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A study of [D-Pro^2, D-Phe^7, D-Trp^9]-substance P and [D-Trp^{7,9}]-substance P as tachykinin partial agonists in the rat colon

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1 Two substance P (SP) analogues, [D-Pro^2, D-Phe^7, D-Trp^9]-SP (DPDPDT) and [D-Trp^{7,9}]-SP (DT79), previously described as tachykinin antagonists, have been shown to contract the rat colon muscularis mucosae preparation. The maximum response exhibited by these analogues was about 30% that of the SP maximum, suggesting that they were acting as partial agonists relative to SP.

2 The responses to DPDPDT were unaffected by pretreatment with mepyramine, methysergide or [Sar^1, Ile^5, Ala^8]-angiotensin II, which abolished the responses to histamine, 5-hydroxytryptamine (5-HT) and angiotensin II, respectively. Methysergide also did not affect the responses to DT79; the other antagonists were not tested against this analogue. Indomethacin and cimetidine also had no inhibitory effect. Atropine (2 μM) was present in all experiments to prevent indirect muscarinic effects.

3 Phenoxybenzamine did not affect the dose-response curves to SP, eledoisin-related peptide (ERP), kassinin, eledoisin or physalaemin, nor did it affect the responses to individual doses of DPDPDT or DT79. However, in the absence of atropine, it shifted the carbachol dose-response curve markedly to the right, and reduced its maximum.

4 The tachykinin antagonist [D-Pro^4, D-Trp^{7,9,10}]-SP$_{4-11}$ reduced the responses to individual matched doses of DPDPDT, DT79 and SP to the same degree, whilst leaving responses to 5-HT or angiotensin II unaffected. This suggested that DPDPDT and DT79 were acting at the same receptor as SP.

5 The inhibitory effects of DPDPDT on responses to SP, ERP and kassinin, and that of DT79 on responses to SP, were analysed. All four combinations yielded data compatible with an interaction at only one receptor, although DPDPDT appeared slightly more potent at inhibiting responses to kassinin.

6 The results are discussed in the light of the proposed existence of multiple tachykinin receptor subtypes. The possible influence of differential metabolism of tachykinin analogues is also considered.

Introduction

Over the past four years, several analogues of substance P (SP) have been shown to inhibit the effects of SP and related tachykinin peptides on isolated smooth muscle preparations (Leban *et al.*, 1979; Folkers *et al.*, 1982; Caranikas *et al.*, 1982, Björkroth *et al.*, 1982; Rosell *et al.*, 1983). Whilst these compounds have been described as specific competitive antagonists, some of the earlier examples exhibited agonist activity which may be transient or maintained (Leban *et al.*, 1979; Leander *et al.*, 1981; Hawcock *et al.*, 1982; Mizrahi *et al.*, 1983). If this agonist activity is mediated through the same mechanism as that of SP, it is possible that desensitization rather than a true competitive antagonism might be responsible for the reduction in agonist responses. This explanation certainly seems appropriate to the apparent SP antagonist activity of [D-Phe^7]-SP (Growcott & Petter, 1980). However, SP analogues which retain the N-terminal basic amino acid residues (Arg^1, Lys^3) are potent histamine releasing agents and several of the antagonist analogues are more active than SP itself in this respect (Fewtrell *et al.*, 1982; Foreman *et al.*, 1982). Indeed, Håkanson *et al.* (1982) have demon-

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strated recently that the apparent agonist activity of [D-Pro^2, D-Trp^{7,9}]-SP on the guinea-pig taenia coli is attributable to its histamine-releasing properties.

In this paper we present evidence that, on the rat colon muscularis mucosae preparation, (Bailey *et al.*, 1982), [D-Pro^2, D-Phe^7, D-Trp^9]-SP (DPDPDT) and [D-Trp^{7,9}]-SP (DT79) behave as true partial agonists at a tachykinin receptor, rather than causing an indirect effect through release of histamine or other mediators.

The interaction of these analogues with some tachykinin full agonists is also described and the results are discussed in the light of recent suggestions concerning the possible existence of multiple tachykinin receptor subtypes.

Methods

A consideration of the various approaches to the study of partial agonists which might be used, and their applicability to the present investigation, is presented in the Appendix.

Preparation of tissue and dosing schedules

Male Sprague-Dawley rats (140–200 g) were stunned and killed by cervical dislocation. A 25 mm length of the distal colon was removed, cleared of any faecal matter, and mounted on a glass rod of maximum diameter 5 mm having a tapered end. The outer muscle layers were stripped off with a moist cotton wool pellet and discarded (see Parsons & Paterson, 1965; Bailey *et al.*, 1982). The remaining tissue retained its tubular conformation and consisted of epithelial tissue and muscularis mucosae; histological slides prepared for light microscopy from sections of colon before and after stripping were similar to the photomicrographs shown in Parsons & Paterson (1965). The colon was mounted in a 1 ml organ bath in a modified Tyrode solution (ionic composition, mM) Na^+ 149.1, K^+ 2.8, Ca^{2+} 1.8, Mg^{2+} 2.1, Cl^- 147.5, $H_2PO_4^-$ 0.3, HCO_3^- 11.9, glucose 5.6) bubbled with 95% O_2, 5% CO_2 at 32°C. All experiments were conducted in the presence of atropine (2 μM)), except when carbachol was used as an agonist. Isotonic contractions were recorded on Servoscribe pen recorders using a 0.5 g counterweight. The dosing schedule for all agonists except kassinin was as follows: agonists were given at 10 min intervals with a contact time of 50–120 s depending on the time taken to reach the maximum response for a given dose. The tissue was washed 3 times with warm gassed Tyrode at this point, and again 3 min after administration of the agonist. Partial agonists were equilibrated with the tissue for 7 min before the relevant agonist doses. This was considered to be sufficient since the agonist response to the partial agonists reached a steady state within 3 or 4 min. The cycle for kassinin was extended as the onset and offset time for this peptide was longer; doses of kassinin were administered every 15 min with a contact time of 5 min.

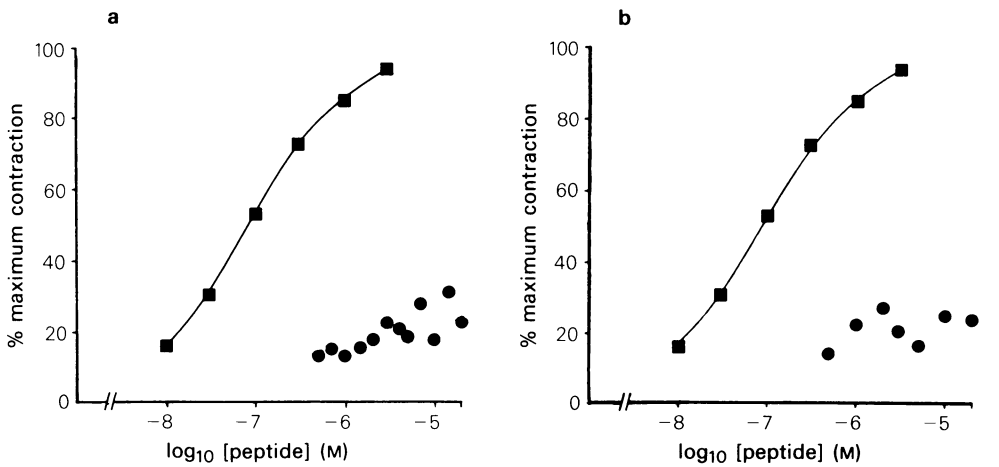

Figure 1 Dose-response curves for the contractile effects of (a) substance P, (SP, (■)), and [D-Pro^2, D-Phe^7, D-Trp^9]-SP (DPDPDT, (●)), and (b) SP, (■) and [D-Trp^{7,9}]-SP, (DT79, (●)) on the rat isolated colon muscularis mucosae preparation. Doses were given at ten min intervals. The time to peak response for each dose was about 90 s for SP, and 3 min for DPDPDT and DT79. All experiments were conducted in the presence of atropine. SP values are the means of 5 determinations; those for DPDPDT and DT79 are the means of 2–10 determinations. Error bars are omitted for clarity, but the s.e.mean never exceeded 10% of the mean for SP, and only rarely so for DPDPDT or DT79.

Adsorption of peptides

Peptide agonists were added to the organ bath by means of an automatic pipette with polypropylene tips. Partial agonists were given in the same way, or by silanized glass Petersen micropipettes. The absorption of SP to unsilanized glass and to polystyrene is by now well known (Cleugh & Gaddum, 1963, Stewart, 1983). Results from this laboratory (S.J. Bailey, unpublished observations) suggest that polypropylene adsorbs SP at least as well as polystyrene, but the process is slow for both plastics (about 1 h to saturation), and thus the few seconds contact between peptide and tip were considered too short for significant depletion of peptide. All glassware used was treated with a solution of dimethyldichlorosilane in 1,1,1-trichlorethane to prevent peptide adsorption.

Effect of phenoxybenzamine pretreatment on responses to peptides and carbachol

Serial dose-response curves were obtained for each full agonist after which the tissue was incubated with 10 μM phenoxybenzamine for 30 min. The preparation was washed throughly and the agonist dose-response curve was re-established. The control dose-response curves were fitted to a logistic function by means of an iterative least squares computer fit. All responses were thus expressed as a percentage of the fitted maximum. Responses to single doses of DPDPDT and DT79 were recorded before and after phenoxybenzamine treatment as above, since insufficient amounts were available to obtain full dose-response curves.

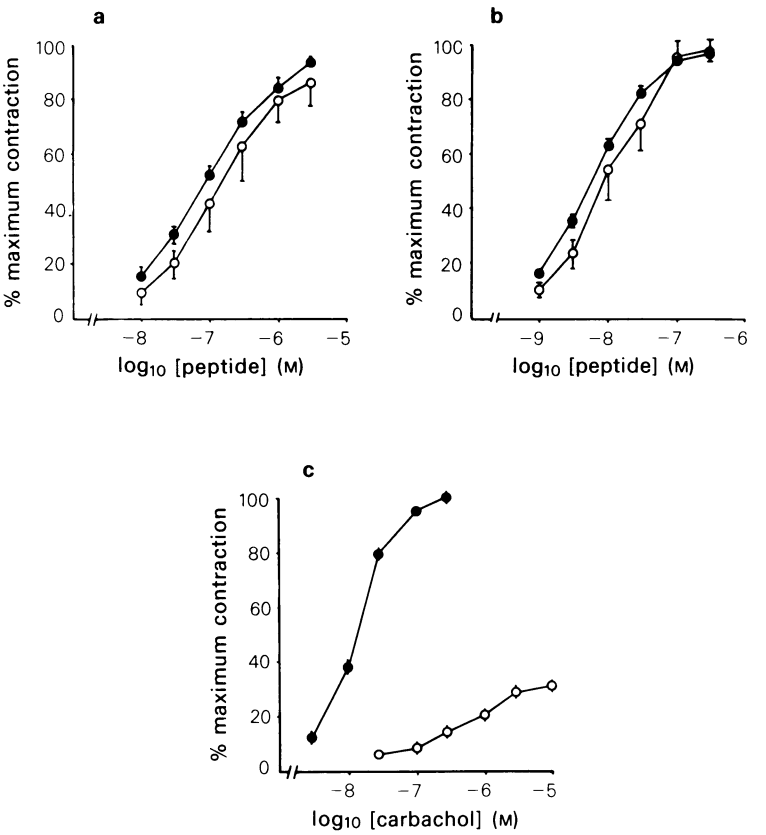

Figure 2 Effect of phenoxybenzamine treatment (10 μM for 30 min) on the dose-response curves to (a) substance P (SP), (b) eledoisin, (c) carbachol on the isolated rat colon muscularis mucosae preparation. Agonist doses were given at 10 min intervals. The time to peak response for each dose was 90 s for SP and carbachol, 3 min for eledoisin. Atropine (2 μM) was present in (a) and (b) only. (●) Control responses, (○) after phenoxybenzamine treatment. SP and eledoisin values are the means of 5 determinations; carbachol values are the means of three determinations.

Effects of some drug antagonists on responses to [D-Pro², D-Phe⁷, D-Trp⁹]-SP (DPDPDT), [D-Trp⁷,⁹]-SP (DT79) and substance P (SP)

Matched doses of SP, DPDPDT or DT79, and the relevant agonist were given before and after administration of mepyramine, methysergide, indomethacin, cimetidine, [Sar¹, Ile⁵, Ala⁸]-angiotensin II or [D-Pro⁴, D-Trp⁷,⁹,¹⁰]-SP_{4-11}.

Interaction of [D-Pro², D-Phe⁷, D-Trp⁹]-SP (DPDPDT) and [D-Trp⁷,⁹]-SP (DT79) with tachykinin full agonists

Serial dose-response curves were obtained to SP, ERP or kassinin. Shorter, incomplete, 5 point dose-response curves were then obtained in the presence of a fixed concentration of DPDPDT or DT79. In some cases, two concentrations of partial agonist were tested on the same preparation. The control dose-response curve was fitted to a logistic function as above. Extrapolation from this curve yielded theoretical concentrations of full agonist which were equieffective with the experimental concentrations of full agonist in the presence of a given concentration of the partial agonist.

Drugs

All peptides used were dissolved in 0.1% acetic acid at a concentration of 1 mM and aliquots were stored at −20°C until required. Phenoxybenzamine was dissolved initially at a concentration of 2 mM in 0.1 M HCl and neutralized with 0.1 M NaOH to a final acid:base ratio of 53:47. This solution was diluted in Tyrode solution as necessary. Indomethacin was dis-

Table 1 Effects of some drug antagonists on agonist induced contractile responses of the rat colon muscularis mucosae

Antagonists		*Agonists*	
Compound	*Concentration*	*Antagonized* (dose ratio > 5)	*Not antagonized* (dose ratio < 2)
Phenoxybenzamine	10 μM (30 min pretreatment)	Carbachol	SP eledoisin physalaemin kassinin ERP DPDPDT DT79
Methysergide	1 μM	5-HT	SP DPDPDT DT79
Mepyramine	1 μM	Histamine	SP DPDPDT
Cimetidine	10 μM		histamine SP DPDPDT
Indomethacin	1 μM (45 min pretreatment)		angiotensin II SP DPDPDT
[Sar¹, Ile⁵, Ala⁸]-angiotensin II	1 μM	Angiotensin II	SP DPDPDT
[D-Pro⁴, D-Trp⁷,⁹,¹⁰]-SP_{4-11}	32 μM	SP DPDPDT DT79	angiotensin II

Abbreviations: SP, substance P; 5-HT, 5-hydroxytryptamine; DPDPDT, [D-Pro², D-Phe⁷, D-Trp⁹]-SP; DT79, [D-Trp⁷,⁹]-SP.

solved at a concentration of 1 mM in deionized water containing sodium carbonate (0.5 g ml^{-1}). Sources of drugs were as follows: substance P (SP) and eledoisin-related peptide (ERP) (Beckman, Switzerland). [D-Pro^2, D-Phe^7, D-Trp^9]-SP, [D-Pro^4, D-Trp^{7,9,10}]-SP_{4-11} and kassinin (Peninsula Laboratories, U.S.A.). [D-Trp^{7,9}]-SP gift from Dr J-C Xu and Professor K. Folkers, Austin, U.S.A.). Physalaemin and eledoisin (gift from Dr R. de Castiglione, Farmitalia, Italy). Angiotensin II, [Sar^1, Ile^5, Ala^8]-angiotensin II, 5-hydroxytryptamine and indomethacin (Sigma). Phenoxybenzamine hydrochloride (Smith, Kline & French). Methysergide bimaleate (Sandoz, Switzerland). Mepyramine maleate (May & Baker). Atropine sulphate monohydrate (Aldrich Chemical Company, U.S.A.). Propranolol hydrochloride (I.C.I.). Phentolamine mesylate (CIBA). Histamine acid phosphate and carbachol (B.D.H.). Cimetidine (S.K. & F.).

Results

Agonist responses to [D-Pro^2, D-Phe^7, D-Trp^9]-SP (DPDPDT) and D-Trp^{7,9}]-SP (DT79)

The dose-response curves to DPDPDT and DT79 are shown in Figure 1. The curves are not well defined, but the slopes of both are less steep than the SP curve when measured at the same level of response (30% of the SP maximum). This, and the reduced maximum response obtained, are characteristic findings with partial agonists. However, the curves are not sufficiently well-defined to employ the double reciprocal plot (see method 1, Appendix), to estimate K_P for either analogue.

Control doses of solvent (dilute acetic acid) did not affect the colon until a concentration of 0.01% was reached. This corresponds to a peptide concentration of 100 μM. Since the maximum peptide concentration reached was 20 μM, solvent effects could thus be ignored.

Effect of phenoxybenzamine and other drug antagonists on responses to tachykinins, [D-Pro^2, D-Phe^7, D-Trp^9]-SP (DPDPDT) and D-Trp^{7,9}]-SP (DT79)

Dose-response curves to SP, physalaemin, eledoisin, kassinin, ERP and carbachol were obtained before and after phenoxybenzamine treatment. Results for SP, eledoisin and carbachol are shown in Figure 2. Whilst the dose-response curve to carbachol was shifted 100–1,000 fold to the right, and the maximum reduced to less than half the control value, responses to the peptides were unaffected by phenoxybenzamine pretreatment.

Individual responses to DPDPDT and DT79 were also unaffected by phenoxybenzamine pretreatment (Table 1). Thus the second method outlined in the Appendix for the estimation of the K_P for a partial agonist was inapplicable, at least with the potential irreversible antagonist at our disposal.

The effects of some other antagonists on responses to SP, DPDPDT and DT79 are summarized in Table 1; examples of 2 experiments, typical of 4 showing inhibition of agonist responses to SP, DPDPDT and DT79 by the tachykinin antagonist [D-Pro,^{4} D-Trp^{7,9,10}]-SP_{4-11} (Caranikas *et al.*, 1982; Bailey *et al.*, 1983) are shown in Figure 3.

Interaction of substance P (SP), eledoisin-related peptide (ERP) and kassinin with partial agonists

Results of experiments where responses to SP, ERP and kassinin were compared in the presence and absence of a range of concentrations of DPDPDT are shown in Figure 4. Corresponding data for the combination SP/DT79 are also shown. The data are shown in the form of a plot of log [(1/m) − 1] against log P. A straight line with a slope not significantly different from unity was obtained in each case. For all four combinations, the expression P.m/(1 − m) was constant with P. Values of K_P obtained by extrapolation from these plots, with 95% confidence limits, are

Table 2 Dissociation constants (μM) for two tachykinin partial agonists, against three full agonists, on the isolated rat colon muscularis mucosae preparation

		Agonist	
Partial agonist	*SP*	*Kassinin*	*ERP*
DPDPDT	5.9 (4.2–8.1)	2.3 (2.1–3.5)	4.1 (3.0–5.4)
DT79	1.4 (1.1–1.9)	1.6 ± 0.1	ND

Abbrevations: N.D., not determined; SP, substance P; ERP, eledoisin-related peptide; DPDPDT, [D-Pro^2, D-Phe^7, D-Trp^9]-SP; DT79, [D-Trp^{7,9}]-SP. Dissociation constants were obtained as the antilog of − intercept on the x axis from the graphs in Figure 4, with slopes constrained to 1. Figures in brackets are 95% confidence limits; the figure for the combination kassin/DT79 is the mean ± s.e.mean of 4 determinations at one concentration only of DT79 (3 μM).

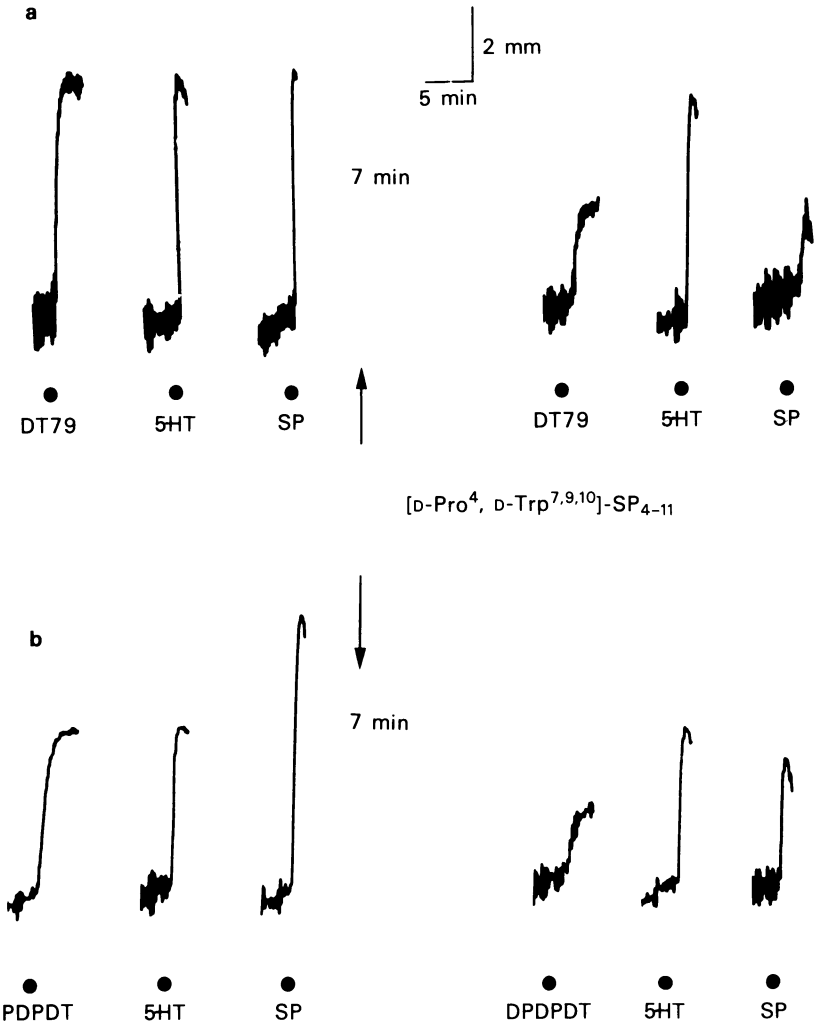

Figure 3 Effect of the tachykinin antagonist [D-Pro^4, D-Trp^{7,9,10}]-SP$_{4-11}$ (32 μM) on contractile responses to substance P (SP), 5-hydroxytryptamine (5-HT), [D-Pro^2, D-Phe^7, D-Trp^9]-SP (DPDPDT), and [D-Trp^{7,9}]-SP (DT79) on the rat isolated colon muscularis mucosae preparation. Traces (a) and (b) are from different preparations. The preparation was washed with the peptide antagonist for 7 min before the agonist doses were given. Doses of agonists were given at 10 min intervals. The time to peak contraction for each dose was 90 s for SP and 5-HT, 3 min for DPDPDT and DT79. Atropine (2 μM) was present throughout. These 2 experiments are typical of 4 showing inhibition of partial agonist responses by [D-Pro^4, D-Trp^{7,9,10}]-SP$_{4-11}$.

shown in Table 2. The value for the combination kassinin/DT79 was obtained using only one concentration of DT79 (3 μM, $n=4$) and the limits shown are mean $\pm$ s.e.mean.

Discussion

The lack of good dose-response relationships for the SP analogues DPDPDT and DT79 precluded estimation of their respective K_Ps by plotting reciprocals of equieffective doses of partial and full agonists. However, it does appear from the shallow slopes of the dose-response curves and their low maximum responses (30% that of SP) that they are behaving as partial agonists, compared to the tachykinins.

That neither DPDPDT nor DT79 are contracting the colon by release of histamine, 5-hydroxytryptamine (5-HT) or arachidonic acid metabolites, or by an interaction with the angiotensin receptor is shown by the data in Table 1. The involvement of a muscarinic component is ruled out since atropine was

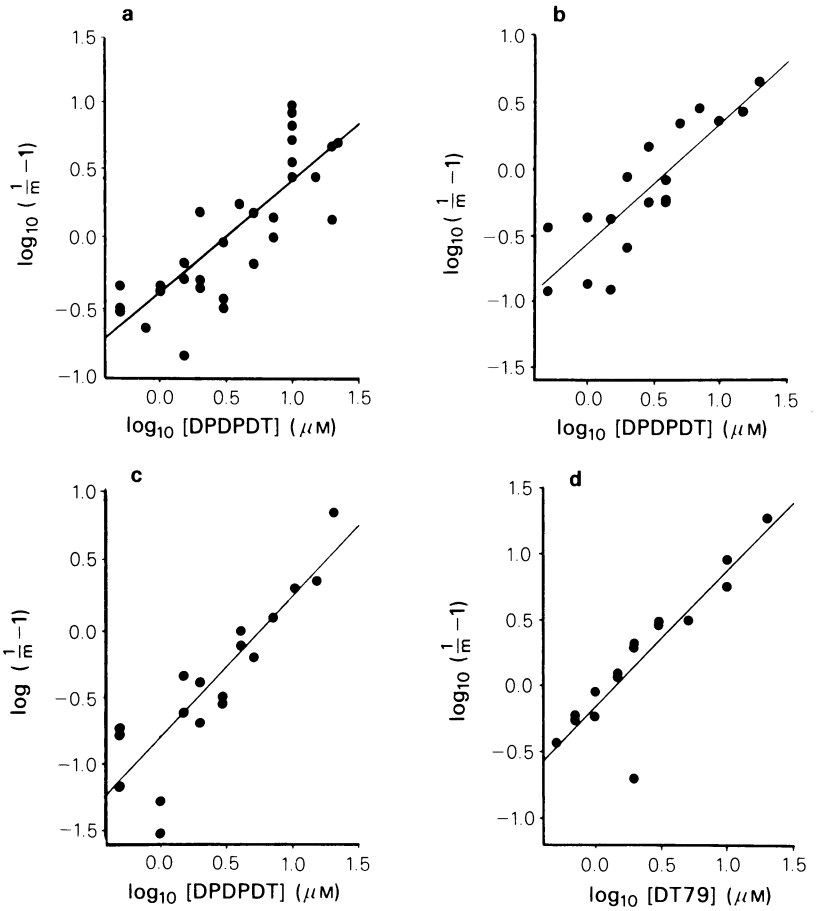

Figure 4 Effect of two tackykinin partial agonists on contractile responses to three tachykinin full agonists in the rat isolated colon muscularis mucosae preparation. (a) [D-Pro^2, D-Phe^7, D-Trp^9]-SP (DPDPDT) and kassinin; (b) DPDPDT and eledosin-related peptide (ERP); (c) DPDPDT and substance P (SP); (d) [D-Trp^{7,9}]-SP and SP. An explanation of the plot is given in the theory section. Briefly, serial dose-response curves were obtained for each agonist, firstly in the absence, and secondly in the presence of a fixed concentration of the relevant partial agonist. These were compared by the method of Lemoine & Kaumann (1982). Dose intervals and contact times for the full agonists were as follows: kassinin 15 min and 5 min, respectively; SP, 10 min and 90 s; ERP, 10 min and 50 s. Doses of partial agonist were administered to the tissue 7 min before the relevant agonist doses. Atropine (2 μM) was present during all experiments. The lines drawn are the best fit linear regression lines for each partial agonist/full agonist combination. In no case did the slope of the line differ significantly from unity, and so a regression line constrained to unit slope was used to estimate the dissociation constant for each partial agonist as antilog − (intercept on x axis) (see Table 2).

present for all experiments in which peptides were used. This does not preclude the possibility that DPDPDT or DT79 may act by release of an, as yet, unidentified transmitter. However, the reduction in response seen in the presence of the specific competitive tachykinin antagonist [D-Pro^4, D-Trp^{7,9,10}]-SP_{4-11} (Caranikas *et al.*; 1982, Bailey *et al.*, 1983) strongly suggests a direct action on a tachykinin receptor.

Agonist responses to DPDPDT have also been observed in the guinea-pig ileum, the rat superior cervical ganglion and the rabbit external jugular vein (Hawcock *et al.*, 1982). Those in the ileum were abolished by atropine, indicating an indirect muscarinic action of DPDPDT. In contrast, the responses of the other two tissues were considered to be mediated directly through the same receptor as SP. From these data, it is indeed possible that DPDPDT is a full agonist, albeit a weak one, on the rat superior cervical ganglion and the rabbit external jugular vein. Although the dose-response curves presented here for DPDPDT and DT79 on the rat colon do not

display a definite maximum, the analogues seem unlikely to reach the same maximum as SP.

The closely related compound [D-Pro^2, D-Trp^{7,9}]-SP also shows some agonist activity on the guinea-pig taenia coli; this has been shown to be due to release of histamine (Håkanson *et al.*, 1982). Although in rat peritoneal mast cell preparations DPDPDT is a potent histamine-releasing agent (Fewtrell *et al.*, 1982), the insensitivity to mepyramine of responses to DPDPDT rules out such a mechanism in the rat colon (Table 1).

The use of indomethacin to block responses to the tachykinins was indicated by the observations of Johns (1981). He showed that indomethacin will inhibit responses to SP in the guinea-pig urinary bladder. This is not the case in the rat colon, where responses to SP, DPDPDT and angiotensin II were refractory to indomethacin.

Growcott *et al.* (1983) have shown phenoxybenzamine (Pbz) to inhibit responses to eledoisin but not those to SP or physalaemin in the isolated guinea-pig bladder. The dose-response curve to eledoisin was shifted about tenfold to the right by a 20 μM dose of Pbz. A similar shift is seen against some tachykinin agonists in the guinea-pig ileum (Lin & Musacchio, 1983; W. Piotrowski, personal communication). In the absence of a more potent selective irreversible tachykinin antagonist, pretreatment with phenoxybenzamine was used in the rat colon in an attempt to abolish agonist responses to DPDPDT and DT79. However, Pbz had no effect on the dose-response curves to SP or eledoisin (Figure 2) or those to physalaemin, kassinin or ERP (data not shown). Furthermore, it had no effect on the responses to single doses of DPDPDT or DT79 (Table 1), although the dose-response curve to carbachol was shifted 100–1,000 fold to the right, and its maximum lowered.

The reason for the discrepancy between the colon on the one hand, and the guinea-pig ileum and bladder preparations on the other, is unresolved. It is clear, however, that Pbz pretreatment does not provide a basis for the analysis of the actions of tachykinin partial agonists on the colon.

Analysis of the interaction between full and partial agonists is thus left as the only feasible method of estimating dissociation constants for tachykinin partial agonists in the rat colon. The slopes of unity for the plot of log [1 m−1] against log P for the four full agonist/partial agonist combinations suggest that only one receptor is mediating the effects of tachykinins on this tissue. This is in accordance with the observation that the analogue [D-Pro^4, D-Trp^{7,9,10}]-SP$_{4-11}$ antagonizes four tachykinin agonists equally on this preparation (Bailey *et al.*, 1983).

The small but significant difference in the K_P for the kassinin/DPDPDT combination compared to that calculated using SP as agonist is not easily explained, but may reflect differences in the receptor reserves for SP and kassinin in this tissue. A similar discrepancy is seen with the ileum where DPDPDT is a pure antagonist (in the presence of atropine), and its calculated K_D when using kassinin as an agonist is roughly half that when using SP (S.J. Bailey unpublished observations).

Two other tachykinin antagonists, [D-Pro^4, D-Trp^{7,9,10}]-SP$_{4-11}$ and [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]-SP also appear more potent against kassinin than SP on the guinea-pig ileum (Bailey *et al.*, 1983; Regoli *et al.*, 1983; S.J. Bailey & R.L. Featherstone, unpublished observations). If there were no pharmacokinetic differences between agonists (that is, loss of peptide due to breakdown or redistribution is either negligible or independent of the agonist), then one would expect the K_D of a given antagonist or partial agonist to be independent of the full agonist used. Results from this laboratory suggest SP to be metabolized faster than kassinin in both the guinea-pig ileum and the rat colon (Bailey & Jordan, 1984). Lee (1982) quotes an IC_{50} for SP and its free acid of roughly 30 μM for inhibiting the breakdown of [^{3}H]-SP in a rat brain homogenate. As we have used concentrations of partial agonist up to 20 μM, and antagonist of up to 32 μM, it is not inconceivable that these analogues could be inhibiting the breakdown of the full agonists. In such a situation, antagonists and partial agonists will appear to have lower K_D's when using more stable agonists, and these will be closer to the 'true' K_D's. Thus, unless stable agonist analogues or inhibitors of peptide breakdown are used, we cannot accept the dependence of antagonist or partial agonist K_D on the agonist used as evidence of multiple tachykinin receptors.

However, it would be inconsistent to invoke this kind of interaction to explain the apparent agonist selectivity observed with DPDPDT, [D-Pro^4, D-Trp^{7,9,10}]-SP$_{4-11}$ and [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]-SP on the ileum, and DPDPDT on the colon, without explaining why such selectivity is not seen on the colon with the compounds [D-Pro^4, D-Trp^{7,9,10}]-SP$_{4-11}$, [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]-SP and DT79.

Another stumbling block to the acceptance of a metabolic interaction to explain our results is the fact that, on the colon, DPDPDT does not exhibit an even higher K_D against ERP than SP, despite the lower stability of ERP compared to SP (Bailey & Jordan, 1984). Also it might be argued that since the degree of protection provided by DPDPDT should increase with increasing concentration of DPDPDT, then the slope of the log [(l/m) − 1] against log P plot above would be reduced to less than unity; this is not seen in practice.

The alternative explanation for the variation of the

K_D of DPDPDT with agonist involves postulation of more than one receptor subtype, but this would be inconsistent with the unit slopes of the double log plots above. In any case, the difference in K_Ds we are discussing is small, which would suggest any possible differences in receptors are in themselves small.

In order to distinguish whether the dependence of the K_D of DPDPDT on the nature of the full agonist used is a reflection of the existence of more than one receptor, a metabolic interaction between partial agonist and full agonist, or different receptor reserves for the full agonists, experiments should be performed using a number of stable analogues of the tachykinins, and/or done in the presence of inhibitors of the enzymatic breakdown of SP. Meanwhile, it is difficult to distinguish between these two models, although we tend to favour a metabolic interaction between partial agonists and full agonists as an explanation for our data. Data such as these showing the dependence of antagonist or partial agonist K_Ds on the agonist used should not therefore be rigidly interpreted as evidence for multiple tachykinin receptor subtypes.

Appendix

Three methods have been described for the estimation of the dissociation constant (K_D) of a partial agonist. A critical review of these can be found in Jenkinson (1979). They are listed here in increasing order of complexity. The first, based on Stephenson's efficacy proposal (Stephenson, 1956), and described in detail by Mackay (1966), Barlow *et al.*, (1967) and Waud (1969), necessitates the determination of dose-response curves to the partial agonist and to a full agonist acting at the same receptor. Extrapolation from these curves yields theoretical concentrations of full agonist (A) giving the same response as the experimental concentrations of partial agonist (P). Waud (1969) demonstrated that a reciprocal plot of 1/A against 1/P fits the linear equation

$$1/A = e_A/(K_A.e_P) + [(e_A.K_P)/(e_P.K_A)] \times 1/P$$

where e_A is the efficacy of the full agonist

e_P is the efficacy of the partial agonist

K_A is the dissociation constant of the full agonist

K_P is the dissociation constant of the partial agonist

The primary assumptions implicit in deriving this equation, and those mentioned later, are that both the full and partial agonists interact with the receptor in accordance with the Law of Mass Action; that only a small fraction of the total receptor population needs to be activated by the full agonist for a maximal response to be generated; and that a state of equilibrium is reached between receptor and ligand(s).

(It is interesting to note Stephenson's own comments on this reciprocal plot (Stephenson, 1975): "Statisticans spurn these reciprocal plots since the experimental points are weighted in an arbitrary and probably incorrect way; they would use a different procedure to extract the information, but I find the graphs a help to understanding." Discussions on how best to weight the points in this kind of plot can be found in Thron (1970), Parker & Waud (1971), Colquhoun (1972) and Marano & Kaumann (1976).)

The second method, described by Furchgott & Bursztyn (1967), involves blockade of a fraction of the receptor pool such that the partial agonist can no longer produce a response. It can then be treated as a pure antagonist, and its affinity constant calculated via a Schild plot (Arunlakshana & Schild, 1959).

The third method, first described by Stephenson (1956), involves determination of a dose-response curve to a full agonist in the presence and absence of a fixed concentration of partial agonist. Extrapolation, as in Method 1, will yield a concentration of full agonist (A) which, if acting alone, would give the same response as the concentration (A') acting in the presence of a fixed concentration of partial agonist. A plot of A against A' is linear with a dimensionless slope m. Stephenson showed that the dissociation constant K_P of the partial agonist is given by the expression

$$K_P = P/((1/m) - 1)$$

where P is the concentration of the partial agonist. Thus K_P estimated in this way should be independent of the full agonist used.

Lemoine & Kaumann (1982) and Kaumann & Marano (1982) have extended this method such that a check can be made as to whether or not the full and partial agonists are interacting with one or more receptor species. They showed that if values for the slope, m, derived as above, corresponding to a range of partial agonist concentrations, are obtained, then a plot of log $[(1/m) - 1]$ against log P has a slope of unity, if only one receptor is involved. A necessary corollary is that the value of $P.m/(1-1)$ doses not vary with P. However, if more than one receptor subtype is activated by both the full and the partial agonist, the slope of the double log plot is less than unity. A plot with unit slope will yield a value for K_P as the antilog of $-$ (intercept on the x axis). The use of the double logarithmic plot in this analysis is analog-

ous to the use of the Schild plot in estimating dissociation constants for competitive antagonists, as noted by Stephenson (1975).

Note that one assumption implicit in this analysis is that the full agonist occupies only a small fraction of the total receptor pool in producing its effect. If this assumption is invalid, the value of the partial agonist K_P thus derived will be an overestimate of the true value. The use of full agonists with different receptor reserves might thus result in differing estimates for the partial agonist K_P, even though a homogeneous receptor population may be present.

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