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Functional changes in the inhibitory effect of spinal cannabinoid (CB) receptor activation in nerve injured rats

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Abstract

Recent interest has focused on the potential of cannabinoids as novel analgesics. The aim of the present study was to investigate the effect of a potent cannabinoid agonist, HU210, on somatosensory transmission in a model of neuropathic pain. Here, the effects of spinal versus systemic administration of HU210 on noxious and innocuous evoked responses of spinal neurones of nerve injured (selective ligation of spinal nerves L5–L6) and sham operated rats were compared 14–17 days post-surgical intervention.

Spinal administration of HU210 (0.5–500 ng/50 μl) significantly reduced the C-fibre mediated post-discharge response of spinal neurones in sham operated, but not nerve injured rats. By contrast, spinal HU210 significantly reduced Aδ-fibre evoked responses of spinal neurones in both sham operated and nerve injured rats.

Systemic administration of HU210 (6–60 μg/kg) significantly reduced C- and Aδ-fibre evoked responses of spinal neurones in sham operated rats. HU210 (60 μg/kg) inhibited the overall C-fibre evoked response (54±8% of control, $p<0.01$), post-discharge response (28±12% of control, $p<0.01$), and Aδ-fibre evoked (48±5% of control $p<0.01$) responses of spinal neurones. In nerve injured rats, systemic administration of HU210 did not significantly reduce C- or Aβ-fibre evoked responses of spinal neurones.

This study demonstrates plasticity of the spinal cannabinoid receptor system following peripheral nerve injury.

Keywords: Cannabinoid receptor; Nerve injury; Spinal cord

1. Introduction

Analgesia is one of the tetrad of behaviours associated with Δ^9-tetrahydrocannabinol (Δ^9-THC) and synthetic cannabinoid receptor agonists (for review see Pertwee, 2001). Cannabinoid CB_1 receptors have been shown to be present in the central nervous system, in particular in areas associated with nociceptive processing such as the superficial laminae of the spinal cord (Herkenham et al., 1991; Tsou et al., 1998). Evidence suggests that cannabinoid receptor binding sites are both pre-synaptic on primary afferent fibres and post-synaptic on dorsal horn neurones of the spinal cord. Autoradiographic studies of dorsal horn CB receptor binding in rats treated with neonatal capsaicin suggest that only 16% of spinal CB receptors is located on C-fibre afferent endings (Hohmann and Herkenham, 1998). Conflicting evidence from studies using dorsal rhizotomy, i.e. deafferentation of both small and large diameter afferents, to evaluate the proportion of pre-synaptic cannabinoid receptor binding sites has been reported (Hohmann et al., 1999a; Farquhar-Smith et al., 2000). An in situ hybridization study of CB_1 receptor mRNA distribution has demonstrated that subpopulations of rat dorsal root ganglion, predominantly medium and large-sized cells, are capable of synthesizing cannabinoid receptors and inserting them on primary afferent terminals (Hohmann and Herkenham, 1999). Recently, we have demonstrated functional CB_1 receptors on adult DRG neurones in culture (Millns et al., 2001). In a recent report a cannabinoid agonist, WIN55,212-2, was shown to pre-synaptically inhibit GABAergic and glycinergic synaptic transmission in the superficial medullary dorsal horn, without having a direct post-synaptic effect on medullary substantia gelatinosa neurones (Jennings et al., 2001). Cannabinoid CB_1

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receptors have been reported to be located predominantly on spinal interneurones (Farquhar-Smith et al., 2000). Currently there is no consensus from anatomical studies; spinal cannabinoid receptors may be located both pre- and post-synaptic to the primary afferent fibres, with pre-synaptic receptors residing on both C-fibre and A-fibre primary afferents.

The ability of cannabinoids to inhibit acute nociceptive processing has been demonstrated with a number of different cannabinoid agonists in behavioural (Lichtman and Martin, 1991; Smith and Martin, 1992) and electrophysiological (Hohmann et al., 1998; Strangman and Walker, 1999; Drew et al., 2000) studies. A functional role of CB_1 receptors located pre-synaptically on C-fibre afferents has been demonstrated in that the endocannabinoid anandamide inhibits capsaicin evoked CGRP release from the dorsal half of the spinal cord (Richardson et al., 1998). We, and others (Strangman and Walker, 1999) have shown that under normal conditions spinal CB receptors predominantly modulate C-fibre transmission, in particular facilitated post-discharge responses of dorsal horn neurones (Drew et al., 2000). Anti-nociceptive effects of spinally administered cannabinoid agonists are at least partly blocked by the selective CB_1 receptor antagonist, SR141716A (Welch et al., 1998; Drew et al., 2000), suggesting that spinally administered cannabinoid agonists act via CB_1 receptors to modulate nociceptive processing. Evidence suggests that the effect of systemically administered cannabinoids on spinal nociceptive processing (Hohmann et al., 1995; Hohmann et al., 1999b) is dependent, at least in part, on descending anti-nociceptive mechanisms (Hohmann et al., 1999b).

To date there have been few studies of the analgesic potential of cannabinoid agonists in models of chronic pain states. Systemically administered cannabinoid agonists have been shown to be effective at reducing touch evoked pain behaviour (allodynia) in animal models of pathophysiological inflammatory (Martin et al., 1999) and neuropathic pain (Herzberg et al., 1997; Bridges et al., 2001; Fox et al., 2001). The aim of the present study was to investigate the analgesic potential of a cannabinoid agonist in the selective spinal nerve (L5–L6) ligation model (Kim and Chung, 1992) of neuropathic pain. In vivo extracellular recordings of A- and C-fibre evoked responses of dorsal horn neurones in nerve injured and sham operated rats were performed; effects of spinal and systemic administration of the potent cannabinoid receptor agonist, HU210, on evoked neuronal responses were measured.

Some of this work has been previously published in abstract form (Chapman, 2000).

2. Methods

2.1. Spinal nerve ligation

Experiments were carried out on 31 male Sprague-Dawley rats (Charles River, UK) weighing 140–150 g at the beginning of the study. Rats were housed five per cage under a 12/12 h day/night cycle for one week prior to surgery. Rats were divided into an experimental group (ligation of the L5–L6 spinal nerves) and a control group (sham operation for spinal nerve ligation). All described procedures were approved by the Home Office and follow the guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

The procedure of ligation of the spinal nerves was performed as previously described by Kim and Chung (1992). Briefly, under halothane anaesthesia (1.5–2% in 66% N_2O–33% O_2) the rat was placed in a prone position and the left paraspinal muscles were separated from the spinous processes at the L4-S2 level. Part of the L6 transverse process was carefully removed and the L4–L6 spinal nerves were identified. The L5–L6 spinal nerves were isolated and tightly ligated, distal to the dorsal root ganglion and proximal to the formation of the sciatic nerve, with 6-0 silk thread. Following complete haemostasis the wound was sutured. The total period of anaesthesia did not exceed 30 min. A separate group of animals was used as sham controls. The procedure of the sham operation for spinal nerve ligation was identical to that of the experimental group, except that the spinal nerves were exposed but not ligated.

2.2. Behavioural studies

After surgery, the rats were maintained under the same conditions as during the pre-operative period. The posture and behaviour of the rats were carefully monitored following recovery from the anaesthesia and then on the first post-operative (PO) day. From PO day 2 onwards, behavioural tests assessing sensitivity to mechanical stimuli were performed for up to 14 days. The weight gain and general behaviour of the rats were monitored throughout the PO period. In addition, posture, foot position, and growth of toe nails were observed.

For behavioural testing, the rats were placed in transparent plastic cubicles on a mesh floored table and a period of acclimatization was allowed prior to testing. The mechanical sensitivity of the ipsilateral and contralateral hindpaws was assessed by measuring the frequency of withdrawal of the feet to normally innocuous mechanical punctate stimuli. Stimuli were applied from underneath the plantar surface of the foot with a 9 g bending force von Frey filament. Each trial consisted of the application of a single von Frey hair 10 times, each for a period of 2–3 s. Testing with ascending consecutive von Frey hairs was separated by a period of at least

5 min. The occurrence of foot withdrawal for each trial was expressed as a percentage response frequency (number of foot withdrawals/10 [number of applications]×100=% response frequency).

2.3. In vivo electrophysiology

Rats were intact, anaesthesia was induced with 2–3% halothane in 66% N_2O–33% O_2 and a tracheal cannula was inserted (Chapman et al., 1994). 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 cord was held rigid by clamps caudal and rostral to the exposed section. Upon completion of surgery the level of halothane was reduced to 1–1.5% which maintained a state of complete areflexia. It has been shown previously that at this depth of anaesthesia blood pressure is in the range of 100–140 mm Hg and electrocorticograms exhibit regular slow waves, with neither parameter being overtly altered by noxious stimuli (Weil-Fugazza et al., 1984). During this time the core body temperature of the rat was monitored and maintained (36.5–37°C) by means of a heating blanket connected to a rectal thermal probe via an automatic feedback control unit.

Extracellular recordings of wide dynamic range dorsal horn neurones were made with parylene coated tungsten electrodes (A-M Systems inc, USA) which were advanced through the cord by a high speed micro-stepper (SCAT-01, Digitimer, UK). Receptive fields of neurones were identified with brush and gentle pinch stimuli and covered one/two toes. The depth of the neurone from the surface of the dorsal horn of the spinal cord was recorded. Data were captured and analysed by a CED 1401 interface (Cambridge Electronic Design, Cambridge, UK) coupled to a Pentium computer with SPIKE 2 software (rate and post-stimulus histogram functions, Cambridge Electronic Design, Cambridge, UK). The responses of neurones following transcutaneous electrical stimulation (fine stimulating needles, 2 ms wide pulses) of the centre of the receptive field were recorded. All selected neurones had a clear short latency Aβ-fibre evoked response followed by a C-fibre evoked response. Responses were elicited by a train of 16 stimuli at three times the threshold for C-fibres at a frequency of 0.5 Hz and a post-stimulus histogram was constructed. The Aβ-fibre evoked responses were taken as the number of action potentials recorded 0–20 ms post-stimulus and the late band of A-fibre activity (20–90 ms post-stimulus) was taken as the Aδ-fibre evoked response. The C-fibre evoked responses were taken as the number of action potentials recorded 90–300 ms post-stimulus. The remaining late neuronal response (300–800 ms post-stimulus), occurring as neurones exhibit hyperexcitability following repetitive stimulation, was taken as the C-fibre mediated post-discharge response of the neurone. The base-line C-fibre evoked neuronal response prior to repetitive stimulation was calculated as the number of action potentials produced by the first stimulation multiplied by the total number of stimuli (16), this response was termed the non-potentiated response.

Following stablization of C-fibre evoked responses (<10% variation of response), the effect of spinal administration (applied topically into a small well over the spinal cord) of HU210 (0.5, 5, 50, 500 ng/50 μl [2.5×10^{-8}–2.5×10^{-5} M], from Tocris, UK) on the electrically evoked responses of dorsal horn neurones of sham (n=6 rats) and nerve injured rats (n=7 rats) was studied. These concentrations of HU210 were chosen on the basis of previous experience (Drew et al., 2000). The effect of each concentration of HU210 was monitored for 60 min at 10 min intervals. HU210 was dissolved in distilled H_2O and ethanol, the final concentration for the highest concentration of HU210 studied <0.3% ethanol. In a separate set of experimental rats the effect of subcutaneous administration of HU210 (6, 30 and 60 μg/kg) on the electrically evoked responses of dorsal horn neurones of sham (n=6 rats) and nerve injured rats (n=5 rats) was studied. Doses of systemic HU210 were chosen on the basis of behavioural studies and are in keeping with those used in a recent behavioural study (Fox et al., 2001). The ability of pre-administration (40 min) of the selective CB_1 receptor antagonist, SR141716A (subcutaneous 1 mg/kg), to block the effect of subcutaneous administration of HU210 (30 μg/kg) on evoked neuronal responses over a 60 min period was studied in sham operated rats (n=7).

Data are presented as mean maximal percentage of the pre-drug control value ± standard error of mean (s.e.m.) unless stated otherwise. Statistical analysis was performed with repeated measures ANOVA and Dunnett's multiple comparison test, or Mann Whitney test where appropriate.

3. Results

3.1. Nerve injured rats develop ipsilateral mechanical allodynia

Following tight ligation of spinal nerves L5 and L6, nerve injured rats (n=12) exhibited normal grooming behaviour and weight gain similar to the sham controls (n=19). The lesioned paw of nerve injured rats was mildly deformed with the toes held together, none of the rats exhibited autotomy. The development of mechanical allodynia was monitored in nerve injured rats over a two-week period. Following the application of innocuous mechanical punctate stimuli (von Frey filament, bending weight 9 g) to the lesioned paw, nerve injured rats exhibited a brisk withdrawal response and in some cases lick-

ing of the lesioned hindpaw. Mechanical allodynia of the lesioned paw was observed as early as PO day 2 and increased over the following two-week period (Fig. 1). Stable levels of mechanical allodynia were observed at PO days 9 and 12. Sham operated rats exhibited negligible levels of mechanical allodynia (Fig. 1). Differences in the level of mechanical allodynia, evoked by the application of a 9 g von Frey filament, of the ipsilateral hindpaw of nerve injured and sham operated rats were significant over this time period. The contralateral hindpaws of nerve injured and sham operated rats did not exhibit mechanical allodynia (data not shown).

3.2. Characteristics of neuronal populations of nerve injured and sham rats

In vivo extracellular recordings of dorsal horn neurones were carried out between PO days 14–17 in nerve injured and sham operated rats. Comparative pharmacological studies of the effect of the cannabinoid agonist HU210 (spinal and systemic administration) were carried out in nerve injured and sham operated rats. The mean depths of neurones, thresholds and latencies of C-fibre evoked responses were similar for nerve injured (803±56 μm, 1.7±0.2 mA, 155±14 ms, respectively) and sham (765±67 μm, 1.7±0.2 mA, 172±15 ms) operated rats used to study the effect of spinal HU210. Similarly, the mean depths of neurones, thresholds and latencies of C-fibre evoked responses were similar for nerve injured (790±78 μm, 2±0.2 mA, 192±25 ms, respectively) and sham (772±57 μm, 1.9±0.2 mA, 173±15 ms) operated rats used to study the effect of systemic HU210. An example trace of a control response of a single dorsal horn neurone following peripheral stimulation of the receptive field in a rat is shown in Fig. 2.

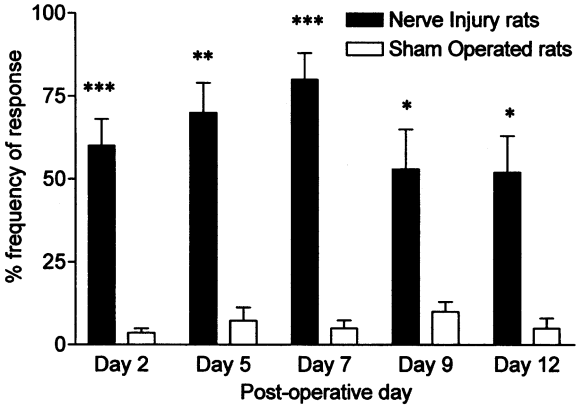

Fig. 1. Temporal development of mechanical allodynia of the ipsilateral hindpaw of nerve injured rats. Mechanical punctate stimuli (von Frey hair, bending force of 9 g) were applied to the ipsilateral hindpaws of nerve injured (n=12) and sham operated (n=19) rats. Significant levels of mechanical allodynia were observed in nerve injured rats as compared to sham operated rats. Data are expressed as mean % frequency response, statistical analysis with Mann Whitney non-parametric test, *p<0.05, **p<0.001, ***p<000.1.

3.3. Differential effects of HU210 on evoked neuronal responses of nerve injured and sham rats

Spinal HU210 (0.5–500 ng/50 μl) did not alter Aβ-fibre evoked neuronal responses in sham (control: 61±9 action potentials) or nerve injured (control: 100±15 action potentials) rats (Fig. 3(A)). However, Aδ-fibre evoked responses of neurones in both sham operated (control: 70±19 action potentials) and nerve injured (54±6 action potentials) rats were significantly reduced by spinal administration of HU210 (Fig. 3(B)).

Spinal HU210 (0.5–500 ng/50 μl) dose-relatedly and significantly reduced the C-fibre mediated post-discharge response of dorsal horn neurones (n=6) in sham operated rats (control: 316±128 action potentials, Fig. 3(C)). In addition, both the overall C-fibre evoked neuronal responses (control: 380±76 action potentials, 72±4% of control, p<0.05) and non-potentiated responses (control: 387±94 action potentials, 62±10% of control) of neurones were reduced HU210 (500 ng/50 μl) in sham operated rats.

In nerve injured rats only the highest concentration of HU210 studied reduced the post-discharge response of dorsal horn neurones, significance was not reached (control: 188±62 action potentials, n=7, Fig. 3(C)). A significant difference between the inhibitory effect of the highest concentration of HU210 on the post-discharge response of neurones of sham and nerve injured rats is reported (p<0.05, Mann Whitney non-parametric test, Fig. 3(C)).

Neither the overall C-fibre evoked neuronal responses (control: 318±28 action potentials, 92±8% of control), nor the non-potentiated responses (control: 357±62 action potentials, 83±18% of control) of neurones were reduced by HU210 (500 ng/50 μl) in nerve injured rats.

A contemporaneous study (Drew et al., 2000) demonstrated that the inhibitory effects of HU210 (500 ng/50 μl) on C-fibre mediated post-discharge responses are significantly reduced (p<0.05, one way ANOVA Kruskal-Wallis post hoc test, n=7) in the presence of the selective CB_1 receptor antagonist SR141716A (spinal administration: 0.01 μg/50 μl).

Systemic administration of HU210 (6–60 μg/kg, s.c.) did not alter Aβ-fibre evoked responses of neurones in sham operated (control: 80±11 action potentials) and nerve injured (control 85±16 action potentials) rats (Fig. 4(A)). By contrast, Aδ-fibre evoked responses of dorsal horn neurones in sham operated rats (control 66±11 action potentials), but not nerve injured (control: 89±27 action potentials) rats, were significantly reduced by systemic administration of HU210 (Fig. 4(B)). A significant difference in the effect of 60 μg/kg of HU210 on Aδ-fibre evoked responses of neurones of nerve injured rats

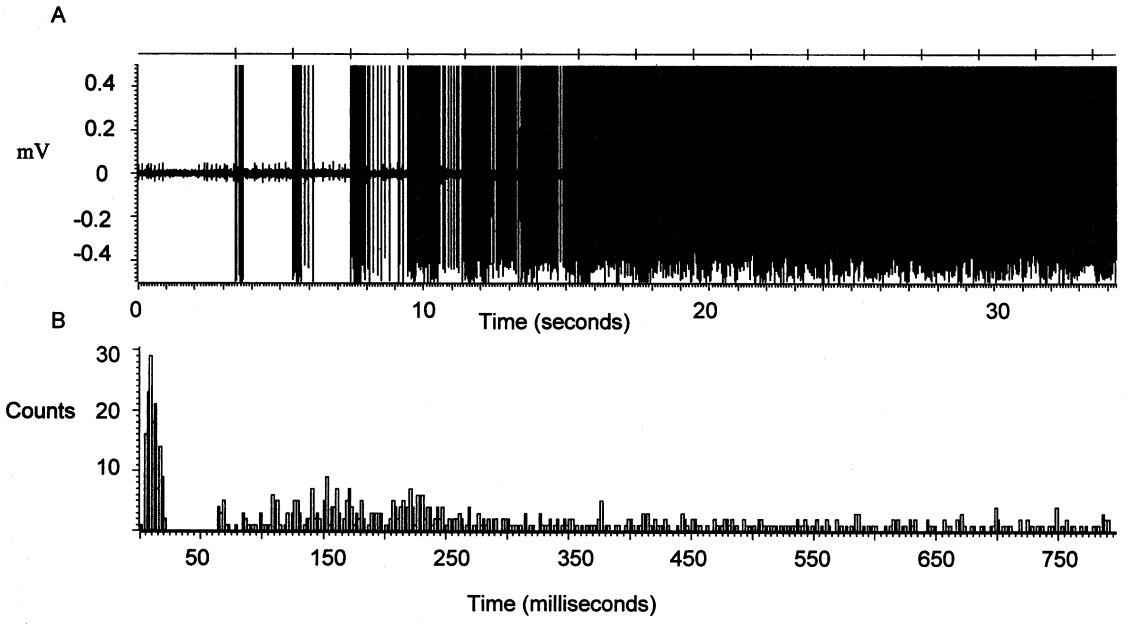

Fig. 2. An example trace recording (A) and post-stimulus histogram (B) of the response of a single dorsal horn neurone following repetitive (16) transcutaneous electrical stimulation of the receptive field (3×C-fibre threshold, frequency of 0.5 Hz). Defined bands of neuronal activity can be identified, including the C-fibre evoked response, 90–300 ms post-stimulus. The neurone exhibits hyperexcitability following repetitive stimulation and a C-fibre mediated late post-discharge response (300–800 ms post-stimulus) is present.

versus sham operated rats is reported ($p<0.01$, Mann Whitney non-parametric test, Fig. 4(B)).

Systemic administration of HU210 dose relatedly and significantly reduced the post-discharge response of dorsal horn neurones in sham operated rats (control: 286±65 action potentials, $n=6$ neurones, Fig. 4(C)), but not in nerve injured rats (control: 430±98 action potentials, $n=5$ neurones, Fig. 4(C)). Significant differences between the effect of 30 μg/kg of HU210 on post-discharge responses of neurones of sham operated and nerve injured rats are reported ($p<0.05$, Mann Whitney non-parametric test, Fig. 4(C)). However, significant differences between the effect of 60 μg/kg of HU210 on post-discharge responses of neurones were not observed between the two groups.

In sham operated rats, the overall C-fibre evoked responses (control 366±33 action potentials) were significantly reduced (54±8% of control, $p<0.01$) by HU210 (60 μg/kg). Non-potentiated responses (control 351±56 action potentials) of dorsal horn neurones in sham operated rats were significantly reduced by 30 and 60 μg/kg of HU210 (63±9% of control, $p<0.05$ and 31±8% of control, $p<0.01$, respectively). However, in nerve injured rats, neither the overall C-fibre evoked responses (control: 421±79 action potentials, 80±4% of control) nor non-potentiated responses (control: 421±52 action potentials, 54±11% of control) of dorsal horn neurones were significantly reduced by HU210 (60 μg/kg).

The effect of systemic HU210 (30 μg/kg) was significantly blocked by pre-administration of SR141716A (1 mg/kg), a selective CB_1 receptor antagonist in sham operated rats. In the presence of SR141716A, HU210 (30 μg/kg) did not significantly alter C-fibre evoked responses of spinal neurones (post-discharge response: 85±7% of control). Significant differences between the effect of HU210 (30 μg/kg) on C-fibre mediated post-discharge responses of neurones, in the presence and absence of SR141716A, are reported ($p<0.05$, Mann Whitney non-parametric test).

4. Discussion

Touch evoked pain (allodynia) is one of the symptoms arising from nerve injury in humans and animal models of neuropathy (Koltzenburg et al., 1994). Following nerve injury, but not sham surgery, rats exhibited a characteristic development of mechanical allodynia of the ipsilateral hindpaw.

In our electrophysiological studies, spinal and systemic administration of the cannabinoid agonist HU210 significantly reduced C-fibre mediated responses of spinal neurones in sham operated rats. In marked contrast, spinal and systemic HU210 did not significantly reduce C-fibre mediated responses of spinal neurones in nerve injured rats. The inhibitory effects of spinal HU210 in sham operated rats are consistent with our previous report of the anti-nociceptive effect of spinal HU210 in rats (Drew et al., 2000). Previously, we reported that inhibitory effects of spinal HU210 are significantly blocked by spinal administration of the CB_1 antagonist SR141716A (Drew et al., 2000). In the present study,

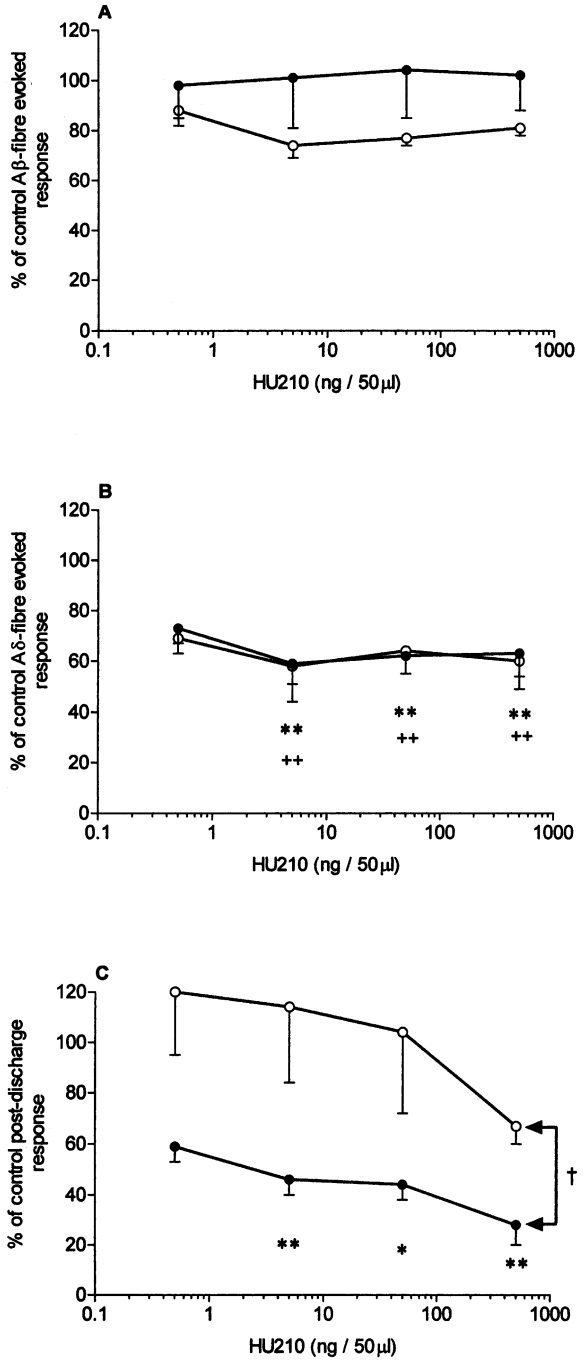

Fig. 3. Effects of spinal administration of HU210 on electrical evoked responses of dorsal horn neurones in sham operated (n=6 neurones in six rats, closed symbols) and nerve injured rats (n=7 neurones in seven rats, open symbols). (A) HU210 did not alter Aβ-fibre evoked responses of spinal neurones in nerve injured or sham operated rats. (B) Aδ-fibre evoked responses of neurones of nerve injured and sham operated rats were significantly reduced by HU210. (C) C-fibre mediated post discharge response of spinal neurones of sham operated rats was significantly reduced by HU210. By contrast, HU210 did not markedly reduce post-discharge responses of neurones in nerve injured rats. Significant differences between the effect of 500 ng/50 µl of HU210 on the post-discharge response of neurones of sham and nerve injured rats are reported. Data are expressed as % of the control evoked response; statistical analysis compared to control used repeated measures ANOVA and Dunnett's multiple comparison test, sham rats $^{*}p<0.05$, $^{**}p<0.001$; nerve injured rats $^{++}p<0.01$. Statistical analysis between effects of HU210 in sham and nerve injured rats used Mann Whitney non-parametric test, $^{\dagger}p<0.05$.

inhibitory effects of systemic HU210 on C-fibre evoked responses in sham operated rats were blocked by systemic administration of SR141716A. The effects of systemic HU210 in sham operated rats reported here confirm the finding of a previous electrophysiological study that systemic cannabinoids inhibit noxious stimulus-evoked responses of spinal neurones in rats (Hohmann et al., 1999b).

Spinal HU210 significantly reduced Aδ-fibre evoked neuronal responses in sham operated and nerve injured rats. However, systemic administration of HU210 only reduced Aδ-fibre evoked neuronal responses in sham operated rats. Aβ-fibre evoked responses of dorsal horn neurones were not influenced by HU210 under any of the conditions studied.

Cannabinoids influence cardiovascular responses, both arterial blood pressure and heart rate have been reported to be decreased by systemic HU210 in the rabbit (Clarke et al., 2001). Blood pressure recordings were not carried out in our studies and therefore cardiovascular effects of systemic HU210 may influence our results. However, sham and nerve injured rats were studied under identical experimental conditions and therefore it is unlikely that the differential effects of systemic HU210 in the two groups of rats arise due to cardiovascular effects. Inhibitory effects of systemic HU210 reported here were blocked by SR141716A, contrasting the report that the effect of HU210 on heart rate is not reversed by SR141716A (Clarke et al., 2001), further dissociating the effect of cannabinoids on nociceptive and cardiovascular events.

A major finding of the present study is the loss of inhibitory effect of spinal HU210 on C-fibre evoked neuronal responses in nerve injured rats, which exhibited a sustained level of mechanical allodynia. Loss of receptor binding sites due to primary afferent deficits is unlikely (Lekan et al., 1997). Recent evidence suggests that CB_1 receptors are also located on post-synaptic excitatory interneurones in the superficial laminae of the spinal cord (Farquhar-Smith et al., 2000). Spinal HU210 had similar effects on Aδ-fibre evoked responses of spinal neurones in nerve injured and sham operated rats, and therefore dramatic changes in post-synaptic receptors are unlikely. The loss of effect of spinal HU210 on C-fibre, but not Aδ-fibre, mediated responses following nerve injury suggests that CB receptors on C-fibres are functionally down-regulated under these conditions, an uncoupling of pre-synaptic spinal CB receptors and asso-

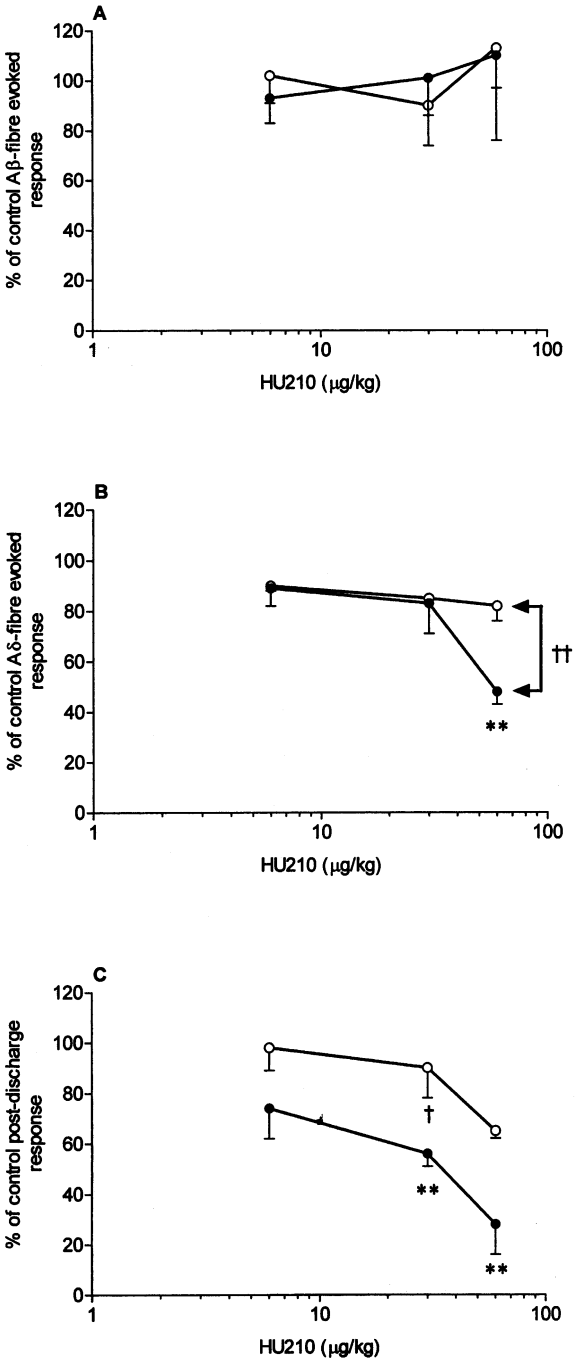

Fig. 4. Effects of systemic administration of HU210 on electrical evoked responses of dorsal horn neurones in sham operated (n=6 neurones in six rats, closed symbols) and nerve injured (n=5 neurones in five rats, open symbols) rats. (A) Systemic administration of HU210 did not influence Aβ-fibre evoked responses of spinal neurones of nerve injured or sham operated rats. (B) Aδ-fibre evoked responses of spinal neurones of sham operated rats were only reduced by the highest dose of systemic administration of HU210 studied. Aδ-fibre evoked neuronal responses of nerve injured rats were not influenced by systemic administration of HU210. Significant differences between the effect of 60 μg/kg of HU210 on Aδ-fibre evoked responses of neurones of sham and nerve injured rats are reported. (C) HU210 significantly and dose-relatedly reduced C-fibre mediated post-discharge responses of neurones of sham operated rats. By contrast HU210 did not markedly influence post-discharge responses of neurones in nerve injured rats. Significant differences between the effect of 30 μg/kg of HU210 on post-discharge responses of neurones of sham and nerve injured rats are reported. Data are expressed as % of the control evoked response; statistical analysis compared to control used repeated measures ANOVA and Dunnett's multiple comparison test, sham operated rats $^{**}p<0.001$. Statistical analysis between effects of HU210 in sham and nerve injured rats used Mann Whitney non-parametric test, $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$.

ciated G-proteins following peripheral nerve damage may contribute to this effect.

Effects of systemic HU210 on spinal C-fibre and Aδ-fibre evoked neuronal responses were also reduced in nerve injured rats, suggesting that the descending control systems underlying supraspinal CB receptor mediated anti-nociception (Hohmann et al., 1999b) are altered following peripheral nerve injury. Supraspinal cannabinoid receptors (Martin et al., 1998) modulate spinal nociceptive transmission via activation of noradrenergic and serotonergic descending inhibitory controls. Functional alteration of these descending controls or the spinal targets activated by the descending inputs (Stone et al., 1999) may underlie the loss of inhibitory effect of systemic cannabinoid reported in nerve injured rats.

Our study demonstrates plasticity of spinal cannabinoid receptor function following peripheral nerve injury, in particular in relation to C-fibre transmission. The sustained effect of spinal HU210 on Aδ-fibre evoked responses of spinal neurones following nerve injury suggests that cannabinoids may reduce some types of spinal somatosensory processing under these conditions. This finding corroborates the results of behavioural studies (Herzberg et al., 1997; Bridges et al., 2001; Fox et al., 2001), which have demonstrated that systemic cannabinoid agonists reduce mechanical allodynia in models of neuropathic pain. Aberrant activity in A-fibres underlies allodynia (Gold, 2000) and evidence suggests that cannabinoid receptors are present on medium sized DRG cell bodies, corresponding to A-fibres (see Section 1), providing a plausible site of action of cannabinoids. Collectively, there is increasing evidence that cannabinoid agonists reduce behavioural allodynia, our results suggest that actions of cannabinoids on Aδ-fibre mediated responses may contribute to these effects.

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