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CHF3381, a novel antinociceptive agent, attenuates capsaicin-induced pain in rats

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Abstract

Here, we have examined the effect of the novel antinociceptive agent CHF3381 on the development of nocifensive behaviour as well as secondary mechanical allodynia and hyperalgesia induced by intraplantar injection of capsaicin in rats.

Vehicle, CHF3381 or gabapentin were orally administered 1 h before capsaicin injection. The duration of nocifensive behaviour was measured during the first 5 min after capsaicin injection. Secondary mechanical allodynia and hyperalgesia were measured at 5 and 15 min after capsaicin injection, respectively.

CHF3381 produced a significant suppression of nocifensive behaviour and completely blocked the development of mechanical allodynia and hyperalgesia at 100 and 200 mg/kg.

Gabapentin weakly inhibited the development of nocifensive behaviour and mechanical allodynia. On the contrary, gabapentin (100 mg/kg) completely prevented the development of mechanical hyperalgesia.

In conclusion, CHF3381 had full efficacy for all the capsaicin-induced pain parameters tested, suggesting that CHF3381 may have a therapeutic utility in the management of pain states involving central sensitisation.

Keywords: CHF3381; NMDA receptor antagonist; MAO-A inhibitor; Antinociceptive effect; Capsaicin

1. Introduction

Intradermal and topically applied capsaicin, a pungent component from chilli peppers, induces a short-lasting nociceptive response as well as allodynia and hyperalgesia in a large area surrounding the application site. Primary allodynia and hyperalgesia to heat and mechanical stimulation occur at the site of capsaicin injection. Secondary allodynia and hyperalgesia to mechanical stimulation are observed in the surrounding, non-injured tissue (Szolcsayni, 1997; Simone et al., 1987, 1989; La Motte et al., 1992; Sun et al., 2003). The short-lasting nociceptive response as well as primary allodynia and hyperalgesia can be explained in terms of peripheral nociceptors sensitisation (Davis et al., 1993). The responsiveness of sensory receptors in the area of secondary allodynia and hyperalgesia is unlikely to be affected by capsaicin injection since they are far enough from the injection site, indicating that the enhanced responses to stimulation in the secondary area must be due to central sensitisation produced by the initial intense nociceptive discharge that follows the capsaicin injection (Willis, 2002; Willis and Coggeshall, 2004). Central sensitisation caused by capsaicin injection involves the activation of different receptor systems in the spinal cord, among which the *N*-methyl-D-aspartate (NMDA) receptor seems to play a crucial role (Willis, 2002).

Behavioural studies have investigated the role of the NMDA receptor in capsaicin-induced nociception in rodents and in primates. NMDA receptor antagonists have been shown to prevent capsaicin-induced nociception after intrathecal or parenteral injection in mice. It is worth noting that the antinociceptive effect of NMDA receptor antagonists

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can be reversed by the spinal co-administration of NMDA (Taniguchi et al., 1997; Sakurada et al., 1998). In unanesthetised nonhuman primates, the noncompetitive NMDA receptor antagonists ketamine and MK-801 prevented capsaicin-induced thermal allodynia at doses that did not induce motor deficits (Butelman et al., 2003).

Intradermal capsaicin has been used extensively as an experimental noxious stimulus also in humans (La Motte et al., 1992). Intravenous infusion of ketamine in human volunteers decreased sensitivity to painful stimuli in primary and secondary hyperalgesic areas following topical application of capsaicin (Andersen et al., 1996). Taken together, these results suggest that the capsaicin-induced pain model is suitable for the evaluation of the antinociceptive activity of new NMDA receptor antagonists both in experimental animals and humans.

CHF3381 is a low-affinity noncompetitive NMDA receptor antagonist and reversible monoamine oxidase-A (MAO-A) inhibitor endowed with antinociceptive activity in experimental models of central sensitisation at doses devoid of obvious side-effects (Villetti et al., 2003). In the present study we investigated the ability of CHF3381 to attenuate capsaicin-evoked nociceptive response as well as counteract secondary mechanical allodynia and hyperalgesia. CHF3381 antinociceptive activity was compared with that of gabapentin, which has been shown to reduce allodynia and hyperalgesia in animal models involving neuronal sensitisation and nerve injury (Field et al., 1997; Hunter et al., 1997) and to suppress cutaneous hyperalgesia following heat-capsaicin sensitisation in healthy volunteers (Dirks et al., 2002).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 150–175 g on day of arrival were supplied from Charles River Laboratories Italy (Calco, Lecco). Animals were group-housed in standard laboratory cages with wood chips as bedding (Lignocel, Harlan Italy, Milan) and acclimated to the local vivarium conditions (temperature: 22±2 °C; relative humidity: 55±15%, 12-h light, 12-h dark cycle from 7.00 a.m. to 7.00 p.m.) for 1 week before the experiments. Standard food (4RF21 produced by Mucedola S.r.l., Milan for Charles River Laboratories Italy) and water were available ad libitum.

All the experiments took place between 10:00 and 17:00 h. No animal was used more than once. The experiments were performed in accordance with EEC Guidelines (86/609/ECC) for the use of laboratory animals. Additionally, all experiments followed the Guidelines on Ethical Standards for Investigation on Experimental Pain in Animals (Zimmermann, 1983).

2.2. Intraplantar injection of capsaicin

Capsaicin (8-methyl-*N*-vanillyl-trans-6-nonenamide) was used in all experiments as its free base. Capsaicin was dissolved in a vehicle containing 7.5% of Tween 80 in distilled water, as previously described (Gilchrist et al., 1996).

Intraplantar injections of the capsaicin solution were given in a volume of 10 μl using a micro-syringe Hamilton fitted with a 30-gauge needle. Rats received a unilateral injection of capsaicin intradermally in the mid-plantar surface of the hind paw. The appearance of a bleb within the injection site indicated a successful injection.

The Tween-80/distilled water vehicle was used for control injection.

2.3. Capsaicin-induced nocifensive behaviour

The day before testing, rats were placed individually into clear plastic cylinders for 30 min to allow adaptation to the new environment. The day of testing, at 1 h after oral administration of the test compounds, rats received a unilateral intraplantar injection with capsaicin (10 μg/paw). Control animals were similarly injected with vehicle only. Animals were placed again into the individual plastic cylinders on a clear plastic table for the behavioural observation. A camera was placed under the plastic table and the experimental sessions were recorded on videotape and analysed off-line.

The total time (in seconds) spent on licking/flinching the capsaicin-injected paw was measured for a period of 5 min, immediately after the intraplantar injection of capsaicin.

2.4. Capsaicin-induced mechanical allodynia

Rats were placed on an elevated screen in a clear testing chamber and allowed to acclimate to the testing environment before any measurements were taken. To assess mechanical allodynia, paw withdrawal thresholds to a non-noxious tactile stimulus were determined using an automatic electronic Von Frey device (Dynamic Plantar Aesthesiometer; Ugo Basile, Italy).

The electronic Von Frey device employs a single nonflexible filament which applies an increasing force (from 0 to 50 g) against the plantar surface of the hind paw over a 20-s period. The end point was taken as nocifensive paw withdrawal, and five thresholds were taken per test and averaged.

To quantify the mechanical sensitivity of the hind paw, baseline withdrawal thresholds were determined before capsaicin injection.

Animals were then stratified into groups based on their baseline withdrawal thresholds, so that the mean baseline did not differ between groups. Animals were then treated with test compounds 60 min before capsaicin (10 μg/paw) injection. Mechanical withdrawal thresholds were then recorded at 5 min after capsaicin injection, as previously reported (Gibbs et al., 2001). Care was taken to stimulate random locations proximal and distal to the injection site on the plantar surface.

2.5. Capsaicin-induced mechanical hyperalgesia

Mechanical nociceptive thresholds were assessed by applying an increasing noxious pressure stimulus to the distal portion of the plantar surface of the hind paw, using an analgesymeter (Ugo Basile, Italy) according to the method of Randall and Selitto (1957). The site of the stimulation was on area of the hind paw between the pads at the base of the third and forth digit, distally to the site of capsaicin injection. A cut-off was set at 500 g to prevent any tissue damage and the endpoint was taken as a complete paw withdrawal.

In preliminary experiments, paw withdrawal thresholds were measured before and up to 15 min after intraplantar injection of 1, 3, 10 or 30 μg of capsaicin in one hind paw. These experiments showed that 30 μg of capsaicin produced a submaximal, reproducible

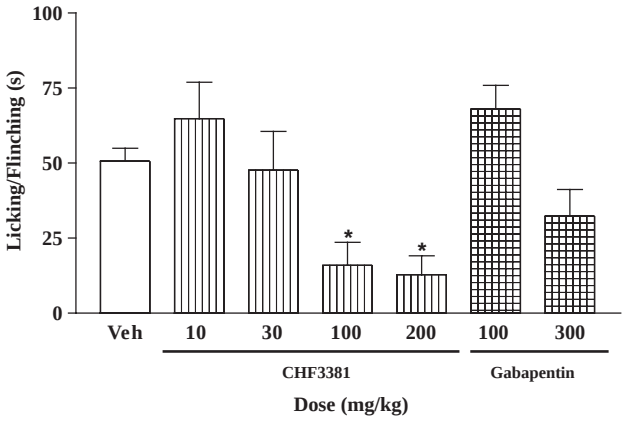

Fig. 1. Effect of CHF3381 and gabapentin on capsaicin-evoked nocifensive behaviour. Nocifensive behaviour was measured for 5 min following capsaicin injection. Vehicle or test compounds were administered by oral route 1 h prior to capsaicin injection. Data are given as the mean±S.E.M. ($n=8$). *$P<0.05$ vs. vehicle-pretreated group.

mechanical hyperalgesia, and this dose was used for further studies. The time of testing, 15 min, is in good agreement with the observations of Gilchrist et al. (1996), which showed that mechanical hyperalgesia peaked between 7 and 15 min after capsaicin injection.

The morning before testing, rats received three training sessions. Baseline paw withdrawal thresholds were defined as the mean of the last two trials to eliminate the large variability found in the initial withdrawal measurement. To examine the inhibition of capsaicin-induced mechanical hyperalgesia, test compounds or vehicle was administered 1 h before capsaicin injection, and withdrawal thresholds were then determined 15 min after capsaicin injection.

2.6. Drugs used

CHF3381 2-[(2,3-dihydro-1H-inden-2-yl)amino]acetamide monohydrochloride was synthesised at the Chiesi Farmaceutici Chemical Synthesis Department. Gabapentin was extracted from Neurontin® (Parke-Davis) tablets. Purity of CHF3381 and gabapentin was >95%. Drugs were dissolved with distilled water and administered by oral route.

2.7. Statistical analysis

Data are expressed as means±S.E.M. For mechanical allodynia and hyperalgesia, data are also expressed as the percentage variation of the nociceptive threshold from baseline values after treatment with test compounds and capsaicin injection.

Data analysis were performed by one-way analysis of variance . On detection of a significant main effect, treatment groups were compared to appropriate control groups using Dunnett's t test.

Differences between vehicle-injected and capsaicin-injected rats were analysed using an independent Student's t test. The level of significance was set at $P<0.05$.

3. Results

3.1. Capsaicin-induced nocifensive behaviour

Intraplantar injection of capsaicin (10 μg) immediately evoked licking and flinching of the paw. Licking/flinching behaviour persisted for 50.6±4.3 s. This behaviour appeared immediately, peaked at 1–3 min and then disappeared by 5 min, before testing for mechanical allodynia and hyperalgesia began (5 min and 15 min after capsaicin injection, respectively).

Oral pretreatment with CHF3381 (10–200 mg/kg) dose-dependently prevented capsaicin-induced nocifensive behaviour, as shown in Fig. 1. The effect was statistically significant at the doses of 100 and 200 mg/kg (licking/flinching duration: 15.9±7.7, 12.8±6.3 s, respectively; $P<0.05$).

In contrast, oral pretreatment with gabapentin (100–300 mg/kg p.o.) was ineffective in this test. A not significant antinociceptive activity was observed with gabapentin 300 mg/kg (flinching/licking duration: 32.3±8.9 s).

3.2. Capsaicin-induced mechanical allodynia

Before capsaicin injection, no differences in mechanical withdrawal thresholds evaluated by the Electronic Von Frey device were observed between groups (mean baseline withdrawal thresholds ranging from 39.6±2.1 to 41.9±1.7 g). Intraplantar injection of capsaicin vehicle (7.5% Tween 80 in distilled water) did not produce significant changes in mechanical withdrawal threshold (data not shown). Following intraplantar injection of capsaicin (10 μg), mechanical withdrawal threshold was significantly reduced to 24.1±1.2 g (40.2±4.4% reduction from baseline) in control animals treated with test compound vehicle ($P<0.01$ vs. capsaicin vehicle-injected animals).

Oral administration of CHF3381 (10–200 mg/kg) produced a dose-related reversal of capsaicin-induced mechanical allodynia; the effect being statistically significant at the doses of 100 and 200 mg/kg ($P<0.01$; Fig. 2). At the highest dose, 200 mg/kg, of CHF3381 reversal of capsaicin-induced allodynia was almost complete (mean withdrawal threshold: 38.8±2.1 g; % reduction from baseline: 1.3±7.8).

Gabapentin at an oral dose of 100 mg/kg had no substantial effect on capsaicin-induced mechanical allodynia, with a mean withdrawal threshold of 28.8±1.7 g (26.3±6.9% reduction from baseline; $P>0.05$).

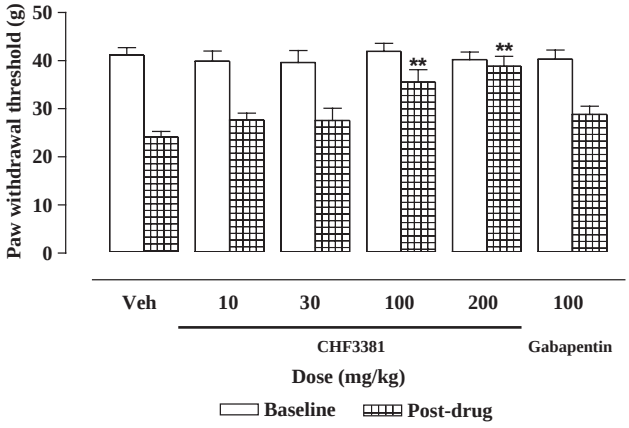

Fig. 2. Effect of CHF3381 and gabapentin on capsaicin-evoked mechanical allodynia. Paw withdrawal thresholds were measured by an electronic Von Frey device prior to vehicle or test compound administration (baseline) and starting 5 min after capsaicin injection (post-drug). Vehicle or test compounds were administered by oral route 1 h prior to capsaicin injection. Data are given as the mean±S.E.M. ($n=7–12$). **$P<0.01$ vs. vehicle-pretreated group.

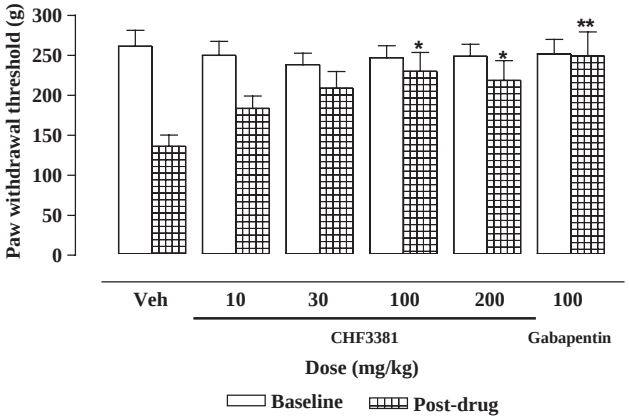

Fig. 3. Effect of CHF3381 and gabapentin on capsaicin-evoked mechanical hyperalgesia. Paw withdrawal thresholds were measured by the paw pressure test prior to vehicle or test compounds administration (baseline) and starting 15 min after capsaicin injection (post-drug). Vehicle or test compound were administered by oral route 1 h prior to capsaicin injection. Data are given as the mean ± S.E.M. ($n = 16–18$). *$P < 0.05$; **$P < 0.01$ vs. vehicle-pretreated group.

3.3. Capsaicin-induced mechanical hyperalgesia

Before capsaicin injection, paw withdrawal thresholds evaluated by the paw pressure test did not differ significantly between groups, ranging from 238.2 ± 14.4 to 261.2 ± 20.0 g. In control rats, capsaicin injection (30 μg) decreased the paw withdrawal threshold to 136.1 ± 14.2 g (46.4 ± 5.4% reduction from baseline). This value was significantly lower than the basal value observed before capsaicin injection ($P < 0.01$).

Oral administration of CHF3381 (10–200 mg/kg) dose-dependently attenuated the development of capsaicin-induced mechanical hyperalgesia, the effect being statistically significant at the doses of 100 and 200 mg/kg ($P < 0.05$; Fig. 3). The maximal effect was observed at the 100 mg/kg dose, with a mean paw withdrawal threshold of 229.8 ± 23.6 g (4.45 ± 9.48% reduction from baseline).

Oral pretreatment with gabapentin (100 mg/kg) completely prevented the development of mechanical hyperalgesia. The mean paw withdrawal threshold in the injected paw of animals pretreated with gabapentin was 249.1 ± 29.9 g (1.74 ± 11.02% reduction from baseline; $P < 0.01$).

4. Discussion

The present study assessed the ability of the novel low-affinity, noncompetitive NMDA receptor antagonist and reversible MAO-A inhibitor CHF3381 to prevent the development of capsaicin-induced pain in rats. The results indicate that CHF3381 attenuated the duration of nocifensive behaviour and blocked the development of secondary mechanical allodynia and hyperalgesia after intraplantar capsaicin injection.

Spontaneous, short-lasting, nocifensive behaviour can be elicited by the local administration of irritants other than capsaicin, such as formalin and adenosine triphospate. However, injections of capsaicin and formalin (first phase) activate different biochemical and receptorial pathways in the central nervous system. This is supported by the evidence that capsaicin-induced nociception is sensitive to the treatment with both excitatory amino acid and tachykinin neurokinin 1 (NK_1) antagonists and can be prevented by desensitisation of sensory nerve terminals achieved by pretreatment with high doses of capsaicin itself (Taniguchi et al., 1997; Sakurada et al., 1993; Szallasi and Blumberg, 1999). In contrast, excitatory amino acid and tachykinin NK_1 receptor antagonists only weakly or partially reduced the nocifensive behaviour observed during the early phase of formalin-induced pain (Quartaroli et al., 1999; Henry et al., 1999). In agreement with the above results, we observed that CHF3381 given at doses (100–200 mg/kg) that almost completely reduced nocifensive behaviour following intraplantar injection of capsaicin was less effective in attenuating the first phase of formalin-induced nociception (maximal effect: 50%; G. Villetti, unpublished observation).

The appearance of a presumed area of secondary mechanical and allodynia after intraplantar capsaicin injection has been described in different studies by different groups (Sluka, 1997; Sun et al., 2003; Yu et al., 2004). In our study, we reproduced the experimental conditions described in these papers, use of a very low volume and dose of capsaicin, application of the testing stimuli outside the area encompassed by capsaicin injection. Therefore, we think that the results of our tests effectively reflect the development of secondary mechanical allodynia and hyperalgesia due to intraplantar capsaicin injection and the effect of test compounds on these parameters.

Importantly, we found that pretreatment with CHF3381 almost completely prevented the development of secondary mechanical allodynia and hyperalgesia following capsaicin injection. To our knowledge, this is the first study to show that a NMDA receptor antagonist and reversible MAO-A inhibitor is orally active in attenuating capsaicin-evoked central sensitisation in experimental animals. In previous studies, NMDA receptor antagonists were administered by spinal infusion or parenteral administration and the effect of test compounds was investigated only on capsaicin-evoked nocifensive behaviour (Taniguchi et al., 1997; Sakurada et al., 1998; Wismer et al., 2003; Soliman et al., 2005). Moreover, present results are consistent with our previous findings that CHF3381 is effective in reversing established mechanical hyperalgesia and allodynia associated with inflammation or nerve injury (Villetti et al., 2003). It is unlikely that CHF3381-induced inhibition of capsaicin-evoked secondary mechanical allodynia and hyperalgesia is attributable to sensorimotor deficits (e.g., inability to detect a mechanical stimulus or to rapidly emit the requested escape response). Indeed, oral administration of CHF3381 at the doses employed in the present study did not impair the performance of rats on the rota-rod test (Villetti et al., 2001).

Preliminary experiments show that CHF3381 potently inhibits capsaicin-induced ventral root depolarisation in an isolated hemisected spinal cord preparation of the newborn

rat (M. Barbieri, unpublished observation). This observation, along with CHF3381-induced inhibition of the wind-up phenomenon (Villetti et al., 2003), supports the hypothesis that CHF3381 acts, at least in part, by inhibiting nociceptive transmission in the spinal cord for producing its antinociceptive activity.

Indeed, CHF3381 has been reported to reversibly and preferentially inhibit MAO-A activity in the same range of concentrations acting on NMDA receptors (IC_{50}=7.2 and 8.8 μM, respectively) (Villetti et al., 2003). MAO-A inhibition could account, at least in part, for the CHF3381 antinociceptive activity observed in this study. The reversible MAO-A inhibitor moclobemide has been reported to be endowed with antinociceptive activity in models of acute and neuropathic pain (Schreiber et al., 1998; Apaydin et al., 2001). A mechanism that may explain the antinociceptive effect observed after treatment with MAO-A inhibitors in experimental pain models is the modulation of descending inhibitory pain pathways originating in the brain and projecting to the spinal dorsal horn through an increase of serotonine and noradrenaline levels, substances that have been implicated as mediators of endogenous analgesic mechanisms (Millan, 2002). However, further experiments that examine the antinociceptive activity of CHF3381 alone and in conjunction with specific noradrenergic and serotonergic antagonists are necessary to clarify the relative involvement of MAO-A inhibition in CHF3381 antinociceptive activity.

Gabapentin was included in the present study for two different reasons. First, in vitro studies have shown that gabapentin inhibits capsaicin-evoked nociceptive spinal transmission (Patel et al., 2001). Second, gabapentin has been reported to suppress cutaneous hyperalgesia following heat-capsaicin sensitisation in healthy volunteers (Dirks et al., 2002). However, we found only a tendency to reduction of capsaicin-induced nocifensive behaviour and secondary mechanical allodynia after oral treatment with gabapentin. On the other hand, gabapentin completely prevented the development of capsaicin-induced secondary mechanical hyperalgesia. The effect on mechanical hyperalgesia is in accordance with our previous report, showing a potent activity of gabapentin in reducing mechanical hyperalgesia induced by carrageenan (Villetti et al., 2003). On the other hand, there are no reports evaluating the activity of gabapentin against capsaicin-induced nocifensive behaviour and mechanical allodynia assessed with the electronic Von Frey. The weak effect of gabapentin on capsaicin-induced nocifensive behaviour is in good agreement with previous studies, reporting that the efficacy of gabapentin in models of acute nociception, such as the hot-plate test and the early phase of formalin-induced pain, is very low (Villetti et al., 2003; Field et al., 1997; Laughlin et al., 2003). As for mechanical allodynia, it has been proposed that mechanical hyperalgesia and mechanical allodynia are mediated by a distinct population of neurones (Woolf et al., 1992). If capsaicin-induced mechanical allodynia and hyperalgesia are mediated by different populations of neurones, it is conceivable that the underlying pharmacology of these symptoms may be separate and that gabapentin has a preferential action on neurones responsible for mechanical hyperalgesia.

In conclusion, our previous and present results with CHF3381 suggest that this compound may have clinical utility in the management of inflammatory and neuropathic pain states involving central sensitisation. On-going clinical trials will elucidate the potential therapeutic utility of CHF3381.

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