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Antinociceptive activity of CP-101,606, an NMDA receptor NR2B subunit antagonist

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1 The analgesic activity of CP-101,606, an NR2B subunit-selective *N*-methyl-D-aspartate (NMDA) receptor antagonist, was examined in carrageenan-induced hyperalgesia, capsaicin- and 4β-phorbol-12-myristate-13-acetate (PMA)-induced nociceptive tests in the rat.

2 CP-101,606 30 mg kg^{-1}, s.c., at 0.5 and 2.5 h after carrageenan challenge suppressed mechanical hyperalgesia without any apparant alternations in motor coordination or behaviour in the rat.

3 CP-101,606 also inhibited capsaicin- and PMA-induced nociceptive responses (licking behaviour) with ED_{50} values of 7.5 and 5.7 mg kg^{-1}, s.c., respectively.

4 These results suggest that inhibition of the NR2B subunit of the NMDA receptor is effective *in vivo* at modulating nociception and hyperalgesia responses without causing the behavioural side effects often observed with currently available NMDA receptor antagonists.

Keywords: CP-101,606; NMDA receptor antagonist; nociception; hyperalgesia

Introduction

Accumulating evidence suggests that the *N*-methyl-D-aspartate (NMDA)-type glutamate receptors play a pivotal role in the transmission of excitatory signals from primary sensory neurones to the brain through the spinal cord (Dickenson, 1990). NMDA receptors mediate Ca^{2+} influx into neurones, and its receptor-gated channel activity is blocked by Mg^{2+} in a voltage-dependent manner. Subunits of the NMDA receptors are classified into two gene families, i.e., NR1 and NR2. A variety of compounds have been designed as antagonists targeting these subunits of the NMDA receptor for the treatment of neurodegenerative disorders, as well as acute and/or chronic pain and hyperalgesia. CP-101,606 is derived from ifenprodil, a well known α_1-adrenoceptor antagonist exhibiting NMDA receptor antagonist activity via interaction with the polyamine modulatatory site (Carter *et al*., 1990). CP-101,606 is an antagonist selective for the NR2B subunit, providing a novel tool to study the function of the NR2B subunit of NMDA receptors *in vitro* (Menniti *et al*., 1997). CP-101,606 exhibited potent neuroprotective activity in hippocampus neurones (Menniti *et al*., 1997) where the NR2B subunit is predominantly expressed (Ishii *et al*., 1993). This compound does not interact with (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) or kainate receptors in the central nervous system (Chenard *et al*., 1995). In the present study, to gain insight into a potential role of the NR2B subunit of NMDA receptors in transmission and/or modulation of pain signals *in vivo*, we have examined the analgesic activity of CP-101,606 in hyperalgesia and nociceptive tests and compared its effect with those of standard NMDA receptor antagonists, MK-801 and memantine.

Methods

Animals

Male 4- to 5-week old Sprague-Dawley rats (100–200 g body weight) were purchased from SLC Japan. Rats were maintained on stock diet and water *ad libitum* in climate-controlled rooms with a 12 h-light/dark cycle until experiments.

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Carrageenan-induced mechanical hyperalgesia

One hundred microlitres of 1.5% carrageenan suspension was injected into the plantar surface of the hind paw of rats. CP-101,606 was subcutaneously (s.c.) administered twice at 0.5 and 2.5 h after carrageenan injection. The nociceptive threshold of mechanical hyperalgesia in the hind paw was measured according to the Randall-Selitto method (Randall & Selitto, 1957).

Capsaicin-induced licking test

The capsaicin-induced licking behaviour, as an index of nociceptive response, was determined according to the method of Sakurada *et al*. (1992). At 0.5 h after s.c. administration of test compounds, 100 μl of capsaicin (0.08 μg μl^{-1}) was intraplantarly (i.pl.) injected in the hind paw of rats. The duration of licking was observed for 5 min immediately after the capsaicin challenge.

PMA-induced licking test

4β-Phorbol-12-myristate-13-acetate (PMA) 1 μg was injected i.pl. into the hind paw of rats. PMA-induced nociceptive response lasted over 15–45 min, much longer than that of capsaicin. Therefore, the number of paw-licking behaviours was counted for 6 s every 30 s from 15 to 45 min following PMA injection. CP-101,606 (1–30 mg kg^{-1}, s.c.) or piroxicam (10 mg kg^{-1}, s.c.) was administered 0.5 h before and dexamethsone (3 mg kg^{-1}, s.c.) was administered 4 h before PMA injection.

Rota-rod test

Motor coordination of rats was examined on the basis of their ability to walk on a rotating rod turning at 7 r.p.m. (model 7750, Ugo Basile, Italy). Animals that were able to walk on the rod for 120 s were selected for subsequent use in the test. Each rat was tested twice and the longer duration was taken as its motor-coordination score. The effect of CP-101,606 on the rota-rod score was determined in the same manner (30 mg kg^{-1}, s.c. injection twice at 0.5 and 2.5 h) as in the carrageenan-induced hyperalgesia experiment. The effects of a

single dose of CP-101,606 (30–60 mg kg^{-1}), MK-801 (0.1 mg kg^{-1}) and memantine (60 mg kg^{-1}) were determined 0.5 h after s.c. administration.

Measurement of drug level

CP-101,606 was administered at 10 mg kg^{-1}, s.c., and after 0.5 h the rats were killed under ether anaesthesia. The cerebral spinal fluid (CSF) was collected with a 1 ml syringe with a 26G needle. The brain and spinal cord were immediately excised, weighed and homogenized in ice-cold H_2O. The sample (40–100 μl) was mixed with 1 ml of 100% acetonitrile. After centrifugation at 1,800 $\times g$ for 10 min, CP-101,606 in the resulting supernatant was analysed by liquid chromatography-linked mass spectroscopy with a Sciex API-III mass spectrometer equipped with a Hewlett-Packard HP1090 HPLC system.

Calculation of ED_{50} values

ED_{50} values are doses required to inhibit the duration of licking or the licking scores by 50%. The ED_{50} values were calculated by computer-assisted linear regression analysis based on dose-response curves.

Chemicals

Methane sulphonate salt of CP-101,606, (1**S**,2**S**)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol and piroxicam were synthesized at Pfizer Central Research. (+)-MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzyl[a,d]cyclohepten-5,10-imine hydrogen maleate), memantine and dexamethasone were purchased from Sigma (St. Louis, MO, U.S.A.). Test compounds were dissolved in 0.1% methyl cellulose-0.9% NaCl. Carrageenan was purchased from Zushikagaku Laboratory Inc. (Japan) and suspended in saline at final concentrations as described below. Capsaicin and PMA from Sigma (St. Louis, MO, U.S.A.) were dissolved in 10% dimethyl sulphoxide-saline and 2.5% acetone/saline solution, respectively.

Statistical tests

Experimental data were analysed by one-way ANOVA and the statistical significance was determined by *post hoc* tests.

Results

Effect of CP-101,606 on carrageenan-induced hyperalgesia

In control rats, carrageenan injection decreased the nociceptive threshold of mechanical hyperalgesia in a time-dependent fashion (Figure 1). The observed thresholds were 73±11, 47±8, and 41±10 g at 2, 3 and 4 h after the carrageenan injection, respectively, and significantly lower than the original value (127±7 g) observed before carrageenan injection. Since CP-101,606 has a short half-life in the rat ($t_{1/2}$=20 min, unpublished data), the compound was administered at 0.5 and 2.5 h after the carrageenan injection, as indicated by arrows in Figure 1. CP-101,606, at a dose of 10 mg kg^{-1}, s.c., inhibited the decrease of the nociceptive threshold (Figure 1). At a dose of 30 mg kg^{-1}, s.c., CP-101,606 almost completely inhibited carrageenan-induced hyperalgesia (Figure 1).

Effects on capsaicin-induced nociceptive response

Injection of capsaicin (8 μg) in a hind paw of rats immediately led to licking behaviour. In the vehicle group, the duration of licking behaviour was 49±7 s, n=5 (Figure 2a). CP-101,606, MK-801 and memantine dose-dependently reduced the licking behaviour with ED_{50} values of 7.5, 0.06 and 14.5 mg kg^{-1}, respectively (Figure 2a and b).

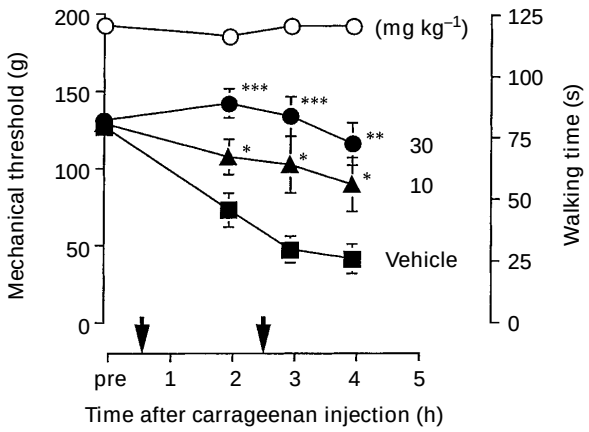

Figure 1 Effect of CP-101,606 on carrageenan-induced mechanical hyperalgesia (solid symbols) and on rota-rod performance (open symbol) in the rat. Hyperalgesia was induced by i.pl. injection of carrageenan at the left hind paw of rats, and vehicle or CP-101,606 (10 mg kg^{-1} and 30 mg kg^{-1}) was administered at 0.5 h and 2.5 h after the carrageenan injection, as described in Methods. The arrows indicate administration of CP-101,606. Rota-rod performance (walking time) was examined by repeated injections of 30 mg kg^{-1} of CP-101,606 with the same dosing protocol for the carrageenan-induced hyperalgesia experiment. Data are expressed as mean and vertical lines show s.e.mean, n=6 to 7. *P<0.05, **P<0.01, ***P<0.001.

Effects on PMA-induced nociceptive response

Like capsaicin, i.pl. injection of PMA (1 μg) into the hind paw of rats caused licking and flinch behaviours, and the pain-associated behaviours were observed for more than 50 min. In the vehicle group, the licking score was 18±3.3, n=5. Figure 2c shows that CP-101,606 significantly reduced the score of licking behaviour (ED_{50} value, 5.7 mg kg^{-1}, s.c.), suggesting antinociceptive activity of this compound against PMA-induced noxious stimulation. Importantly, piroxicam (10 mg kg^{-1}, s.c.), a cyclo-oxygenase inhibitor, and dexamethasone (3 mg kg^{-1}, s.c.) were inactive in this test (Figure 2d).

Effects on motor coordination

CP-101,606 (30 mg kg^{-1}, s.c.) was administered at 0.5 and 2.5 h after carrageenan challenge, and its effect on motor coordination was examined by the rota-rod test (Figure 1). CP-101,606 did not show any detectable effect on the rota-rod performance over the time period observed (up to 4 h). Likewise, no side effects were observed with a single administration of CP-101,606 up to 60 mg kg^{-1}, s.c., in the rota-rod test. In contrast, MK-801 (0.1 mg kg^{-1}, s.c.) and memantine (60 mg kg^{-1}, s.c.) significantly reduced the walking time period from 120 s for control to 54±33 s (P<0.001, n=6) and 19±30 s (P<0.001, n=6), respectively (data not shown).

CP-101,606 levels in plasma, CSF, brain and spinal cord

Thirty minutes after s.c. administration at 10 mg kg^{-1}, the concentration of CP-101,606 in CSF was 62±8 ng ml^{-1}, that was slightly lower than the plasma level, 142±17 ng ml^{-1}. Drug concentrations in the brain and spinal cord tissues were 1,680±125 ng g^{-1} and 2,060±159 ng g^{-1}, respectively.

Discussion

Our present study demonstrates that CP-101,606 has anti-nociceptive activity in carrageenan-induced mechanical hyperalgesia (Figure 1), and capsaicin and PMA-induced licking tests (Figure 2). In all three tests, CP-101,606 did not produce any

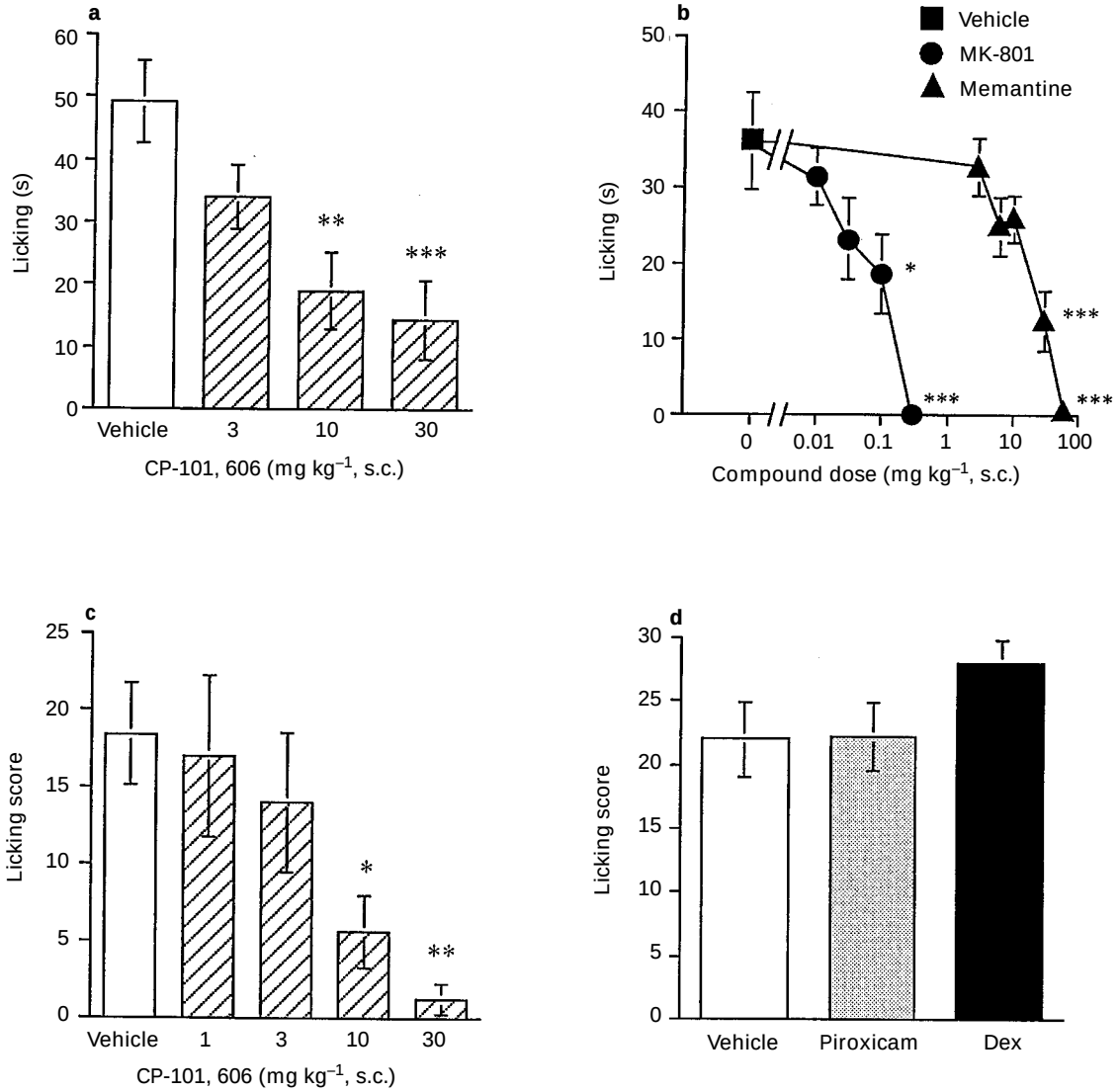

Figure 2 Effect of CP-101,606 on capsaicin-induced (a and b) and PMA-induced licking response (c) in the rat. In the capsaicin test (a and b), CP-101,606 (3–30 mg kg^{-1}), MK-801 (●, 0.01–0.3 mg kg^{-1}) and memantine (▲, 3–60 mg kg^{-1}) were s.c. administered at 0.5 h before capsaicin injection. (c) In the PMA test, CP-101,606 was administered at 1 to 30 mg kg^{-1}, s.c. (d) Effects of piroxicam (10 mg kg^{-1}, s.c.) and dexamethasone (Dex, 3 mg kg^{-1}, s.c.) on PMA-induced licking test. Data are expressed as mean ± s.e., $n=5$. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

observable behavioural alterations such as ataxia, sedation, hyperactivity or circling behaviour over the time course of the experiments. Furthermore, CP-101,606 up to 60 mg kg^{-1}, s.c., did not cause any apparent CNS-related motor incoordination in the rota-rod test. Previously, Pagnozzi *et al.* also showed that CP-101,606 did not cause hyperactivity at doses up to 56 mg kg^{-1}, s.c., or 100 mg kg^{-1}, i.p. (Pagnozzi *et al.*, 1995).

Although other NMDA receptor antagonists, such as MK-801 and memantine, exhibit potent analgesic activities, they are known to produce significant behavioural alterations in animals. We confirmed that MK-801 and memantine significantly reduced capsaicin-induced duration of licking at 0.1 and 30 mg kg^{-1}, s.c., respectively. However, in the rota-rod test, 0.1 mg kg^{-1} of MK-801 or 60 mg kg^{-1} of memantine caused motor incoordination (data not shown).

CP-101,606 was derived from ifenprodil, a compound that exhibited NMDA receptor antagonist activity. Interaction of ifenprodil with the NMDA receptor is selective to the NR2B subunit (Williams *et al.*, 1993; Williams, 1995). However, compared to ifenprodil, CP-101,606 possesses greatly improved selectivity toward the NR2B subunit of the NMDA receptor (Chenard *et al.*, 1995). [^{3}H]-CP-101,606 binding site was found to be most dense in the hippocampus and cortex (White *et al.*, 1995), and *in situ* hybridization revealed that NR2B subunit mRNA was expressed at high levels in these areas (Ishii *et al.*, 1993). Menniti *et al.* have recently demonstrated that glutamate-induced cell death of primary culture of rat hippocampal neurones was dose-dependently inhibited by CP-101,606 with an IC_{50} value of 11 nM. Taken together, it is strongly suggested that CP-101,606 is an NR2B-selective antagonist. Drug concentrations of CP-101,606 0.5 h after s.c. administration at 10 mg kg^{-1} in the brain and the spinal cord tissues were 1,680 ± 125 ng g^{-1} and 2,060 ± 159 ng g^{-1}, respectively. Thus, CP-101,606 distributed in the brain and spinal cord at relatively high levels. Therefore, the analgesic activity of CP-101,606 shown in the present study is suggested to be evoked via blockade of NR2B subunit of NMDA receptor within the CNS.

In summary, CP-101,606, the NR2B subunit-selective NMDA receptor antagonist, has potent analgesic activity in rat hyperalgesia and nociceptive tests at doses causing no behavioural abnormality. The NR2B subunit of the NMDA receptor is considered to be a potential molecular target for the discovery of novel analgesics.

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