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The non-peptide tachykinin antagonist, CP-96,345, is a potent inhibitor of neurogenic inflammation

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1 Release of the tachykinin, substance P, from the peripheral terminals of polymodal afferent C-fibres is thought to be largely responsible for the vasodilatation and plasma protein extravasation described as neurogenic inflammation. The effects of CP-96,345, a non-peptide antagonist at the substance P (NK_1) receptor, on these vascular reactions were investigated in the rat.

2 Intravenously (i.v.) injected CP-96,345 (0.4–3.0 μmol kg^{-1}) prevented the drop in blood pressure, a measure of the peripheral vasodilatation, evoked by substance P and neurokinin A in a dose- and time-dependent manner, but did not affect that elicited by the non-tachykinin peptides calcitonin gene-related peptide and vasoactive intestinal polypeptide.

3 Plasma protein extravasation evoked by i.a. infusion of substance P, antidromic stimulation of the saphenous or the vagus nerve, and stimulation of cutaneous afferent nerves with mustard oil, were each significantly inhibited by CP-96,345 (3.0–9.0 μmol kg^{-1}, i.v.). Furthermore, CP-96,345 was orally active in blocking mustard oil-induced plasma extravasation with an ED_{50} of 10 μmol kg^{-1}.

4 The inhibition of substance P-induced vasodilatation and of neurogenic plasma extravasation by CP-96,345 was stereospecific as the inactive isomer CP-96,344 (**2R**, **3R** enantiomer of CP-96,345) had no effect.

5 Thus CP-96,345 is a specific, highly potent, long-acting and orally active inhibitor of tachykinin-mediated neurogenic inflammation.

Keywords: Neurogenic plasma extravasation; vasodilatation; substance P; neurokinin A; calcitonin gene-related peptide; vasoactive intestinal polypeptide; substance P antagonist; oral activity

Introduction

Polymodal afferent C-fibres contain the vasoactive tachykinins, substance P and neurokinin A, as well as calcitonin gene-related peptide (CGRP), peptides which are released following nerve stimulation at the central and peripheral terminals of these neurones. Release of these peptides from their central terminals induces autonomic reflexes, neuroendocrine regulations and the sensation of pain (for reviews see Lembeck, 1987; 1988). When the peripheral terminals of these fibres are stimulated by chemical, thermal or mechanical stimuli, the release of neuropeptides from these terminals induces vasodilatation and plasma protein extravasation in the skin (Lembeck & Holzer, 1979) and in the gastric mucosa (Holzer *et al.*, 1991), as well as oedema and bronchoconstriction in the airways (Lundberg & Saria, 1987; Barnes *et al.*, 1988). Since it has been proposed that substance P and neurokinin A, among these peptides, play a pathophysiological role in neurogenic inflammation in the skin and airways (Holzer, 1988), there seems to be a definite therapeutic potential for the use of metabolically stable tachykinin antagonists in these acute inflammatory conditions.

Recently the non-peptide compound CP-96,345, or (2S,3S)-*cis*-2-(diphenylmethyl)-N-((2-methoxyphenyl)methyl)-1-azabicyclo [2.2.2]octan-3-amine, has been shown to displace substance P and substance P analogues from binding to cell membranes in the CNS and has been classified as a selective NK_1 receptor antagonist (Snider *et al.*, 1991; McLean *et al.*, 1991; Rouissi *et al.*, 1991). The compound has been shown to inhibit selectively the effects of substance P on smooth muscle preparations and on locus coeruleus neurones *in vitro* and to block the salivary secretion evoked by substance P *in vivo* (Snider *et al.*, 1991; McLean *et al.*, 1991).

The present study was designed to examine whether CP-96,345 would also inhibit the effects of endogenous substance P released from peripheral terminals of afferent nerve fibres *in vivo*, and, since the compound does not have a peptide structure, to assess its oral activity.

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Methods

Female Sprague-Dawley rats (body weight 200–250 g) were used for all the experiments. All animals were anaesthetized with an i.p. injection of sodium pentobarbitone (50 mg kg^{-1}). One jugular vein was cannulated for the i.v. injection of drugs, and in experiments in which arterial blood pressure and heart rate were recorded, one carotid artery was cannulated.

To evoke peptide-induced hypotension, a dose of 30 pmol kg^{-1} substance P corresponding to its ED_{50} (Maggi *et al.*, 1987) was injected i.v. at 5 min intervals to give a reproducible brief fall in mean arterial blood pressure of about 20 mmHg. A similar reproducible fall in blood pressure was also induced by neurokinin A (150 pmol kg^{-1}), CGRP (45 pmol kg^{-1}) and vasoactive intestinal polypeptide (VIP, 90 pmol kg^{-1}) injected at 5 min intervals. Each peptide dose was injected in 0.1 ml of saline during a time period of 20 s.

The tachykinin antagonist CP-96,345 or its inactive isomer CP-96,345 (**2R**, **3R** enantiomer of CP-96,345) was slowly injected i.v. at the appropriate concentration in 1 ml of saline over a time period of 1 min, 5 min before the third injection of the peptide agonists.

To evoke plasma protein extravasation into the skin of the hind paw, the superficial epigastric artery was cannulated in one set of experiments for retrograde i.a. infusion of substance P (3.7 pmol min^{-1} during 5 min) into the femoral artery. In another set of experiments the saphenous nerve was cut in the thigh, and its distal part placed on bipolar platinum electrodes and stimulated for 5 min (10 V, 1 ms, 5 Hz). In a third set of experiments the dorsal skin of the hind paw was painted with 5% mustard oil in paraffin oil (w/w) twice during a time period of 10 min (for details of these methods see Lembeck & Holzer, 1979). To induce plasma protein extravasation in the trachea, the right cervical vagus nerve was exposed and cut

and its distal end was electrically stimulated for 5 min (10 V, 0.2 ms, 10 Hz; Lundberg & Saria, 1982).

Evans Blue (50 mg kg^{-1}), used as a marker for extravasated proteins, was injected i.v. 7 min before the stimulation period whereas CP-96,345, CP-96,344 or saline were injected i.v., as described above, 5 min before the stimulation period. After the end of the stimulation experiments in the paw, the rats were killed by bleeding and the dorsal paw skin was removed. Immediately after stimulation of the vagus nerve, the thorax was opened and the animals were perfused with 50 ml saline during 30 s via the aorta. A portion of the trachea (1 cm) adjacent to the stem bronchi was excised. All the tissues were weighed, Evans Blue was extracted with formamide and quantitated photometrically (Lembeck & Holzer, 1979).

To determine oral activity of CP-96,345 or CP-96,344 against mustard oil-induced plasma protein extravasation, the compounds were dissolved in 0.1% methyl cellulose/water and dosed orally 1 h before the mustard oil challenge. In these experiments a slightly different protocol was employed. The whole foot below the ankle was painted with 5% mustard oil in paraffin oil and 20 min later the foot was amputated, frozen in liquid nitrogen, pulverized and the Evans Blue dye extracted. For this set of experiments the basal dye content in the foot painted only with the vehicle paraffin oil is given for comparison. Basal dye content accounts for the blood volume left in larger vessels and an increase above basal levels represents mustard oil-induced dye extravasation and increased intravascular volume.

Substances

Compound CP-96,345, or (2S,3S)-*cis*-2-(diphenylmethyl)-N-((2-methoxyphenyl)methyl)-1-azabicyclo[2.2.2]octan-3-amine, and CP-96,344 (2**R**,3**R** enantiomer of CP-96,345) were generously provided by Dr Michael Snider from Pfizer Central Research Division (U.S.A.); substance P, neurokinin A, CGRP and VIP were obtained from Peninsula (U.S.A.), and Evans Blue was obtained from Fluka (Switzerland).

Results

To assess the potency and duration of action of CP-96,345 we chose to calibrate its effect against the hypotensive action of substance P. The i.v. injection of CP-96,345 (0.4–3.0 μmol kg^{-1}) inhibited the substance P-induced fall in blood pressure in a dose-dependent fashion (Figure 1). The largest dose of CP-96,345 used in this assay (3.0 μmol kg^{-1}) caused an almost complete inhibition of the hypotensive effect of substance P for up to 30 min. The inactive isomer CP-96,344 (2**R**, 3**R** enantiomer of CP-96,345) had no effect when tested also at a dose of 3.0 μmol kg^{-1} ($n = 3$, results not shown). Whereas CP-96,345, injected i.v. in a dose of 3.0 μmol kg^{-1}, caused also almost complete inhibition of the hypotensive action of another tachykinin, neurokinin A, for up to 30 min, it did not affect the hypotensive actions of the unrelated peptides CGRP and VIP in the time period 5 min to 30 min after its i.v. injection. As an example, the hypotensive responses of the four vasoactive peptide agonists investigated 10 min after 3 μmol kg^{-1} CP-96,345 are given for comparison in Figure 2. The i.v. injection of 3.0 μmol kg^{-1} of CP-96,345 alone had a slight depressant effect on blood pressure (-10 ± 1 mmHg below the pre-injection level which was 136 ± 3 mmHg, $n = 20$) and on heart rate, which fell from 400 ± 10 b.p.m. to 360 ± 10 b.p.m. ($n = 20$), effects not related to any substance P-antagonistic action because they were shared by the inactive isomer CP-96,344 ($n = 5$).

The effect of CP-96,345 on the plasma protein extravasation evoked by three different means of sensory nerve stimulation (mustard oil, antidromic saphenous or efferent vagus nerve stimulation) was compared with that observed on substance P-induced plasma extravasation (close i.a. infusion into the femoral artery). A 3.0 μmol kg^{-1} dose of CP-96,345 was sufficient to inhibit significantly the effect of mustard oil whereas a 9.0 μmol kg^{-1} dose of the substance P antagonist was required for a complete blockade (Figure 3). Plasma protein extravasation induced by substance P or by saphenous or vagus nerve stimulation was also markedly inhibited by 3.0 μmol kg^{-1} of CP-96,345 (Figure 3). The inactive isomer CP-96,344 was tested for its action against plasma protein extravasation following an antidromic saphenous nerve stimulation and was found to have no antagonistic action ($n = 3$, results not shown).

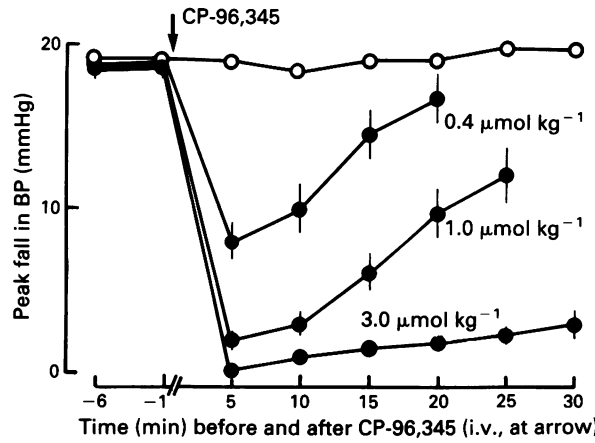

Figure 1 Inhibition of the fall in blood pressure (BP) induced by substance P (30 pmol kg^{-1}, i.v.) in pentobarbitone-anaesthetized rats. Substance P caused a short fall in BP which was of constant magnitude when the peptide was injected at 5 min intervals (○). Following the i.v. injection of CP-96,345 (0.4–3.0 μmol kg^{-1}, ●), but not that of saline used as a control (○), the fall in BP was reduced; both the magnitude and duration of the effect of CP-96,345 were dose-dependent. Whereas a dose of 0.4 μmol kg^{-1} of CP-96,345 blocked only the next two applications of substance P, 3.0 μmol kg^{-1} of CP-96,345 abolished almost completely the substance P responses for the next 30 min ($P < 0.05$, Quade test). Means of 4–5 experiments with s.e.mean shown by vertical lines.

The efficiency of the oral administration of CP-96,345 was examined in the mustard oil-induced plasma extravasation test in the rat foot. CP-96,345 inhibited this reaction in a dose-dependent manner with an oral ED_{50} of 10 μmol kg^{-1} (Figure 4). The inhibition was stereospecific and the inactive isomer

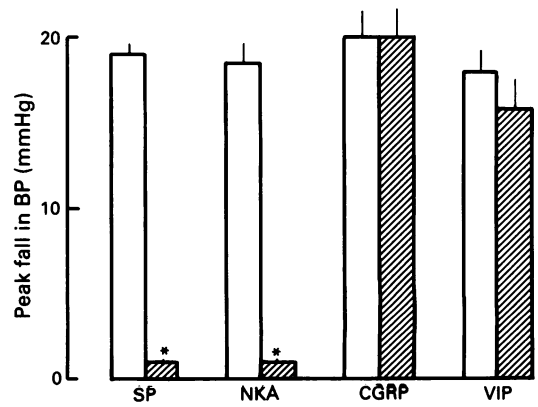

Figure 2 Fall in blood pressure (BP) in pentobarbitone-anaesthetized rats following the i.v. injections of substance P (SP, 30 pmol kg^{-1}), neurokinin A (NKA, 150 pmol kg^{-1}), calcitonin gene-related peptide (CGRP, 45 pmol kg^{-1}) and vasoactive intestinal polypeptide (VIP, 90 pmol kg^{-1}). The fall in blood pressure observed with all four peptides was of similar size before the injection of CP-96,345 (open columns). Ten min after CP-96,345 injection (3.0 μmol kg^{-1}, i.v., hatched columns) the effects of substance P and neurokinin A were almost abolished (* $P < 0.05$, t test), those of CGRP and VIP remained unchanged. Means of 4–5 experiments; vertical lines show s.e.mean.

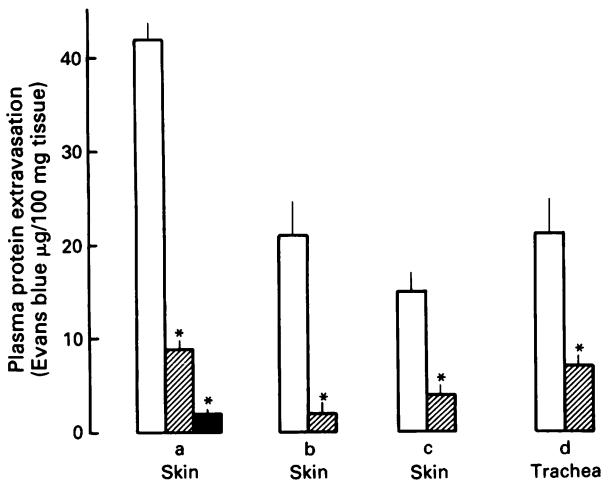

Figure 3 Plasma protein extravasation (measured by Evans blue content of tissues) in pentobarbitone-anaesthetized rats. Controls = open columns. CP-96,345 (3.0 μmol kg^{-1} = hatched columns, 9.0 μmol kg^{-1} = solid column) was injected i.v. 5 min before the following treatments: (a) Plasma protein extravasation in the skin of the hind paw following painting the paw with 5% mustard oil in paraffin oil. (b) Plasma protein extravasation in the skin of the hind paw following antidromic stimulation of the saphenous nerve (10 V, 1 ms, 5 Hz for 5 min). (c) Plasma protein extravasation in the skin of the hind paw following close i.a. infusion of substance P (3.7 pmol min^{-1}) into the femoral artery. (d) Plasma protein extravasation in the trachea following stimulation (10 V, 0.2 ms, 10 Hz for 5 min) of the centrally divided right vagus nerve. Means of $n = 4$–6 for each group; vertical lines show s.e.mean. * $P < 0.05$ compared to the appropriate control by t test or ANOVA.

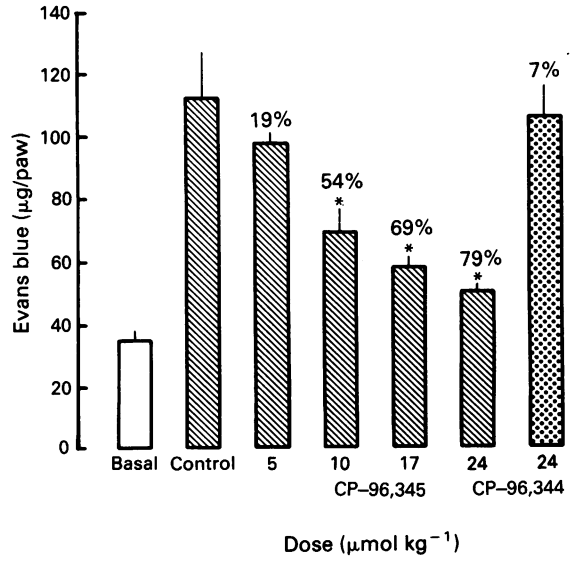

Figure 4 Dose-dependent inhibition of mustard oil-induced Evans blue accumulation in the paw of anaesthetized rats. CP-96,345 (hatched columns) and the inactive enantiomer CP-96,344 (stippled column) were dissolved in 0.1% methyl cellulose-water and given orally 1 h before the mustard oil challenge as in Figure 3. Each column represents the mean of 5–6 rats (s.e.mean shown by vertical lines) and the number above indicates % inhibition after subtracting the basal (open column, without mustard oil, see methods). * indicates $P < 0.01$ compared with control by 1 way ANOVA.

CP-96,344 had no effect up to 24 μmol kg^{-1}. Basal dye content of the foot (see methods) was not changed by the highest dose of CP-96,345 (24 μmol kg^{-1}) employed ($n = 5$, results not shown).

Discussion

CP-96,345 exhibited stereospecific antagonistic effects against tachykinin-induced hypotension as well as against substance P-induced, or several types of neurogenic plasma protein extravasation. Earlier investigations have demonstrated that the hypotensive responses to the i.v. injection of substance P (Burcher *et al.*, 1977), neurokinin A (Hua *et al.*, 1984; Maggi *et al.*, 1987), CGRP (Lappe *et al.*, 1987) and VIP (Said & Mutt, 1970) can be regarded as a correlate of peripheral vasodilatation. The tachykinin-induced hypotension (vasodilatation) has been characterized as a tachykinin effect mediated by the NK_1 receptor (Maggi *et al.*, 1987). Although the compound CP-96,345 has been classified as selective NK_1 receptor (substance P receptor) antagonist, this does not exclude its antagonistic action against neurokinin A which can also interact with the NK_1 receptor. However, the antagonistic effects of CP-96,345 were observed only against tachykinins and not against unrelated peptides such as CGRP or VIP (as demonstrated here with their hypotensive action) nor against bradykinin-, leukotriene D_4-, histamine-, or PAF-induced plasma protein extravasation (Nagahisa, personal communication).

The specific substance P-antagonistic effects of CP-96,345 were clearly separable from its unspecific, slightly depressant, effects on blood pressure and heart rate, because the latter were also observed after administration of the inactive isomer, CP-96,344.

The almost complete inhibition of the various types of neurogenic plasma protein extravasation in skin and trachea in the rat would indicate that such phenomenon predominantly resulted from the release of substance P and neurokinin A. However, vasodilatation induced by the coreleased CGRP, itself unaffected by CP-96,345, could certainly have remained. The present findings also do not eliminate a synergy of oedema formation in the skin between CGRP and tachykinins (Brain & Williams, 1985; Gamse & Saria, 1985), since inhibition of either component may significantly modulate oedema formation.

The main advantages of CP-96,345 are its oral activity and long duration of action. The peptide-antagonists of the tachykinins developed so far were metabolically unstable and have thus, apart from a few exceptions (Lundberg *et al.*, 1983; Maggi *et al.*, 1991), only been applied locally in the tissue (Lembeck *et al.*, 1982; Xu *et al.*, 1991). Although the effects of CP-96,345 have still to be explored on the different functions of substance P-containing neurones in the intestinal intrinsic nerve plexus and in the CNS under *in vivo* conditions, the potent effects on afferent neurones characterizes the compound as a useful and practical tool for the study of tachykinin-mediated neurogenic inflammation.

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