Differences in the effects of NK₁-receptor antagonists, (±)-CP 96,345 and CP 99,994, on agonist-induced responses in guinea-pig trachea

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1 The effects of the NK₁-receptor antagonists, (±)-CP 96,345 and CP 99,994, on NK₁-agonist evoked contractions were compared in isolated rings of guinea-pig tracheal smooth muscle.

2 (±)-CP 96,345 and CP 99,994 were similarly effective in antagonizing responses evoked by septide, whereas CP 99,994 was more effective than (±)-CP 96,345 in inhibiting responses evoked by [Sar⁹Met¹¹(O₂)] substance P.

3 These results suggest that responses to septide and [Sar⁹Met¹¹(O₂)] substance P may be operated via different populations of NK₁-receptors.

Keywords: NK₁-receptor subtypes; guinea-pig trachea; (±)-CP 96,345; CP 99,994

Introduction

The development of non-peptide selective NK₁-receptor antagonists has shown that species differences in NK₁-receptors exist (Snider *et al.*, 1990; Beresford *et al.*, 1991; Garret *et al.*, 1991). It has recently been suggested that subtypes of NK₁-receptors may exist in different tissues from the same species, based on the differential effects of NK₁-receptor antagonists (Carruette *et al.*, 1992; Beresford *et al.*, 1992). Thus CP 96345 was less effective in inhibiting NK₁-receptor evoked responses in guinea-pig trachea than in guinea-pig ileum (Carruette *et al.*, 1992; Beresford *et al.*, 1992). However, identification of receptor subtypes by comparison of antagonist pA₂ values between tissues can be misleading, since values for the same antagonist may vary considerably between tissues (Kenakin, 1990).

The use of different NK₁-receptor agonists may aid the identification of NK₁-receptor subtypes. It has been suggested that a new neurokinin receptor subtype (possibly belonging to the NK₁-receptor family) may exist in guinea-pig ileum, since responses evoked by septide and [APA⁹⁻¹⁰] substance P (SP) are more susceptible to inhibition by the peptide NK₁-receptor antagonists GR 71251 and [D-Pro⁹, Pro¹⁰, Trp¹¹]SP than are responses evoked by [Pro⁹]SP (Petitet *et al.*, 1992; Chassaing *et al.*, 1992).

In the present experiments we compared the effects of two NK₁-receptor antagonists ((±)-CP 96,345 and CP 99,994) on contractile responses evoked by two supposedly selective agonists (septide and [Sar⁹Met¹¹(O₂)]SP) in guinea-pig tracheal smooth muscle. RP 67580, an NK₁-receptor antagonist which is known to have a different pharmacological profile (i.e. higher affinity for NK₁-receptors in rat tissues than for NK₁-receptors in guinea-pig tissues), was used as a comparison (Garrett *et al.*, 1991).

Methods

Tracheal rings (approximately 5 mm) were obtained from male Dunkin Hartley guinea-pigs (Interfauna, 300–400 g). The tissues were mounted for tension recording in siliconized organ baths containing Krebs physiological salt solution (PSS (mM): NaCl 118, KCl 4.8, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2 and glucose 11.1) maintained at 37°C, pH 7.4, gassed with 95% O₂, 5% CO₂. The PSS also

contained atropine sulphate (1 µM), indomethacin (1 µM), mepyramine maleate (1 µM) and methysergide (1 µM) to eliminate any indirect/non NK₁-receptor-mediated responses. The tissues were placed under a 1 g resting load. Following a 60 min equilibration period, SR 48968 (30 nM) an NK₂-receptor antagonist was added to the tissue bath to eliminate any effects of the agonists at NK₂-receptors; 15 min later, enzyme inhibitors (bestatin 1 µM, phosphoramidon 1 µM, bacitracin 0.04 g l⁻¹) were added: higher concentrations of these inhibitors attenuated responses to the agonists, an effect which may result from an interaction with NK₁-receptors (Fong, unpublished observation). A further 15 min later a cumulative concentration-effect curve to septide or to [Sar⁹Met¹¹(O₂)]SP was obtained. The tissues were then washed. Following equilibration (60 min) with either (±)-CP 96,345, CP 99,994 or RP 67580, the concentration-effect curve to the appropriate agonist was repeated again in the presence of SR 48968 and the enzyme inhibitors (see above). Each tissue was exposed to either [Sar⁹Met¹¹(O₂)]SP or septide and to a single concentration of the antagonist under test. Appropriate vehicle- and time-matched control tissues were used.

Analysis of data

Responses were expressed as a percentage of the maxima of the initial concentration-effect curve. For each individual tissue, dose-ratios (DR) were calculated and were used to construct full Schild plots according to the method of Arunlakshana & Schild (1959) or to estimate the pK_B value (i.e. log dissociation constant) using the following equation where:

$$\text{estimated } pK_B = \frac{\log([\text{concentration of antagonist}])}{(\text{DR} - 1)}$$

In order to allow direct comparisons of antagonist affinity, the slope of each Schild plot was constrained to unity and the pK_B values were then calculated (Jenkinson, 1991). Concentration-effect curves were fitted to the data by least squares analysis of variance to the equation $Y = Y_{\max}/(1 + (EC_{50}/\text{agonist concentration})^{nH})$, where EC₅₀ is the half maximally effective concentration and nH is the Hill coefficient, using an iterative procedure on a VAX computer with Research System 1 Software (BBN Software Products Corporation).

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Drugs and solutions

The following substances were used: septide ([pGlu⁶,Pro⁹]SP (6–11)), bestatin, phosphoramidon, bacitracin, atropine sulphate, indomethacin, mepyramine maleate (all from Sigma); [Sar⁹,Met¹¹(O₂)]SP (CRB); methysergide maleate (RBI). SR 48968 ((S)-N-methyl-N[4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide), (±)-CP 96,345 (racemic(2S,3S/2R,3R) (cis-2)-diphenylmethyl-N-(2-methoxyphenyl)methyl-1-azabicyclo(2.2.2)octan-3-amine), CP 99,994 ((+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine)] and RP 67580 ((3aR,7aR)-7,7-diphenyl-2(1-imino-2-(2-methoxyphenyl)ethyl)perhydroisoindole)) were synthesized by Merck Sharp & Dohme Research Laboratories. The neurokinin agonists were dissolved in water (stock solutions of 10 mM) and neurokinin antagonists were dissolved in dimethylsulphoxide (DMSO; Fluka, final bath concentration not greater than 0.1%).

Table 1 pK_B values (with 95% confidence limits) for (±)-CP 96,345, CP 99,994 and RP 67580

Agonist	Antagonist	pK _B	95% CL
[Sar ⁹ Met ¹¹ (O ₂)] substance P	(±)-CP 96345	6.9*	6.7–7.1
	CP 99994	7.7	7.6–7.8
	RP 67580	6.4*	6.1–6.7
Septide	(±)-CP 96345	8.4	8.3–8.6
	CP 99994	8.5	8.2–8.7
	RP 67580	7.0	6.8–7.2

Values were determined from Schild analysis where the slope has been constrained to unity except * where pK_B values are estimates obtained from a single antagonist concentration (CP 96,345 = 300 nM; RP 67580 = 1 μM; n = 8).

Results

Figure 1 shows the effects of (±)-CP 96,345, CP 99,994 and RP 67580 on the concentration-effect curves to septide and [Sar⁹Met¹¹(O₂)]SP. In vehicle- and time-matched control tissues the dose-ratios were less than two. (±)-CP 96,345, CP 99,994 and RP 67580 produced parallel shifts in the position of the concentration-effect curves with little or no effect on the maximum of the curve, suggesting competitive antagonism. The slopes of the Schild plots were not significantly different from unity supporting the assumption of the competitive nature of the antagonism. The pK_B values (calculated from constrained Schild plots) for (±)-CP 96,345, CP 99,994 and RP 67580 are shown in Table 1. It should be noted that due to the weak antagonist effects of (±)-CP 96,345 and RP 67580 on [Sar⁹Met¹¹(O₂)] SP-evoked responses, the pK_B values are estimates obtained with a single antagonist concentration.

Discussion

In the present study we compared the effects of (±)-CP 96,345 and CP 99,994 on NK₁-receptor-mediated responses in guinea-pig trachea evoked by two NK₁-receptor agonists. (±)-CP 96,345 and CP 99,994 are selective NK₁-receptor antagonists which have been shown to exert similar effects in both binding and functional studies. Thus both (±)-CP 96,345 and CP 99,994 inhibit [¹²⁵I]-labelled BH substance P in hIM-9 cells with K_i values of 0.6 and 0.3 nM respectively, they inhibit substance P-induced increases in firing rate in guinea-pig locus coeruleus neurones (IC₅₀ values of 90 and 50 nM respectively) and reverse [Sar⁹Met¹¹(O₂)]SP-induced locomotor activity in guinea-pigs (ED₅₀ values 1.9 and 0.6 mg kg⁻¹, s.c. Desai *et al.*, 1992; McLean *et al.*, 1992; Snider *et al.*, 1991).

In the present study, (±)-CP 96,345 and CP 99,994 were similarly effective in inhibiting responses evoked by septide (pK_B values were 8.4 and 8.5 respectively) in guinea-pig trachea. The pK_B value for RP 67580 was approximately 1.5 log units less. The effects of the antagonists on septide-evoked responses are consistent with their previously reported actions on NK₁-receptor-mediated responses in guinea-pig isolated tissue (Carruette *et al.*, 1992).

When [Sar⁹Met¹¹(O₂)]SP was used as the agonist, differences in the effects of the antagonists were observed. Firstly, (±)-CP 96,345, CP 99,994 and RP 67580 were less effective in antagonizing responses evoked by [Sar⁹Met¹¹(O₂)]SP than in antagonizing responses evoked by septide. Secondly, when using different agonists there was a difference in the rank order of effectiveness of the antagonists. In the case of septide, the rank order was (±)-CP 96,345 = CP 99,994 > RP 67580. However, when [Sar⁹Met¹¹(O₂)]SP was used as the agonist, the rank order was CP 99,994 > (±)-CP 96,345 > RP 67580. Guinea-pig trachea is known to contain a mixed

Figure 1 Left hand panel: effects of (a) (±)-CP 96,345 (● control, ○ 300 nM), (b) CP 99,994 (● control, ○ 30 nM, ■ 100 nM, □ 300 nM) and (c) RP 67580 (● control, ○ 1 μM) on [Sar⁹Met¹¹(O₂)]SP-induced contractile responses. Right hand panel: effects of (d) (±)-CP 96,345 (● control, ■ 10 nM, □ 30 nM, ▲ 100 nM), (e) CP 99,994 (● control, ○ 10 nM, ■ 30 nM, □ 100 nM, ▲ 300 nM) and (f) RP 67580 (● control, ○ 30 nM, ■ 1 μM, □ 3 μM) on septide-induced contractions. Each point, expressed as a percentage of the maximum response obtained in the initial control curve, is the mean value with s.e.mean (n = 4–8). For reasons of clarity not all s.e. bars are shown. Curves were fitted by least squares analysis of variance (see text for details).

population of NK₁- and NK₂-receptors (Ireland *et al.*, 1991). However, since septide and [Sar⁹Met¹¹(O₂)]SP are selective NK₁-receptor agonists and experiments were carried out in the presence of SR 48968 (a NK₂-receptor antagonist), it is unlikely that differences in the effects of the NK₁-receptor antagonists result from activation of NK₂-receptors.

It has been shown recently that only two amino acid residues in the binding site of the rat and human NK₁-receptors are responsible for the large differences in the affinity of these receptors for RP 67580 and (±)-CP 96,345 respectively, leading to the suggestion that rat and human NK₁-receptors are species variants of the same receptor (Fong *et al.*, 1992a). In the present report, the rank order for (±)-CP 96,345 and RP 67580 were preserved, regardless of the agonist used. These observations cannot be explained on the basis of a 'rat-type NK₁-receptor' being present in guinea-pig trachea.

It is possible that the differences in the effects of (±)-CP 96,345 and CP 99,994 may reflect that in guinea-pig

trachea, two subtypes of NK₁-receptors exist, one of which is activated by septide and shows equal sensitivity to the effects of (±)-CP 96,345 and CP 99,994, and a second subtype which is activated by [Sar⁹Met¹¹(O₂)]SP and shows differential sensitivity to these antagonists. Alternatively, it has been reported that the human NK₁ receptor can exist in alternative spliced variants (Fong *et al.*, 1992b) where each isoform is coupled to a separate second messenger system. It is possible that in guinea-pig trachea different agonists activate different isoforms of NK₁-receptors and differences in second messenger coupling can influence measured antagonist affinity constants (Kenakin, 1990).

In conclusion, in guinea-pig trachea there are differences in the effects of (±)-CP 96,345 and CP 99,994 which may reflect the existence of two populations of NK₁-receptors.

We thank Pamela Taylor for excellent technical assistance.

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(Received May 11, 1993
Revised November 19, 1993
Accepted January 4, 1994)