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SPECIAL REPORT

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Non-specific activity of (±)-CP-96,345 in models of pain and inflammation

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The non-peptide NK_1 receptor antagonist, CP-96,345, and its **2R,3R** enantiomer CP-96,344, which is not an NK_1 receptor antagonist ($IC_{50} > 10\,\mu M$), were evaluated for antinociceptive and anti-inflammatory activities in several classical models of pain and inflammation in the rat. Both CP-96,345 and CP-96,344 reduced carrageenin-induced paw oedema and hyperalgesia, and attenuated the second phase of formalin-induced paw licking with equal potency. These results indicate that NK_1 antagonism is not responsible for the activity of (±)-CP-96,345 in the above animal models.

Keywords: CP-96,345; non-peptide NK_1 receptor antagonist; pain; inflammation

Introduction The discovery of non-peptide NK_1 receptor antagonist, CP-96,345 [(2S,3S)-*cis*-2-(diphenylmethyl)-N-[(2-methoxyphenyl)methyl)-1-azabicyclo[2.2.2]octan-3-amine] (Snider *et al.*, 1991) has been a major breakthrough in our ability to investigate the role of substance P (SP) and NK_1 receptor responses in a variety of *in vitro* and *in vivo* biological systems.

Recently, several investigators studied CP-96,345 in well-established models of pain and inflammation and implicated NK_1 receptor responses in mustard oil- (Lembeck *et al.*, 1992) and capsaicin-induced (Nagahisa *et al.*, 1992) neurogenic plasma extravasation in carrageenin-induced foot oedema and hyperalgesia (Birch *et al.*, 1992), and in formalin- (Birch *et al.*, 1992), acetic acid- (Nagahisa *et al.*, 1992) and hot plate- (Lecci *et al.*, 1991) induced nociceptive responses. However, a number of these studies were carried out using racemic (±)-CP-96,345 (Birch *et al.*, 1992; Lecci *et al.*, 1991).

In other pharmacological studies, CP-96,345 has been shown to lower blood pressure in intact anaesthetized dogs (Constantine *et al.*, 1992). Since CP-96,344, the **2R,3R** enantiomer of CP-96,345, which is not a SP antagonist, was equally potent, the hypotensive activity CP-96,345 seems to be unrelated to SP antagonism.

In order to ascertain that the previous observations made with racemic (±)-CP-96,345 are indeed due to NK_1 antagonism, we evaluated CP-96,345 and CP-96,344 in several models of pain and inflammation.

Methods *Binding studies* Binding experiments were conducted on brain and spinal cord membrane preparations from rat, essentially according to previously described procedures (Snider *et al.*, 1991). Briefly, tissue preprations (0.1–0.2 mg membrane protein per assay tube) were incubated with test compounds and radiolabelled SP (0.36 nM [^{125}I]-SP and 1 nM [^{3}H]-SP for brain and spinal cord, respectively) at 25°C for 1 h. Non-specific binding was determined in the presence of 1 μM SP.

Carrageenin-induced foot oedema and hyperalgesia in rat Male Sprague-Dawley rats (90–125 g) were used for all experiments. Rat foot inflammation was induced by subplantar injection of 0.1 ml of a 1% solution of carrageenin in saline. To determine the extent of oedema, volumes of the injected foot were measured at various time points with a water-displacement plethysmometer (model TK-101, Unicom). Hyperalgesia was assessed by measuring nociceptive pressure thresholds with an analgesymeter (Ugo Basile). CP-96,345 and CP-96,344 (dihydrochloride salts) were dissolved in 0.1% methyl cellulose-water or saline and given orally 60 min before or subcutaneously 30 min before the carrageenin injection, respectively. Results were compared against vehicle control.

Formalin-induced paw licking in rat Formalin-induced paw licking was induced by intraplantar injection with 50 μl of 5% formalin in saline (Dubuisson & Dennis, 1977). CP-96,345 and CP-96,344 were dissolved in methyl cellulose-saline and given s.c. 30 min before formalin injection. Animals were placed in 2 l clear beakers (1 per beaker) and duration of licking behaviour was determined for 15–25 min (for second phase).

Materials Dihydrochloride salts of CP-96,345 [(2S,3S)-*cis*-2-(diphenylmethyl)-N-[(2-methoxyphenyl)methyl)]-1-azabicyclo [2.2.2]octan-3-amine] and CP-96,344 [(2R,3R)-*cis*-2-(diphenylmethyl)-N-[(2-methoxyphenyl)methyl)]-1-azabicyclo[2.2.2] octan-3-amine] were synthesized in the Central Research Division, Pfizer Inc., as previously described (Lowe *et al.*, 1992). Carrageenin was purchased from Zushikagaku Laboratory Inc., Japan. Substance P was purchased from Peptide Institute, Japan, and [^{3}H]-SP and [^{125}I]-SP were from New England Nuclear. Male Sprague-Dawley rats were purchased from Oriental Yeast Company, Japan.

Results *Binding studies* CP-96,345 potently inhibited SP binding in membrane preparations from brain and spinal cord of rat with IC_{50} values (95% confidence limits) of 45 (8–81) and 160 (86–220) nM, respectively. The binding of CP-96,345 was stereospecific in that the **2R,3R** enantiomer, CP-96,344, was inactive up to 10 μM. The results are consistent with an earlier finding in which bovine caudate tissue was used (Snider *et al.*, 1991).

Carrageenin-induced foot oedema in rat Intraplantar injection of carrageenin caused a rapid increase in the paw volume, reaching a maximum 3 h after injection. Both CP-96,345 and CP-96,344, inhibited foot oedema in a dose-dependent manner, with statistically significant effects observed at 30 and 45 mg kg^{-1}, s.c. (62 and 93 μmol kg^{-1}) (Figure 1a). Essentially equivalent effects were observed when CP-96,345 and CP-96,344 were administered orally.

Carrageenin-induced hyperalgesia in rat Intraplantar injection of carrageenin caused a reduction in the nociceptive

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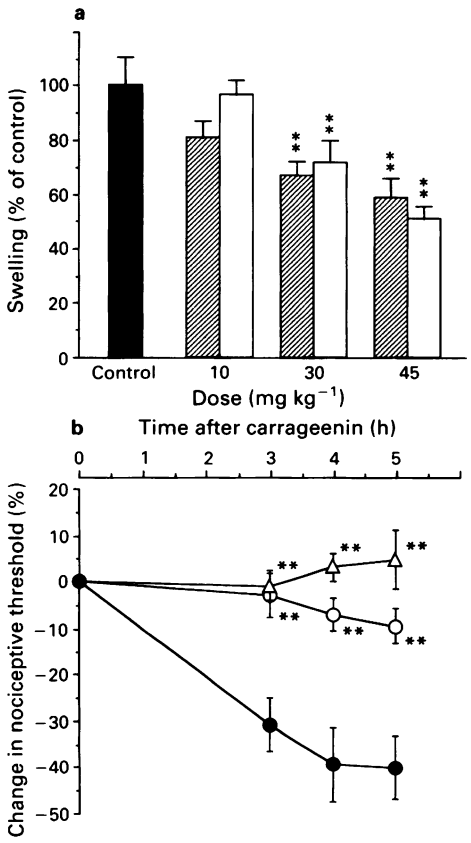

Figure 1 (a) Effects of CP-96,345 (hatched columns) and CP-96,344 (open columns) on carrageenin-induced foot oedema in rat. Solid column indicates vehicle-treated animals. The foot volumes were measured at 3 h after intraplantar injection of 1% carrageenin. Test compounds were given subcutaneously 30 min before carrageenin. Each column represents mean data from 5 animals; vertical lines show s.e.means. (b) Effects of CP-96,345 and CP-96,344 on carrageenin-induced hyperalgesia in rats. Symbols indicate animals treated with 45 mg kg^{-1} s.c. of CP-96,345 (○) and CP-96,344 (△) or vehicle control (●). Each point represents mean data from 8 animals; vertical lines show s.e.means. $^{**}P<0.01$ significantly different from vehicle control by 1-way ANOVA, Dunnett's test.

pressure threshold 3, 4 and 5 h after injection. CP-96,345 and CP-96,344 completely blocked the hyperalgesic response at 45 mg kg^{-1}, s.c. (Figure 1b).

Formalin-induced paw licking in rat Intraplantar injection of formalin caused a characteristic biphasic licking response. The duration of the second phase of the response was 217 ± 14 s (mean ± s.c.). CP-96,345 and CP-96,344 inhibited the second phase of the response in a dose-dependent fashion with equal potency. Statistically significant effects were observed at 30 and 45 mg kg^{-1} s.c. (Figure 2).

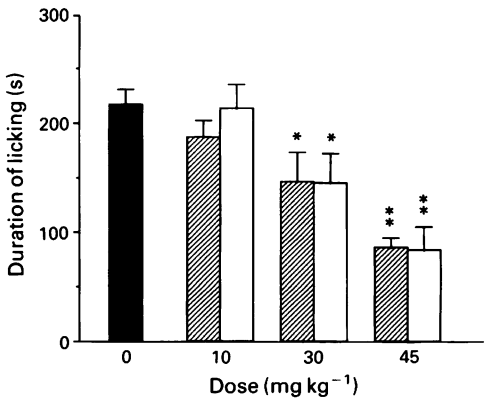

Figure 2 Effects of CP-96,345 (hatched columns) and CP-96,344 (open columns) on the second phase of the formalin-induced paw licking response in rats. Solid column indicates vehicle-treated animals. Each column represents mean data from 5–6 animals; vertical lines show s.e.means. $^{*}P<0.05$ and $^{**}P<0.01$ significantly different from vehicle control by 1-way ANOVA, Dunnett's test.

Discussion The racemic mixture of CP-96,345 has been reported to show antinociceptive and anti-oedema activity in the carrageenin-induced hyperalgesia and foot oedema models in the rat, as well as to inhibit the formalin-induced licking response in the rat (Birch *et al.*, 1992). Based on these results, it was suggested that SP is a mediator of the effects observed in these two well-characterized models of inflammation and pain. In our studies, CP-96,344, the **2R,3R** enantiomer of CP-96,345, which is inactive as an NK_1 antagonist ($IC_{50}>10\ \mu M$) in rat brain and spinal cord, reduced carrageenin-induced paw oedema and hyperalgesia, and attenuated the second phase of the formalin-induced paw licking response, with potency equal to CP-96,345. These results do not suggest a mediator role of SP in the above models.

In contrast, we have previously shown that CP-96,345 stereoselectivity inhibited mustard oil-induced rat foot oedema in rats and acetic acid-induced writhing in mice (Lembeck *et al.*, 1992; Nagahisa *et al.*, 1992). Thus, NK_1 receptor responses appear to play a role in these animal models of pain and inflammation.

Recently, both CP-96,345 and CP-96,344 have been shown to interact with Ca^{2+} channel binding sites (Schmidt *et al.*, 1992). In our hands, verapamil (30 mg kg^{-1} p.o.) inhibited carrageenin-induced rat foot oedema and hyperalgesia as well as the formalin-induced response (data not shown) suggesting that the Ca^{2+} antagonist activity might account for the non-specific pharmacological effects of CP-96,345 and CP-96,344.

We conclude that studies in which the racemic mixture of CP-96,345 are used may lead to erroneous conclusions.

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