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THE MECHANISM OF ACTION OF A SUBSTANCE P ANTAGONIST (D-Pro^2, D-Trp^{7,9})-SP

R. HÅKANSON, J. HÖRIG & S. LEANDER

Department of Pharmacology, University of Lund, Lund, Sweden and Ferring, Kiel, FRG

1 A newly synthesized substance P (SP) analogue, (D-Pro^2, D-Trp^{7,9})-SP, specifically antagonizes the contractile effects of SP on the guinea-pig isolated taenia coli. In addition, previous studies had indicated that the SP analogue *per se* is capable of contracting this preparation. The results of the present study on the guinea-pig taenia suggest that the smooth muscle contractions produced by the SP analogue are due to histamine release. No contractions were observed following blockade of histamine H_1-receptors by mepyramine or following pretreatment with the histamine liberating agent, compound 48/80.

2 Analysis of the inhibition of SP-induced contraction by the analogue suggests that the inhibition is of the competitive type; pA_2 was calculated to be 6.1.

3 We conclude that (D-Pro^2, D-Trp^{7,9})-SP is a competitive SP antagonist with histamine-releasing properties.

Introduction

A newly synthesized analogue of substance P (SP), (D-Pro^2, DTrp^{7,9})-SP, has been found to exercise a specific antagonism to SP (and related tachykinins) on various target organs (Holmdahl, Håkanson, Leander, Rosell, Folkers & Sundler, 1981; Leander, Håkanson, Rosell, Folkers, Sundler & Tornqvist, 1981). It inhibits neuronally mediated non-cholinergic, non-adrenergic contractions of the isolated guinea-pig taenia coli and rabbit iris sphincter (Leander *et al.*, 1981) and also the inflammatory response to trauma in the eye (Holmdahl *et al.*, 1981). The SP analogue was thought to act as a competitive antagonist to SP (Leander *et al.*, 1981). A partial agonism was tentatively suggested because the analogue produced smooth muscle contraction. However, SP is known to release histamine from mast cells (Johnson & Erdös, 1973; Erjavec, Lembeck, Florjanc-Irman, Skofitsch, Donnerer, Saria & Holzer, 1981) and it was conceivable that release of histamine contributed to the smooth muscle contraction produced by the analogue. The present paper provides more detailed information on the mode of action of (D-Pro^2, D-Trp^{7,9})-SP.

Methods

Guinea-pig taenia coli preparations, consisting of longitudinal smooth muscle with the attached myenteric plexus (Burnstock, Campbell & Rand, 1966), were placed in Krebs solution, kept at 4°C for about 1 h and then mounted vertically on a Perspex holder in a 7 ml organ bath at 35°C. One end was attached to a rigid support and the other to a lever connected via a spring to a Grass FT03 force displacement transducer or to a photoelectric transducer for isotonic registration of mechanical activity. The load on the muscle was set at 0.2 g. Platinum ring electrodes were placed around the muscle with a constant electrode distance of 5 mm and the electrodes were connected to a Grass S4C stimulator for field stimulation with square wave pulses (15 V over the electrodes, 0.5–1 ms duration). The preparations were stimulated with trains of pulses lasting 3 s and with a frequency of 3 Hz, the resting period between stimulations being at least 2 min. The mechanical activity of the preparation was continuously recorded on a Grass model 7 or model 5 polygraph. The bathing fluid was a modified Krebs solution of the following composition (mM): NaCl 133, $NaHCO_3$ 16.3, KCl 4.7, $MgCl_2$ 1.0, NaH_2PO_4 1.4, $CaCl_2$ 2.5 and glucose 7.8. The solution was bubbled with a gas mixture of 7% CO_2 in O_2 giving a pH of 7.2–7.3.

Electrical stimulation was carried out in the presence of atropine (10^{-6} M) and guanethidine (5×10^{-6} M). The contractile response to SP and to (D-Pro^2, D-Trp^{7,9})-SP was examined with or without the histamine H_1-receptor antagonist mepyramine (10^{-6} M) in the bath and with or without pretreatment (for 15–30 min) of the taenia coli preparation with the histamine-releasing agent compound 48/80 (30 μg per ml in the bath). Also the effects of the

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5-hydroxytryptamine antagonist, methysergide (10^{-5} M) and the histamine H_2-receptor antagonist, metiamide (10^{-5} M) were examined.

Dose-response curves were constructed by adding step-wise increasing amounts of SP to the bath. Below 10^{-7} M three concentrations of SP were tested on each muscle; at and above 10^{-7} M only one concentration was tested per muscle. SP was added in a volume of 200 μl, the SP analogue in 40 μl. Between each addition the bath was rinsed until the muscle tension had returned to base-line (usually three rinses, in all 5–10 min). The responses were expressed as percentage of the contractions produced by carbachol (10^{-5} M). By using carbachol rather than maximally effective concentrations of SP the problem of desensitization can be avoided. pA_2 for (D-Pro^2, D-Trp^{7,9})-SP was calculated according to Schild (Tallarida, Cowan & Adler, 1979).

Drugs

(D-Pro^2, D-Trp^{7,9})-SP was synthesized as previously described (Folkers, Hörig, Rosell & Björkroth, 1981; Rosell, Björkroth, Hörig, Xu & Folkers, 1981) and SP was purchased from Peninsula, San Carlos, Ca., U.S.A. In addition the following drugs were used: atropine (ACO, Stockholm, Sweden), carbachol (Merck, Darmstadt, FRG), compound 48/80 and guanethidine (Sigma, St Louis, Mo., U.S.A.), mepyramine and metiamide (SKF, Welwyn Garden City), methysergide (Sandoz, Basle, Switzerland).

Results

The contractile responses produced by (D-Pro^2, D-Trp^{7,9})-SP and small amounts of SP ($< 10^{-9}$ M) were reduced upon repeated administration (Figure 1). With high doses of SP (10^{-7} M and above) the response was not reduced upon repeated administration. The contractile response to (D-Pro^2, D-Trp^{7,9})-SP was completely abolished by mepyramine or compound 48/80 (Figure 2), while the initial and subsequent responses to small doses of SP became

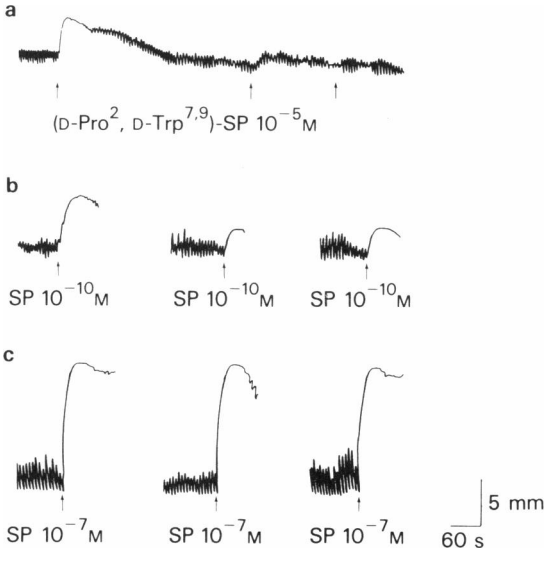

Figure 1 Contractile responses of the guinea-pig isolated taenia coli produced by (D-Pro^2, D-Trp^{7,9})-SP (a) and by low (10^{-10} M) (b) and high (10^{-7} M) concentrations of substance P (SP) (c). Repeated administration of (D-Pro^2, D-Trp^{7,9})-SP and of low concentrations of SP produced a a reduced contractile response compared to that observed after the initial administration. With SP the reduction was 56.4% ± 8.8 (mean ± s.e.mean, $n = 6$). High concentrations of SP produced the same contractile response upon repeated administrations. Rinsing after each addition of SP was necessary because even with low concentrations of SP the contraction lasted for about 10 min. The tracings shown are typical examples of 4–6 experiments.

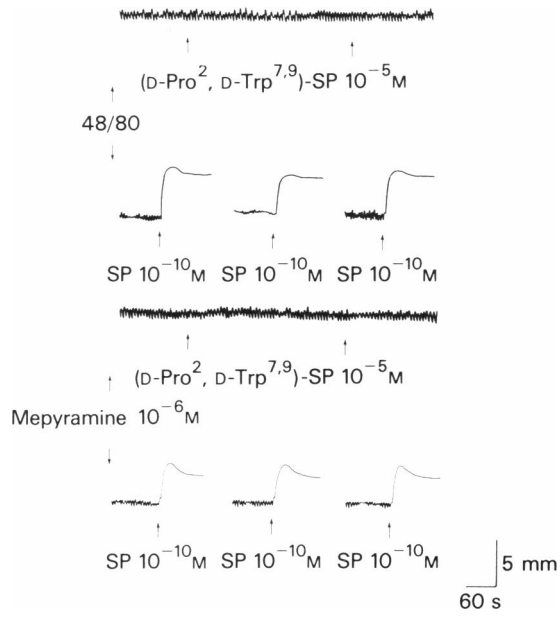

Figure 2 Pretreatment of the guinea-pig taenia coli with the histamine-liberating agent, compound 48/80 (upper pair of tracings) or with the histamine H_1-receptor antagonist mepyramine (lower pair of tracings) abolished the contractions produced by (D-Pro^2, D-Trp^{7,9})-SP. Under these circumstances low concentrations of substance P (SP), (10^{-10} M) produced the same contractile response upon repeated administration. The preparation was rinsed after each addition of SP. The mepyramine dose used effectively blocked the effect of histamine (10^{-6} M) (see Figure 3). The tracings shown are typical examples of 4–6 recordings.

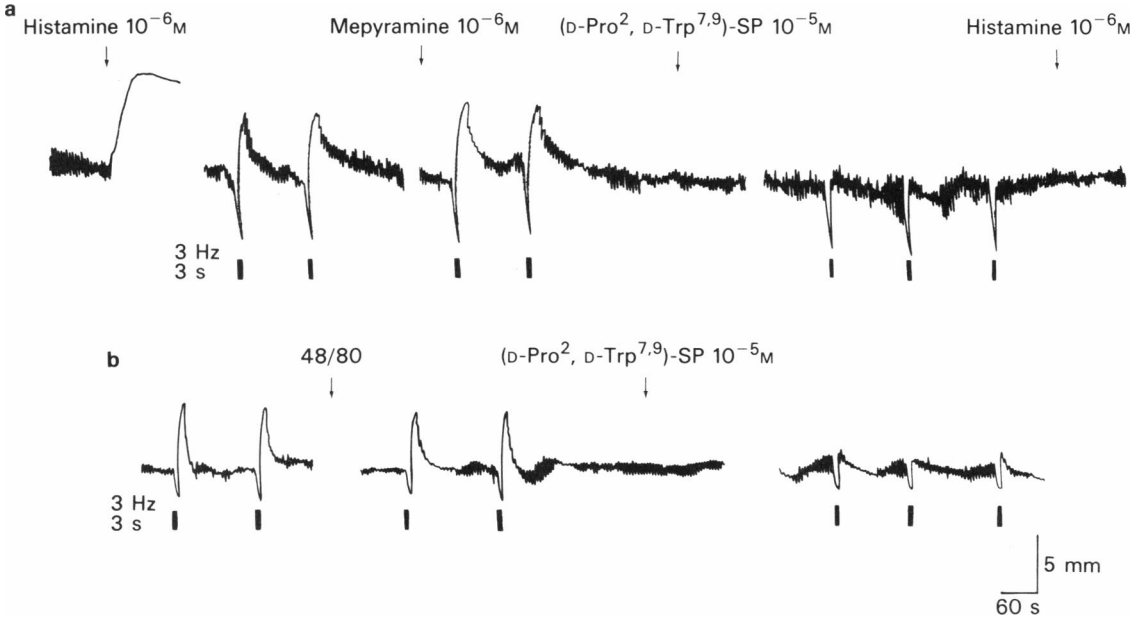

Figure 3 Effect of histamine and of electrical stimulation (15 V, 3 Hz, 3 s) on the tension of the taenia with atropine (10^{-6}M) and guanethidine (5×10^{-6}M) in the bath. Addition of the histamine H_1-receptor antagonist mepyramine (a) abolished the response to histamine but not to electrical stimulation. Subsequent addition of (D-Pro^2, D-Trp^{7,9})-SP abolished the contractile response to electrical stimulation, leaving only the relaxation. Administration of the histamine-liberating agent, compound 48/80 (b) did not affect the response to subsequent electrical stimulation, whereas addition of (D-Pro^2, D-Trp^{7,9})-SP abolished the contraction. The results shown ar typical examples from a series of 4–6 experiments.

identical. The response to high doses of SP was unaffected by mepyramine and by compound 48/80 (not shown in figure). The addition of mepyramine or compound 48/80 to the bath did not affect the tension of the smooth muscle. Neither methysergide nor metiamide affected the contractile responses produced by the two peptides, and metiamide was without effect on the contractile response to histamine (10^{-6}M). Mepyramine blocked the histamine-induced contraction (Figure 3) but was without effect on the contractile response to 5-hydroxytryptamine, whereas methysergide blocked the response to 5-hydroxytryptamine but not that to histamine.

Electrical stimulation of the taenia coli in the presence of atropine and guanethidine produced relaxation followed by contraction upon cessation of stimulation. The response to electrical stimulation was not affected by addition of mepyramine or pretreatment with compound 48/80. The contraction could be abolished by (D-Pro^2, D-Trp^{7,9})-SP, leaving only the relaxation response (Figure 3).

(D-Pro^2, D-Trp^{7,9})-SP inhibited the contractile response produced by SP and shifted the dose-response curve of SP to the right, the pA_2 being 6.1. (Figure 4). The estimated slope of the Schild plot (Tallarida *et al.*, 1979) was − 1.0.

Discussion

SP, which is known to release histamine from mast cells (Johnson & Erdös, 1973), probably releases histamine also in the isolated taenia coli preparation, since histamine H_1-receptor blockade or pretreatment with the mast-cell-degranulating and histamine-releasing agent, compound 48/80, reduced the contractile response to low concentrations of SP. However, the contractile response to high concentrations were notably unaffected by H_1-receptor blockade and histamine depletion. Blockade of histamine H_2-receptors and 5-hydroxytryptamine receptors was without effect. Interestingly, histamine release thus seems to play a role in the contractile response only to small doses of SP; with large doses of SP the contribution made by histamine was relatively unimportant. Also the SP analogue, (D-Pro^2, D-Trp^{7,9})-SP, seems to have the capacity to release histamine since the contractile response to this agent at all dose levels could be completely abolished by mepyramine or compound 48/80 pretreatment. Previously, (D-Pro^2, D-Trp^{7,9})-SP was described as a partial agonist (Leander *et al.*, 1981). The present results suggest that the contraction produced by this SP analogue is due to histamine

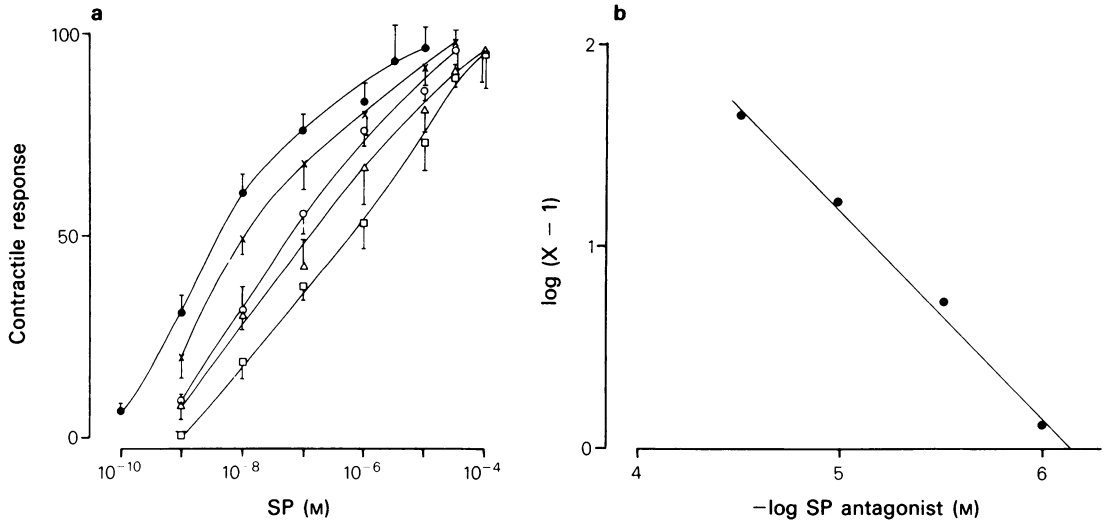

Figure 4 Dose-response curves showing the effects of substance P (SP) alone (●) and of SP in the presence of increasing concentrations of (D-Pro^2, D-Trp^{7,9})-SP ($\times 10^{-6}$ M; ○ 3×10^{-6} M; △ 10^{-5} M; □ 3×10^{-5} M) on the tension of the guinea-pig taenia expressed as percentage of the contraction produced by carbachol (10^{-5} M). Each value is the mean of 4–40 experiments. Vertical bars give s.e.mean. The results were identical in the presence of 10^{-6} M mepyramine (not shown) except that the contractile response to 10^{-10} M SP was somewhat lower. (b) A Schild plot embodying the results of experiments with four concentrations of (D-Pro^2, D-Trp^{7,9})-SP. × is the dose ratio (Tallarida *et al.*, 1979). pA_2 (the intercept on the abscissa scale) is 6.1. The slope of the line was calculated to be −1.0, $r = 0.997$. The slope had a 95% confidence range of −0.77 to −1.28, giving a 95% confidence range for A_2 of 3.92×10^{-7} to 1.28×10^{-6} M.

release rather than to interaction with SP receptors on the smooth muscle.

The SP antagonism displayed by (D-Pro^2, D-Trp^{7,9})-SP on taenia coli was analyzed by constructing SP concentration-response curves in the absence or presence of the SP analogue. The results show a parallel shift to the right of the dose-response curve with increasing concentrations of the SP analogue. The slope of the Schild plot conformed to the theoretical value for competitive inhibition (−1), supporting the view that the inhibition is competitive in nature (Leander *et al.*, 1981).

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