μM) on innocuous (A)- and noxious (B)-evoked responses of dorsal horn neurones in non-inflamed rats ($n=6$). Minimum effective concentration of ACEA was 0.27 nM for all von Frey hair weights, $P<0.05$ (8 and 12 g) and $P<0.001$ (21, 45 and 80 g). All higher concentrations of ACEA produced significant inhibitions of mechanically evoked responses of dorsal horn neurones, with the exception of 2.7 nM on 8-g-evoked response. Data presented as mean maximal % of pre-drug control response ± S.E.M. Statistical analysis performed with repeated measures analysis of variance (ANOVA) and Dunnett's post hoc test.

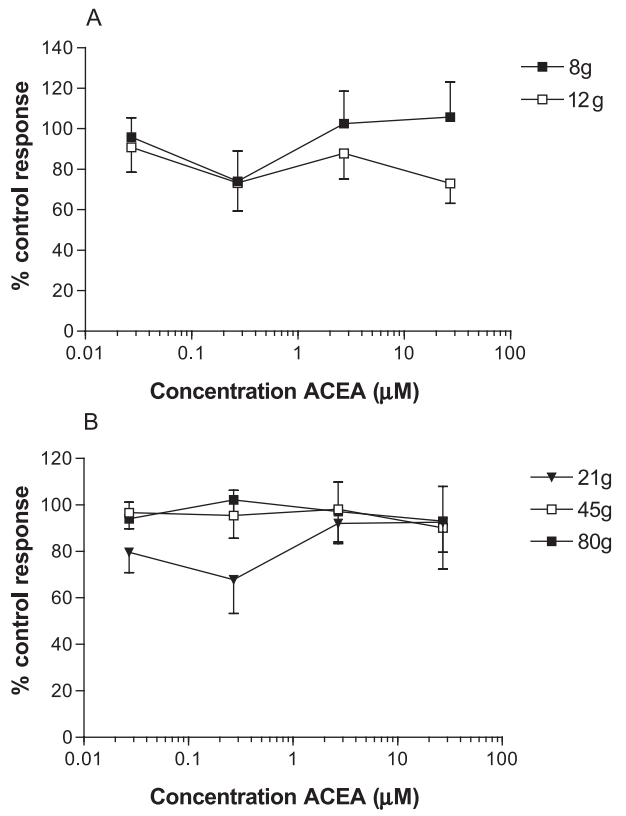

Fig. 3. Effects of spinal administration of ACEA (27 nM–27 μM) on innocuous (A)- and noxious (B)-evoked responses of dorsal horn neurones in rats with hindpaw carrageenan-induced inflammation ($n=6$). ACEA did not significantly alter mechanically evoked responses of dorsal horn neurones in rats with hindpaw carrageenan-induced inflammation. Data presented as mean maximal % of pre-drug control response ± S.E.M.

neurones in non-inflamed rats ($n=6$, Fig. 2B). Inhibitory effects of ACEA on mechanically evoked responses were maintained over the period of the study (Fig. 4). In rats with hindpaw carrageenan-induced inflammation, spinally administered ACEA (0.27 nM–27 μM) did not significantly

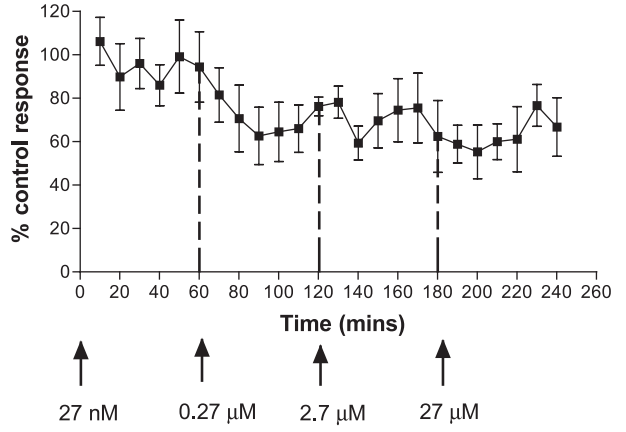

Fig. 4. Time-course of the effect of spinal administration of ACEA (27 nM–27 μM) on noxious 80-g-evoked responses of dorsal horn neurones in non-inflamed rats; note that desensitisation to the inhibitory effect of ACEA was not observed.

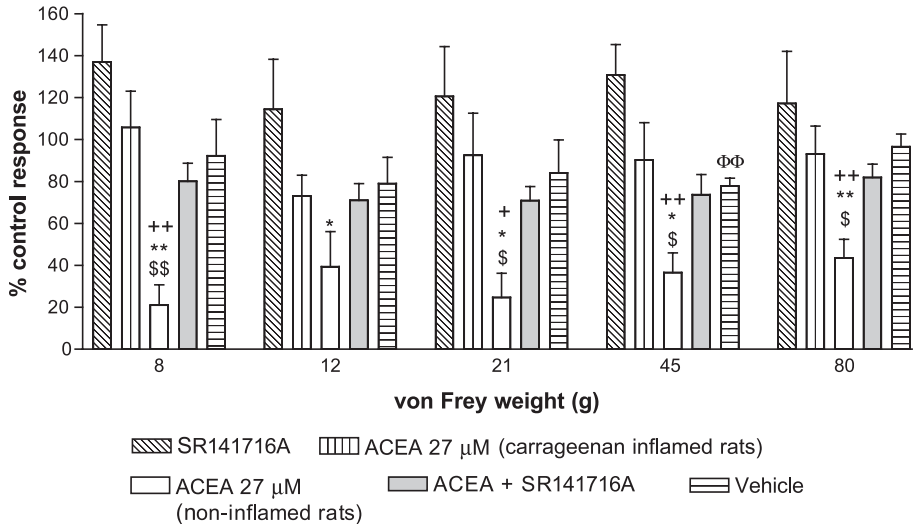

Fig. 5. Spinal administration of ACEA (27 μM) significantly inhibited mechanically evoked responses of dorsal horn neurones in non-inflamed rats, but not rats with hindpaw carrageenan-induced inflammation. Pre-administration of spinal SR141716A (0.43 μM) significantly attenuated inhibitory effects of spinal ACEA (27 μM) ($n=7$) on mechanically evoked responses of dorsal horn neurones in non-inflamed rats. Spinal administration of SR141716A (0.43 μM) alone and vehicle (0.2% ethanol) ($n=6/5$) had minor effects on mechanically evoked responses of dorsal horn neurones in non-inflamed rats. Data presented as mean maximal % of pre-drug control response ± S.E.M. Statistical comparison between vehicle and ACEA performed with Mann–Whitney test, $^{+}P<0.05$, $^{++}P<0.01$. Comparison between effects of ACEA in non-inflamed rats and in rats with peripheral carrageenan inflammation performed with Mann–Whitney test, $^{\$}P<0.05$, $^{\$\$}P<0.01$. Comparison between effects of ACEA alone and ACEA and SR141716A in non-inflamed rats performed with Mann–Whitney test, $*P<0.05$, $**P<0.01$. Statistical analysis of the effect of vehicle on pre-drug control responses performed with paired *t*-test, $^{\Phi\Phi}P<0.01$.

alter noxious 21-, 45- and 80-g-evoked responses of dorsal horn neurones (Fig. 3B).

Comparison of data for the highest concentration of ACEA studied revealed significant differences between inhibitory effects of ACEA in non-inflamed rats and rats with hindpaw carrageenan-induced inflammation (Fig. 5). Spinal administration of the CB_1 receptor antagonist SR141716A (0.43 μM) prior to administration of ACEA (27 μM) significantly attenuated the inhibitory effects of ACEA on mechanically evoked responses of dorsal horn neurones in non-inflamed rats, compared to vehicle (Fig. 5). Spinal administration of SR141716A (0.43 μM) alone produced a nonsignificant increase in mechanically evoked responses of dorsal horn neurones in non-inflamed rats (Fig. 5). Spinal administration of vehicle (0.2% ethanol in distilled H_2O) had minor inhibitory effects on mechanically evoked responses of dorsal horn neurones in non-inflamed rats (Fig. 5).

4. Discussion

Spinal administration of the selective CB_1 receptor agonist, ACEA, significantly inhibited innocuous and noxious mechanically evoked responses of dorsal horn neurones in non-inflamed rats. In rats with hindpaw carrageenan-induced inflammation, spinally administered ACEA did not significantly alter mechanically evoked responses of dorsal horn neurones of the spinal cord. Spinal administration of vehicle had minor inhibitory effects on mechanically evoked responses of dorsal horn neurones in non-inflamed rats.

Both innocuous (8 and 12 g) and noxious (21, 45, and 80 g) mechanically evoked responses of dorsal horn neurones were significantly inhibited by ACEA in non-inflamed rats, compared to control pre-drug values and vehicle. These data suggest that activation of spinal CB_1 receptors can inhibit both innocuous A- and noxious C-fibre-evoked responses of dorsal horn neurones in the spinal cord. This finding supports our previous study, which demonstrated that spinal administration of ACEA significantly inhibits electrically evoked Aδ-fibre responses, as well as C-fibre-evoked responses of dorsal horn neurones of the spinal cord in non-inflamed rats (Kelly and Chapman, 2001). Spinal CB_1 receptors are present at both pre- and post-synaptic sites within the spinal cord and there is evidence for CB_1 receptors on small and large diameter dorsal root ganglia neurones, corresponding to C- and A-fibres (see Introduction). The mechanism by which spinally administered ACEA reduces mechanically evoked responses of dorsal horn neurones may involve activation of pre- (Hohmann and Herkenham, 1998; Hohmann et al., 1999; Morisset and Urban, 2001) and/or post-synaptic (Farquahr-Smith et al., 2000; Salio et al., 2002) CB_1 receptors, leading to the reduction of transmitter release and post-synaptic hyperpolarisation through the modulation of ion channel function (for review, see Pertwee, 2001).

ACEA is a high affinity agonist at CB_1 receptors (Hillard et al., 1999) and the inhibitory effects of ACEA on innocuous and noxious mechanically evoked responses were blocked by pre-administration of the CB_1 receptor antagonist SR141716A. The concentration of SR141716A applied to the surface of the spinal cord is reported to be selective for CB_1 receptors (Rinaldi-Carmona et al., 1994),

however, the concentration of SR141716A at spinal CB_1 receptors in this in vivo preparation is unknown. Previously, we have demonstrated that the same concentration of SR141716A attenuates the inhibitory effects of spinal ACEA on Aδ- and C-fibre electrically evoked responses of dorsal horn neurones (Kelly and Chapman, 2001). It should be noted, however, that SR141716A has inverse agonist properties in vitro (Maclennan et al., 1998). Potential inverse agonist properties are difficult to prove in vivo, nevertheless, the possibility that this may contribute to some of the in vivo observations reported here cannot be discounted. Concentrations (1 μM) of SR141716A, higher than those used in the present study, have been reported to act antagonistically at vanilloid (VR1) receptors (De Petrocellis et al., 2001). In the present study, spinal administration of SR141716A alone produced a nonsignificant facilitation of mechanically evoked responses of dorsal horn neurones, compared to pre-drug control values. Previously, we have demonstrated inhibitory effects of spinal administration of the VR1 receptor antagonist capsazepine on Aδ- and C-fibre-evoked responses of spinal neurones (Kelly and Chapman, 2002), thus, it is unlikely that the concentration of SR141716A used in the present study is acting at VR1 receptors.

The finding that spinal administration of SR141716A alone produces a nonsignificant facilitation of mechanically evoked responses of dorsal horn neurones in non-inflamed rats corroborates our previous study, which demonstrated that spinal administration of SR141716A facilitates C-fibre-evoked responses of dorsal horn neurones (Chapman, 1999). Similarly, behavioural studies have demonstrated that spinal administration of SR141716A enhances thermal hyperalgesia in mice (Richardson et al., 1997). Collectively, electrophysological and behavioural studies suggest that there is tonic control of spinal nociceptive processing by endocannabinoids acting at CB_1 receptors.

Three hours following peripheral carrageenan inflammation, there were no marked changes in the mean magnitude of mechanically evoked responses of dorsal horn neurones, compared to non-inflamed rats. This finding corroborates previous evidence that innocuous and noxious mechanically evoked responses of spinal neurones are not altered following hindpaw carrageenan-induced inflammation, compared to non-inflamed rats (Carpenter et al., 2000). Previously, it has been reported that the mean population response of spinal neurones to electrical stimulation is not altered following carrageenan injection, individual neurones, however, exhibit either increased or decreased evoked responses (Stanfa et al., 1992). In the present study, evoked responses were not followed during the development of inflammation. We have, however, observed bi-directional changes in electrically and mechanically evoked responses of spinal neurones during the development of carrageenan inflammation in a separate study (unpublished observation). Although spontaneous firing rates of dorsal root ganglia neurones are increased following carrageenan inflammation (Xu et al., 2000), we and others (Stanfa et al., 1992) have not observed an increase in spontaneous activity of dorsal horn neurones following carrageenan inflammation in vivo in halothane anaesthetised rats. Sensitisation of wide dynamic range neurones to brush, pinch and pressure and enlargement of peripheral receptive fields have been reported following carrageenan inflammation in nembutal anaesthetised rats (Ma and Sluka, 2001). Electrophysiological studies of hemisected spinal cord from carrageenan treated rats have shown that resting membrane potentials and input resistances of spinal neurones are similar in control- and carrageenan-treated rats (Ackley et al., 2001). Thus, the majority of electrophysiological data suggests that there are not marked changes in the response properties of spinal neurones following peripheral carrageenan inflammation. Nevertheless, behavioural studies have reported lowered mechanical withdrawal thresholds at 3 h following carrageenan inflammation (Ackley et al., 2001; Hargreaves et al., 1988; Sluka and Chandran, 2002), which fits well with the reported enlargement of peripheral receptive fields and sensitisation of wide dynamic range neurones following peripheral inflammation (Ma and Sluka, 2001).

In the present study, spinal administration of ACEA did not significantly alter mechanically evoked responses of dorsal horn neurones in rats with established hindpaw carrageenan-induced inflammation. Indeed effects of ACEA were similar to those produced by spinal administration of vehicle. Previous studies have reported plasticity of receptor systems following peripheral carrageenan inflammation, most notably changes in cholecystokinin modulation of opioid-mediated inhibition (Stanfa and Dickenson, 1993), over a similar time course. The basis for the loss of inhibitory effect of spinally administered ACEA remains to be determined, but may involve changes in the rate of break-down of spinal ACEA under these conditions, or functional down-regulation of CB_1 receptors. Following peripheral inflammation, up-regulation of spinal protein kinase A (Malmberg et al., 1997) and/or protein kinase C (Martin et al., 1999a, Yashpal et al., 1995) may result in increased protein kinase A/protein kinase C-dependent phosphorylation of pre- and/or post-synaptic spinal CB_1 receptors (Garcia et al., 1998, Huang et al., 2002) and a functional down-regulation of spinal CB_1 receptors. Such a putative mechanism may account for the loss of inhibitory effects of spinal ACEA on mechanically evoked responses of spinal neurones in rats with hindpaw carrageenan-induced inflammation reported here.

Loss of inhibitory effects of spinally administered ACEA following peripheral hindpaw inflammation is not representative of other mixed CB_1/CB_2 cannabinoid receptor agonists, since a behavioural study has shown enhanced inhibitory effects of spinal anandamide in rats with peripheral inflammation (Richardson et al., 1998). Furthermore, we have shown that anandamide (Harris et al., 2000) and the CB_1/CB_2 synthetic cannabinoid agonist 6*a*(*R*)-*trans*-3-(1,1-

Dimethylheptyl)-6a, 7, 10, 10*a*-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[*b*,*d*]pyran-9-methanol (HU210) (Drew et al., 2000) inhibit electrically evoked Aδ- and C-fibre responses of dorsal horn neurones in non-inflamed rats and rats with hindpaw carrageenan-induced inflammation, effects blocked by SR141716A. Thus it is clear that there are marked differences between the spinal effects of non-selective cannabinoid agonists and the CB_1 receptor agonist ACEA under conditions of inflammation. One possible explanation for these differences is that previous electrophysiological experiments used electrical stimulation, which would activate all primary afferent fibres, whereas the present study used mechanical stimuli, which activates a mechanically sensitive population of primary afferent fibres. Alternatively, the selective loss of the inhibitory effects of ACEA, but not anandamide or HU210, under conditions of peripheral inflammation may arise due to these mixed agonists acting at another novel spinal cannabinoid receptor, the determination of which would require the development of selective cannabinoid-based drugs for these receptors. Recent studies of mice with mutated CB_1 receptor genes ($CB_1^{-/-}$ mice) have provided evidence for novel cannabinoid receptors in the central nervous system. Pharmacological effects of anandamide and *R*-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphtalenylmethanone mesylate (WIN55,212-2), but not Δ^9-tetrahydrocannabinol, on locomotor activity and analgesia have been demonstrated in $CB_1^{-/-}$ mice (Di Marzo et al., 2000). This finding is supported by evidence in vitro that both anandamide and WIN55,212-2 stimulate GTPγS binding via activation of a non-CB_1 receptor in brain membranes taken from $CB_1^{-/-}$ mice (Breivogel et al., 2001). Further evidence for a novel cannabinoid receptor has been provided by electrophysiological studies of hippocampal slices (Hajos and Freund, 2002). In this previous study, SR141716A was unable to distinguish between CB_1 receptors and the new CB receptor identified in the hippocampus (Hajos and Freund, 2002). This finding resembles our report that SR141716A is able to block the inhibitory effects of both HU210 and ACEA at the level of the spinal cord in non-inflamed rats.

The results of the present study demonstrate that activation of spinal CB_1 receptors inhibits both innocuous and noxious mechanically evoked responses of spinal neurones in non-inflamed rats. Following peripheral inflammation, we report a loss of effect of spinally administered ACEA, which may reflect a change in the break-down of ACEA or functional loss of CB_1 receptors.

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