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(±)-CP-96,345, a selective tachykinin NK_1 receptor antagonist, has non-specific actions on neurotransmission

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1 The non-specific effects of the non-peptide tachykinin receptor antagonist (±)-CP-96,345, were assessed in several smooth muscle-nerve preparations. The preparations were the iris sphincter muscle of the rabbit and the taenia coli, vas deferens and seminal vesicle of the guinea-pig.

2 (±)-CP-96,345 concentration-dependently inhibited the elctrically evoked, tachykinin-mediated contractile responses of the iris sphincter and the taenia coli. The pIC_{50} values were 5.4 ± 0.2 (mean ± s.e.mean) and 5.7 ± 0.08 respectively.

3 (±)-CP-96,345 also inhibited non-tachykinin-mediated contractile responses to electrical stimulation of the iris sphincter, taenia coli, vas deferens and seminal vesicle. The pIC_{50} values were 4.3 ± 0.02, 4.8 ± 0.03, 4.7 ± 0.02 and 4.4 ± 0.05 respectively. These values differ significantly from the pIC_{50} values of the inhibition of the tachykinin-mediated response in the iris sphincter and taenia coli.

4 (±)-CP-96,345 was without effect on carbachol- and noradrenaline-evoked contractions of the iris sphincter but inhibited carbachol- and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-evoked contractions of the taenia coli.

5 We suggest that (±)-CP-96,345, apart from its NK_1 receptor blocking activity, induces non-specific suppression of neurotransmission, exerted at both pre- and post-junctional sites.

Keywords: NK_1 receptor antagonist; non-specific action on neurotransmission; rabbit iris; guinea-pig taenia coli; guinea-pig vas deferens; guinea-pig seminal vesicle

Introduction

The mammalian tachykinins, substance P, neurokinin A and neurokinin B, exert numerous biological activities in the central nervous system and in peripheral organs. The available evidence to date indicates that three tachykinin receptors, named NK_1, NK_2 and NK_3 receptors, mediate the biological effects of these peptides in mammalian tissues (for reviews see Maggio, 1988; Guard & Watson, 1991).

The characterization of different tachykinin receptor subtypes has been based mainly on work with selective tachykinin receptor agonists (Regoli *et al.*, 1988; Brunelleschi *et al.*, 1990; Hagan *et al.*, 1991; Hall *et al.*, 1991). Selective and potent tachykinin receptor antagonists are much needed to corroborate current concepts concerning tachykinin receptors. Very recently, a non-peptide antagonist of NK_1-receptors, CP-96,345 has attracted considerable interest (Snider *et al.*, 1991). Using various isolated organs and smooth muscle preparations, (±)-CP-96,345 was found to be a competitive NK_1-receptor antagonist while being devoid of activity at NK_2 or NK_3 receptors (Snider *et al.*, 1991; Rouissi *et al.*, 1991; Håkanson *et al.*, 1991; Lecci *et al.*, 1991; Gitter *et al.*, 1991). Also, results of *in vivo* experiments have confirmed that (±)-CP-96,345 acts as a selective NK_1 receptor antagonist (Snider *et al.*, 1991; Lecci *et al.*, 1991; Radhakrishnan & Henry, 1991).

However, some effects of (±)-CP-96,345 which are not related to the antagonism to tachykinins have been observed (Boyle *et al.*, 1991; Lecci *et al.*, 1991). In the present study, a series of *in vitro* experiments was performed to investigate whether (±)-CP-96,345 exerted non-specific effects on neurotransmission.

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Methods

General

Adult pigmented rabbits of either sex (1.5–3.0 kg) and male guinea-pigs (200–250 g) were used. The animals were killed by a blow on the neck and exsanguinated. The iris sphincter muscle of the rabbit and the vas deferens and seminal vesicle of the guinea-pig were prepared and mounted vertically on a Perspex holder in a 8 ml organ bath as described elsewhere (Stjernquist *et al.*, 1983; Wahlestedt *et al.*, 1985). One end of the preparation was attached to a rigid support and the other to a Grass FT03 force displacement transducer. The preparation was stretched with a force of 1.5 mN (iris sphincter) or 5 mN (vas deferens and seminal vesicle). The modified Krebs solution (Wahlestedt *et al.*, 1985) was bubbled with a gas mixture of 7% CO_2 in O_2 giving a pH of 7.2–7.3 at 37°C.

The guinea-pig taenia coli preparation, consisting of longitudinal smooth muscle with the attached myenteric plexus (Leander *et al.*, 1981), was placed in modified Krebs solution and kept at 4°C for about 60 min. The preparation was then mounted vertically on a Perspex holder in a 8 ml organ bath maintained at 33°C. One end of the preparation was attached to a rigid support and the other to a lever connected via a spring to a Grass FT03 force-displacement transducer. The load on the muscle was set at 0.2 g.

Two preparations from the same animal were mounted in separate baths, one being exposed to the antagonist and the other being exposed to the vehicle (as control preparation).

Studies of electrically evoked smooth muscle contractions

The mechanical activity of each preparation was recorded continuously on a Grass model 7 polygraph. Before the start of each experiment the preparation was allowed to equilibrate for 60–90 min. Electrical stimulation with square wave

pulses (25 V, voltage drop 14–17 V over the electrodes, 0.3–1 ms duration) was applied by means of platinum electrodes connected to a Grass S4C stimulator. The preparations were stimulated either with single pulses or with trains of pulses lasting 3–20 s, the pulse frequency varying from 1–20 Hz. All electrically evoked responses were abolished by 10^{-6} M tetrodotoxin (TTX). (±)-CP-96,345 was given in a cumulative manner. The preparations were exposed to each concentration of the antagonist for 10 min before the electrical stimulation.

Studies of drug-evoked smooth muscle contractions

We investigated the effects of (±)-CP-96,345 on the contractions produced by carbachol or noradrenaline in the iris sphincter muscle and by carbachol or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the taenia coli. Repeated applications (30 min intervals) of each of carbachol, noradrenaline and $PGF_{2\alpha}$ caused reproducible contractions in the preparation tested. (±)-CP-96,345 was applied 10 min before the application of the agonist.

Drugs

(±)-CP-96,345 was a gift from AB Astra Pain Control, Södertälje, Sweden. It is a racemic mixture containing both [(2**R**, 3**R**)-*cis*- and (2**S**,3**S**)-*cis*-2-(diphenylmethyl)-*N*-(2-methoxyphenyl)-methyl]-l-azabicyclo[2.2.2]octan-3-amine. Only the (2**S**,3**S**)-*cis*- form is thought to possess antagonistic potency (Snider *et al.*, 1991). Atropine was from Alcon, TX, U.S.A. and guanethidine from CIBA-Geigy, Basel, Switzerland. TTX, which is a blocker of nerve conduction (Kao, 1966), was from Sankyo, Japan. Carbachol and noradrenaline were purchased from Sigma, MO, U.S.A. (±)-CP-96,345 was dissolved in 0.1 N acetic acid (10^{-2} M solution).

Analysis of results

Concentration-response curves were constructed and the pIC_{50} values (the negative logarithm of the molar concentration of the antagonist producing 50% inhibition of the electrically evoked contraction) were estimated by linear regression analysis of the results in the 10–90% response interval. Statistical analysis was made by Student's *t* test. A probability of $P < 0.05$ was considered statistically significant.

Results

Studies of tachykinin antagonism

In the presence of 10^{-6} M atropine and 5×10^{-6} M guanethidine, the contractile response of the rabbit iris sphincter to electrical stimulation (20 Hz, 25 V, 10 s) is mediated by tachykinins (Wahlestedt *et al.*, 1985). (±)-CP-96,345 inhibited the tachykinin-mediated contraction concentration-dependently (Figure 1a). The pIC_{50} value is given in Table 1.

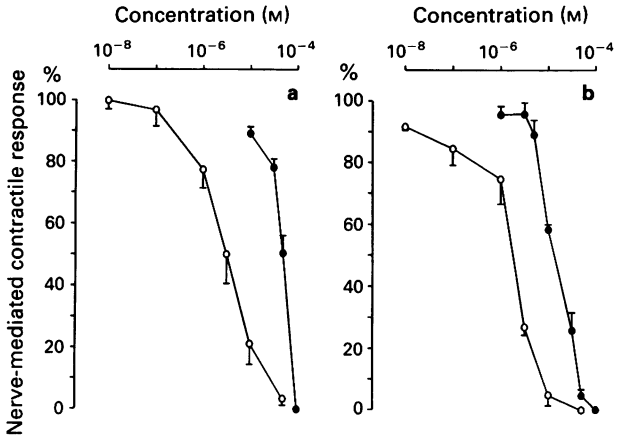

Figure 1 (±)-CP-96,345 concentration-dependently inhibited both tachykinin-mediated (○) and cholinergic (●) contractile responses of the rabbit iris sphincter (a) and guinea-pig taenia coli (b) to electrical stimulation. The contractile responses to electrical stimulation before the application of (±)-CP-96,345 were considered as 100%. Means of 5–6 experiments. Vertical bars give s.e.mean.

Also, electrical stimulation (3 Hz, 25 V, 3 s) of the atropinized guinea-pig taenia coli induces a contractile response that is mediated by tachykinins (Leander *et al.*, 1981). (±)-CP-96,345 inhibited the tachykinin-mediated contraction concentration-dependently (Figure 1b). The pIC_{50} value is given in Table 1.

Studies of non-specific actions of neurotransmission

The iris sphincter muscle responds to single pulse stimulation (1 pulse/60 s, 25 V) with a twitch-like contraction. This contraction can be blocked by 10^{-6} M atropine (Leander & Håkanson, 1985). (±)-CP-96,345 inhibited the cholinergic contractile response concentration-dependently (Figures 1a, 2a). At 10^{-4} M, (±)-CP-96,345 abolished the contraction. The pIC_{50} is given in Table 1; it was about 1 log unit lower than the pIC_{50} of the inhibition of the tachykinin-mediated response ($P < 0.01$). After extensive washing, the contractile response to electrical stimulation showed complete recovery (not shown).

The taenia coli responds to electrical field stimulation (1 Hz, 25 V, 3 s) with a contraction that reflects the release of acetylcholine from cholinergic nerve fibres (Leander *et al.*, 1981). This contraction was abolished by 10^{-6} M atropine (not shown). (±)-CP-96,345 inhibited the cholinergic contractile response concentration-dependently (Figure 1b, 2b). At 10^{-4} M, (±)-CP-96,345 abolished the contraction. The pIC_{50} is given in Table 1; it was about 1 log unit lower than the pIC_{50} of the inhibition of the tachykinin-mediated response ($P < 0.01$). After extensive washing, the contractile response to electrical stimulation showed complete recovery (not shown).

Table 1 Effect of (±)-CP-96,345 on contractile responses of various smooth muscle preparations to electrical stimulation

	pIC_{50}	
	Tachykinin response	*Non-tachykinin response*
Rabbit iris sphincter muscle	5.4 ± 0.20 (5)**	4.3 ± 0.02 (6)
Guinea-pig taenia coli	5.7 ± 0.08 (6)**	4.8 ± 0.03 (6)
Guinea-pig vas deferens	–	4.7 ± 0.02 (4)
Guinea-pig seminal vesicle	–	4.4 ± 0.05 (4)

Means ± s.e.mean. Numbers in parentheses indicate the number of experiments (animals).
**Indicates $P < 0.01$ (difference between pIC_{50} for tachykinin and non-tachykinin-mediated responses).

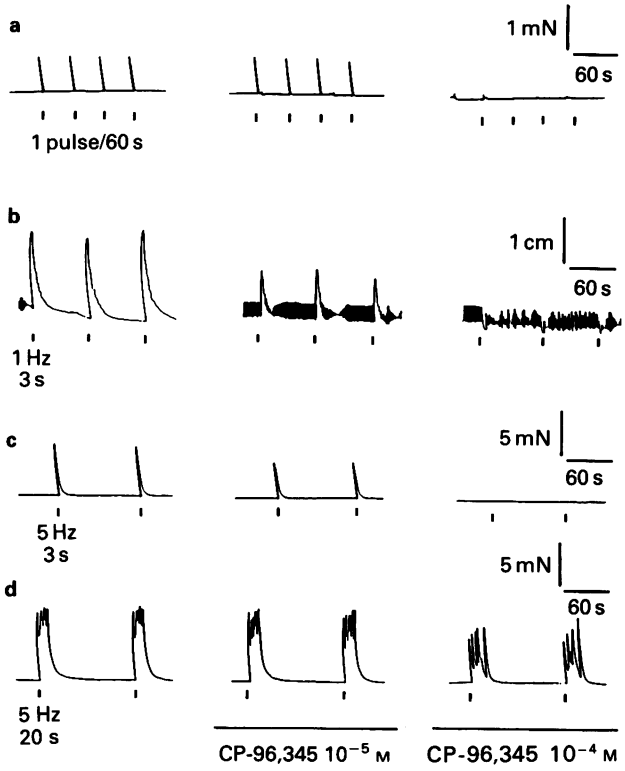

Figure 2 Original tracings showing the inhibitory effect of (±)-CP-96,345 on the contractile non-tachykininergic responses to electrical stimulation of different smooth muscle preparations: (a) rabbit iris sphincter; (b) guinea-pig taenia coli; (c) guinea-pig vas deferens; (d) guinea-pig seminal vesicle. The application of (±)-CP-96,345 increased the spontaneous activity of the guinea-pig taenia coli; the vehicle used to dissolve (±)-CP-96,345 had same effect (not shown). In the other preparations, the vehicle was without effect on the contractile responses (not shown).

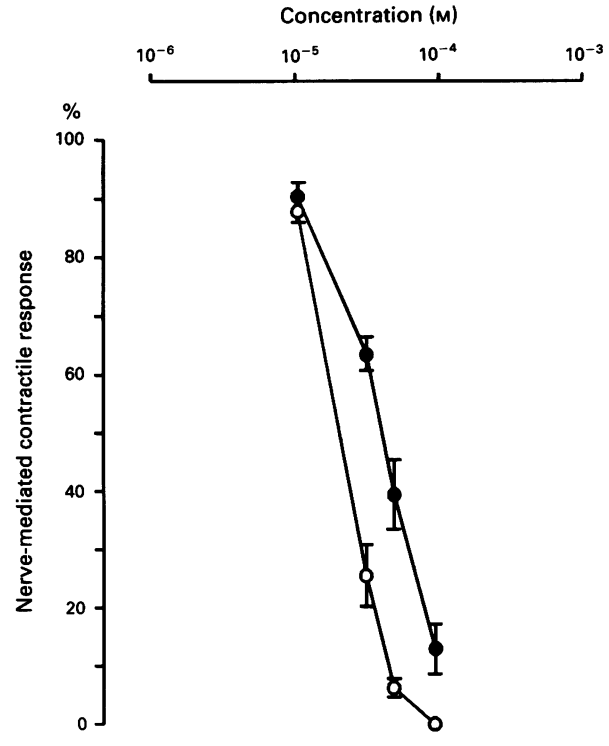

Figure 3 (±)-CP-96,345 concentration-dependently inhibited the non-tachykininergic contractile responses of the guinea-pig vas deferens (○) and seminal vesicle (●) to electrical stimulation. The contractile responses to electrical stimulation before the application of (±)-CP-96,345 were considered as 100%. Means of 4 experiments. Vertical bars give s.e.mean.

The vas deferens responds to low frequency stimulation (5 Hz, 25 V, 3 s) with a twitch-like contraction (Stjernquist *et al.*, 1983). This contraction was unaffected by 10^{-6} M atropine but abolished by 5×10^{-6} M guanethidine (not shown). (±)-CP-96,345 inhibited the contraction concentration-dependently (Figure 2c, 3); the contraction was abolished at 10^{-4} M. The pIC_{50} is given in Table 1. After extensive washing, the contractile response to electrical stimulation showed complete recovery (not shown).

The seminal vesicle preparation responds to electrical stimulation (5 Hz, 25 V, 20 s) with a contractile response which is unaffected by atropine and guanethidine. The neurotransmitter involved has not been identified (Stjernquist *et al.*, 1983). (±)-CP-96,345 concentration-dependently inhibited the 'tonic' component of the contractile response (Figures 2d, 3). The twitch contractions that were superimposed on the 'tonic' contraction were not suppressed

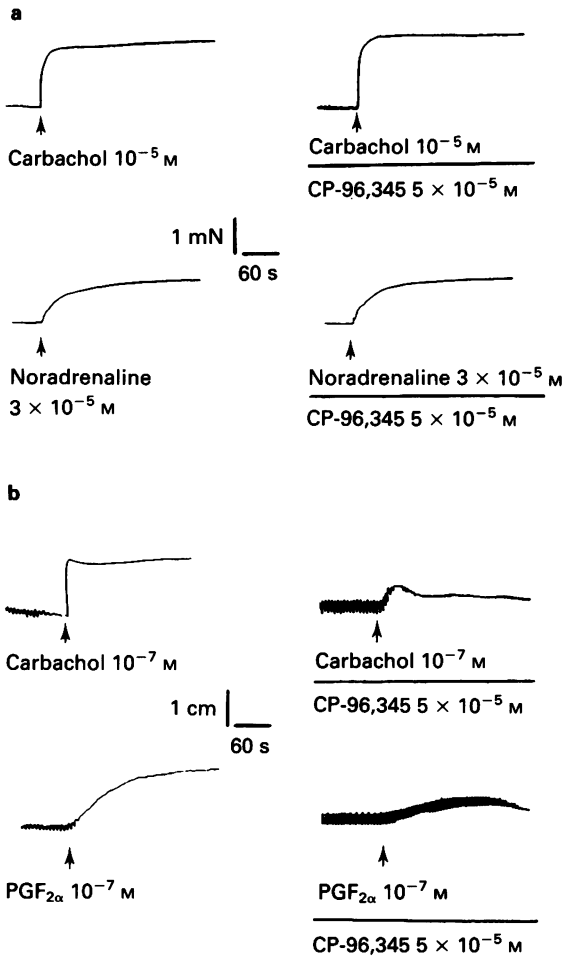

Figure 4 The tracings illustrate the effects of (±)-CP-96,345 on the contractions evoked by carbachol (10^{-5} M) and noradrenaline (3×10^{-5} M) in the rabbit iris sphincter (a) and on the contractions evoked by carbachol and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (10^{-7} M of each) in the guinea-pig taenia coli (b). The concentrations of carbachol, noradrenaline and $PGF_{2\alpha}$ are submaximal.

(Figure 2d). The pIC_{50} is given in Table 1. After extensive washing, the contractile response showed partial recovery (not shown).

Studies of the effect of (±)-CP-96,345 on drug-evoked contractions

Both carbachol (10^{-5} M) and noradrenaline (3×10^{-5} M) contracted the rabbit iris sphincter muslce. (±)-CP-96,345 5×10^{-5} M, was without effect on the contraction produced by either carbachol or noradrenaline (Figure 4a).

Both carbachol and $PGF_{2\alpha}$ (10^{-7} M each) contracted the guinea-pig taenia coli; 5×10^{-5} M (±)-CP-96,345 inhibited both contractile responses greatly (Figure 4b).

Discussion

Snider *et al.* (1991) claimed that (±)-CP-96,345 is the most potent antagonist of the NK_1 receptor identified to date. In addition, the non-peptide nature of CP-96,345 makes it an attractive tool with which to study the pharmacotherapeutic potential of selective NK_1 receptor antagonists. In this context, it would be of interest to identify additional, non-specific actions of the drug. Some properties of (±)-CP-96,345 other than the antagonism of tachykinins have been described previously, e.g. its ability to produce unspecific depression of contractility in the guinea-pig ileum and an unspecific smooth muscle relaxing activity in the rabbit pulmonary artery, hamster trachea and rat portal vein (Boyle *et al.*, 1991; Lecci *et al.*, 1991).

In the present study, (±)-CP-96,345 was found to inhibit tachykinin-evoked contractile responses of the rabbit iris sphincter muscle and guinea-pig taenia coli. It was also found to inhibit non-tachykinin-mediated contractile responses to electrical stimulation of the iris sphincter muscle of the rabbit and of the taenia coli, vas deferens and seminal vesicle of the guinea-pig. In all instances, the non-specific action of (±)-CP-96,345 was concentration-dependent and reversible. However, the concentration of (±)-CP-96,345 needed to inhibit non-tachykinin responses was ten times higher than that needed to inhibit tachykinin-mediated responses. (±)-CP-96,345 did not affect the contractions produced by carbachol and noradrenaline in the iris sphincter but inhibited the contractions produced by carbachol and $PGF_{2\alpha}$ in the taenia coli. Hence, the possibility cannot be excluded that it acts at both pre- and post-junctional sites. Our results suggest that racemic (±)-CP-96,345 exerts non-specific effects on neurotransmission, perhaps related to a local anaesthetic action. It remains to be shown if these non-specific effects are associated with the active or with the inactive enantiomer or both.

Note added in proof

Recently, the active enantiomer of (±)-CP-96,345 was placed at our disposal through the courtesy of Dr R.M. Snider, Pfizer Inc. The ability of this agent to inhibit tachykinin-mediated and non-tachykinin-mediated responses was very similar to that of the racemate. An example: the pIC_{50} value for the tachykinin-mediated response of the rabbit iris sphincter muscle was 5.6 ± 0.01 and for the non-tachykinin-mediated response 4.3 ± 0.02.

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