Biochimica et Biophysica Acta, 972 (1988) 37–44
Elsevier

BBA 12346

An analogue of substance P with broad receptor antagonist activity

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(Received 1 June 1988)

Key words: Substance P; Receptor antagonist; (Guinea pig)

[DPro^4,DTrp^{7,9,10}]Substance P-4-11 functions as a substance P receptor antagonist in several different systems. Because some analogues of substance P can function as receptor antagonists for bombesin as well as substance P, we tested [DPro^4,DTrp^{7,9,10}]substance P-4-11 for its ability to modify the interaction of various pancreatic secretagogues with their receptors in dispersed acini from guinea pig pancreas. [DPro^4,DTrp^{7,9,19}]Substance P-4-11 did not stimulate amylase secretion and did not alter the stimulation of amylase secretion caused by secretin, vasoactive intestinal peptide, calcitonin gene-related peptide or carbachol, but did inhibit the stimulation of amylase secretion caused by substance P, bombesin or cholecystokinin. With substance P, bombesin and cholecystokinin, [DPro^4,DTrp^{7,9,10}]substance P-4-11 caused a parallel rightward shift in the dose-response curve for stimulation of amylase secretion with no change in the maximal response. Schild plots of these results gave straight lines with slopes that were not significantly different from unity. [DPro^4,DTrp^{7,9,10}]Substance P-4-11 inhibited binding of ^{125}I-labeled substance P, ^{125}I-[Tyr^4]bombesin and ^{125}I-cholecystokinin octapeptide over the same range of concentrations as that in which it inhibited biologic activity of each of these peptides. Half-maximal inhibition of binding of ^{125}I-substance P occurred with 4 μM, of ^{125}I-[Tyr^4]bombesin with 17 μM and of ^{125}I-cholecystokinin octapeptide with 5 μM. With each radiolabeled peptide the value of K_i for inhibition of binding by [DPro^4,DTrp^{7,9,10}]substance P-4-11 was not significantly different from the corresponding value of K_i calculated from the appropriate Schild plot. The present results indicate that [DPro^4,DTrp^{7,9,10}]substance P-4-11 is a competitive antagonist at receptors for substance P, for bombesin and for cholecystokinin. Thus, these receptors must share a common peptide recognition mechanism even though they interact with agonists that have no obvious structural similarity.

Introduction

Several analogues of substance P have been reported to function as substance P receptor antagonists [1,2]. Interestingly, these analogues have also been reported to inhibit the action of bombesin in vitro [3–5] and under some [6,7], but not all [8–10], circumstances in vivo. Although it has been shown that some of these analogues do

Abbreviations: CCK, cholecystokinin; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. 8-Br-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphate; CGRP, calcitonin gene-related peptide; VIP, vasoactive intestinal peptide; CCK-8, cholecystokinin octapeptide; ^{125}I-CCK-8, ^{125}I-Bolton-Hunter labeled CCK-8; ^{125}I-substance P, ^{125}I-Bolton-Hunter-labeled substance P; TFA, trifluoroacetic acid.

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not inhibit the action of bradykinin [11], these substance P-related peptides have not been examined extensively for actions affecting other biologically active compounds.

In the present study, we examined the ability of [DPro^4,DTrp^{7,9,10}]substance P-4-11, a substance P receptor antagonist [12], to modify the actions of various secretagogues on dispersed acini prepared from guinea pig pancreas. Our results indicate that [DPro^4,DTrp^{7,9,10}]substance P-4-11 is a specific, reversible receptor antagonist for substance P receptors, for bombesin receptors and for cholecystokinin (CCK) receptors.

Materials and Methods

Materials

Male NIH-strain guinea pig (175–225 g) were obtained from the Small Animal Section, Veterinary Resources Branch, National Institutes of Health. HEPES was from Boehringer Mannheim Biochemicals, Indianapolis, IN; purified collagenase (type CLSPA, 399 units/mg) was from Worthington Biochemical, Freehold, NJ; 8-bromoadenosine 3′,5′-cyclic monophosphate (8-Br-cAMP), carbamylcholine (carbachol), soybean trypsin inhibitor, theophylline and bacitracin were from Sigma Chemical, St. Louis, MO; basal medium (Eagle's) amino acids (100-times concentrated) were from Grand Island Biochemical, Grand Island, NY; essential vitamin mixture (100-times concentrated) was from Microbiological Associates, Bethesda, MD; Phadebas amylase test was from Pharmacia Diagnostics, Piscataway, NJ; bovine plasma albumin (fraction V) was from Miles Laboratories, Elkhart, IN; bombesin, secretin, substance P-4-11, substance P, C-terminal octapeptide of CCK (CCK-8), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), [Tyr^4]bombesin and [DPro^4,DTrp^{7,9,10}]substance P-4-11 were from Peninsula Laboratories, Belmont, CA; ^{125}I-Bolton-Hunter labeled CCK-8 (^{125}I-CCK-8; 2200 Ci/mmol), Na^{125}I and ^{125}I-Bolton-Hunter labeled substance P (^{125}I-substance P; 2200 Ci/mmol) were from New England Nuclear, Boston, MA.

Unless stated otherwise, the standard incubation solution contained: 24.5 Hepes (pH 7.4)/98 mM NaCl/6 mM KCl/2.5 mM NaH_2PO_4/5 mM sodium pyruvate/5 mM sodium fumarate/5 mM sodium glutamate/11.5 mM glucose/0.5 mM $CaCl_2$/2 mM glutamine/0.1% (w/v) soybean trypsin inhibitor/1% (v/v) amino acid mixture/1% (w/v) albumin/1% (v/v) vitamin mixture. The incubation solution was equilibrated with 100% O_2, and all incubations were performed with 100% O_2 as the gas phase.

Methods

Tissue preparation. Dispersed acini from guinea pig pancreas were prepared according to the modifications [13] of the procedure published previously [14].

TABLE I

EFFECT OF SUBSTANCE P-4-11 AND [DPro^4,DTrp^{7,9,10}] SUBSTANCE P-4-11 ON AMYLASE SECRETION STIMULATED BY VARIOUS SECRETAGOGUES

Pancreatic acini were incubated with the agents indicated for 30 min at 37° C. Values for amylase secretion are expressed as percent of amylase activity in acini at the start of incubation that was released into extracellular medium during incubation. In each experiment, each value was determined in duplicate and results given are means from at least four separate experiments.

Secretagogue	Amylase secretion (%)		
	alone	plus substance P-4-11 (30 μM)	plus [DPro^4,DTrp^{7,9,10}]-substance P-4-11 (30 μM)
None	2.9±0.7	7.7±1.8 *	4.0±1.0
CCK-8 (100 pM)	25.5±1.8	25.6±1.9	9.6±2.1 **
Bombesin (1 nM)	23.0±3.0	23.8±4.0	9.8±1.3 **
Substance P (3 nM)	8.1±0.7	8.9±1.3	4.3±0.3 **
Carbachol (30 μM)	25.6±2.8	25.7±1.8	29.7±3.9
VIP (1 nM)	20.3±1.0	23.5±1.3 *	20.9±5.0
Secretin (1 μM)	20.6±2.2	24.9±2.3 *	20.2±4.5
8-Br-cAMP (1 mM)	20.3±1.3	24.9±2.3 *	19.8±4.1
CGRP (100 nM)	6.2±1.5	10.9±2.0 *	6.1±1.9

* Significantly greater ($P < 0.05$) than the corresponding value for the secretagogue alone, by Student's paired t-test.

** Significantly less ($P < 0.05$) than the corresponding value for the secretagogue alone, by Student's paired t-test.

Amylase Secretion. Amylase secretion was measured using the procedure published previously [15]. Acini from one pancreas were suspended in 100 ml of standard incubation solution containing 5 mM theophylline. Incubations contained 250 μl of cell suspension and were at 37 °C for 30 min unless otherwise indicated. Amylase activity was determined using the Phadebas reagent as described previously [15]. Amylase secretion was calculated as the percentage of the amylase activity in the acini at the beginning of the incubation that was released into the extracellular medium during the incubation.

Preparation of ^{125}I-[Tyr^4]bombesin. Iodo-Gen (1 mg) was dissolved in 5 ml of chloroform and 5 μl of this solution (1 μg Iodo-Gen) was transferred to a vial and dried under N_2. Then 50 μl of 0.5 M K H_2PO_4 (pH 7.4), [Tyr^4]bombesin (6 μg in 6 μl H_2O) and Na^{125}I (1 mCi) were added and the mixture was incubated at 4 °C for 6 min. The reaction was stopped by adding 100 μl of 0.1% (v/v) aqueous TFA and then immediately leading the mixture on a Sep-Pak cartridge. The Sep-Pak was eluted with 0.1% (v/v) aqueous TFA prepared in 50% (v/v) acetonitrile/water. The eluate was incubated with dithiothreitol for 60 min at 80 °C and then loaded onto a fresh Sep-Pak and eluted with 50% acetonitrile: 0.1% (v/v) aqueous TFA. The eluate was placed under a gentle stream of N_2 for 30 min at 4 °C to remove acetonitrile and the ^{125}I-[Tyr^4]bombesin was purified using reverse-phase HPLC as described by Scemama et al. [16]. The purified ^{125}I-[Tyr^4]bombesin (spec. act. 2200 Ci/mmol) was adjusted to pH 7.4 using 0.2 M Tris (pH 9.5).

Binding of ^{125}I-CCK-8, ^{125}I-substance P and ^{125}I-[Tyr^4]bombesin. Dispersed acini from one pancreas were suspended in 5 ml of standard incubation solution. Acini (250 μl) were incubated with 41 pM ^{125}I-CCK-8 (100 000 cpm/ml), 41 pM ^{125}I-[Tyr^4]bombesin (100 000 cpm/ml) or 82 pM ^{125}I-substance P (200 000 cpm/ml) together with the appropriate concentrations of unlabeled peptide for 30 min at 37 °C. Samples (90 μl) were taken and the acini were washed three times by alternate centrifugation and resuspension as described previously [17]. Nonsaturable binding of ^{125}I-CCK-8, ^{125}I-substance P or ^{125}I-[Tyr^4]bombesin was the amount of radioactivity associated with the acini when the incubations contained 41 pM ^{125}I-CCK-8 plus 10 μM CCK-8, 82 pM ^{125}I-substance P plus 3 μM substance P or 41 pM

TABLE II

EFFECT OF FIRST INCUBATING PANCREATIC ACINI WITH [DPro^4,DTrp^{7,9,10}]SUBSTANCE P-4-11 ON THE SUBSEQUENT STIMULATION OF AMYLASE SECRETION CAUSED BY CCK-8 OR BOMBESIN

Pancreatic acini were suspended in standard incubation solution and amylase secretion was first measured during a 20-min incubation at 37 °C with the indicated agents (first incubation). Acini were then washed, resuspended in fresh incubation solution and incubated at 37 °C for 20 min with or without 0.1 nM CCK-8 or 1 nM bombesin (second incubation). Values for amylase secretion are expressed as percent of amylase activity in the acini at the start of the incubation that was released into the extracellular medium during incubation. In each experiment each value was determined in duplicate and results given are means ±1 S.D. from at least five separate experiments SP analogue, [DPro^4,DTrp^{7,9,10}]substance P-4-11.

1st Incubation		2nd Incubation	
additions	amylase secretion (%)	additions	amylase secretion (%)
None	2.8±0.9	None	2.1±0.7
		CCK-8 (100 pM)	17.1±1.8
		bombesin (1 nM)	15.0±1.0
CCK-8 (100 pM)	16.1±2.1	–	–
Bombesin (1 nM)	14.6±1.6	–	–
SP analogue (30 μM)	2.7±0.7	none	2.3±0.6
		CCK-8 (100 pM)	19.0±1.5
		bombesin (1 nM)	18.1±0.9
SP analogue (30 μM) +CCK-8 (100 pM)	7.1±1.3 *	none	2.0±0.5
		CCK-8 (100 pM)	16.4±3.4
SP analogue (30μM) +Bombesin (1 nM)	8.8±1.4 *	none	2.5±0.9
		bombesin (1 nM)	16.9±4.5

*Significantly less ($P < 0.05$) than the corresponding value for bombesin or CCK-8 alone by Student's paired *t*-test.

^{125}I-[Tyr^4]bombesin plus 10 μM bombesin. All values in the present paper are for saturable binding, i.e., binding measured with the labeled peptide alone (total binding) minus nonsaturable binding. In all experiments, nonsaturable binding was less than 20% of total binding.

Results

[DPro^4,DTrp^{7,9,10}]Substance P-4-11 did not stimulate amylase secretion, but inhibited the stimulation of amylase secretion caused by CCK-8, bombesin or substance P (Table I). [DPro^4, DTrp^{7,9,10}]Substance P-4-11 did not alter the stimulation of amylase secretion caused by carbachol, VIP, secretin, 8-Br-cAMP or CGRP (Table I). In contrast to [DPro^4,DTrp^{7,9,10}]substance P-4-11, substance P-4-11 stimulated amylase secretion, but with substance P-4-11 plus CCK-8, bombesin, substance P or carbachol, the value for amylase secretion was the same as it that with CCK-8 alone, bombesin alone, substance P alone or carbachol alone, respectively (Table I). The increase in amylase secretion caused by substance P-4-11 added to the increase caused by VIP, 8-Br-cAMP or CGRP (Table I).

Previous studies [1] have shown that certain analogues of substance P will inhibit the action of substance P and that this inhibitory action is reversible. In the present study, we examined the reversibility of the ability of [DPro^4,DTrp^{7,9,10}]substance P-4-11 to inhibit the action of CCK-8 or bombesin on amylase secretion. As illustrated in Table II, [DPro^4, DTrp^{7,9,10}]substance P-4-11 inhibited the stimulation of amylase secretion caused by CCK-8 or bombesin. When the acini were washed and then reincubated with CCK-8 or bombesin, the stimulation of amylase secretion was fully restored (Table II).

With increasing concentrations of substance P, amylase secretion increased and reached a plateau (Fig. 1A). Increasing concentrations of [DPro^4,

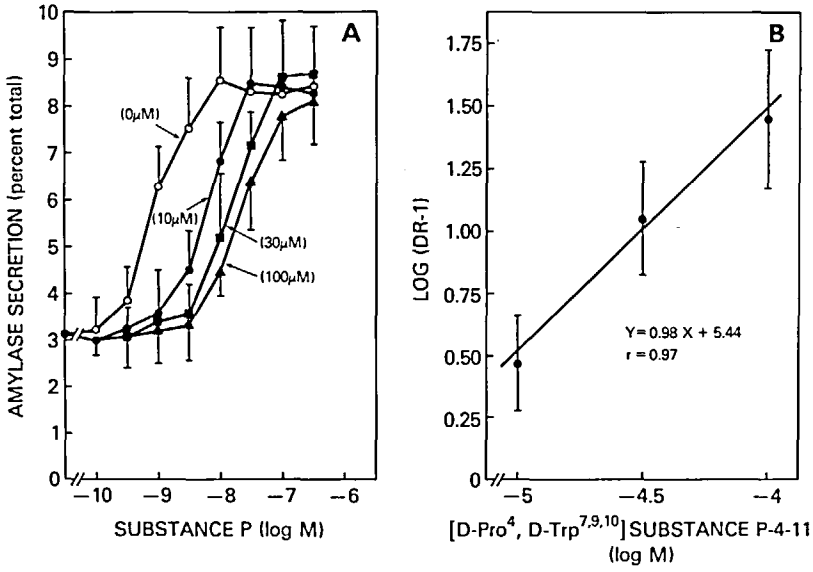

Fig. 1. (A) Effect of various concentrations of [DPro^4,DTrp^{7,9,10}]substance P-4-11 on the dose-response for substance P-stimulated amylase secretion. Acini were incubated with the concentrations of [DPro^4,DTrp^{7,9,10}]substance P-4-11 given in parentheses plus the indicated concentrations of substance P. Amylase secretion was measured during a 30-min incubation at 37 °C and is expressed as the percentage of amylase activity in the acini at the start of the incubation released into the extracellular medium during the incubation. In each experiment, each value was determined in duplicate and results given are means from five separate experiments. Vertical bars represent 1 S.D. (B) Results from the left panel are plotted in the form described by Schild [18]. DR, dose-ratio, i.e., the ratio of the concentration of substance P required to give half-maximal stimulation of amylase secretion in the presence of a given concentration of [DPro^4,DTrp^{7,9,10}]substance P-4-11 to the concentration of substance P required to give half-maximal stimulation of amylase secretion without [DPro^4,DTrp^{7,9,10}]substance P-4-11. The best fit to the line ($Y = aX + b$) and the correlation coefficient (r) were calculated by least-squares analysis. Vertical bars represent ± 1 S.D.

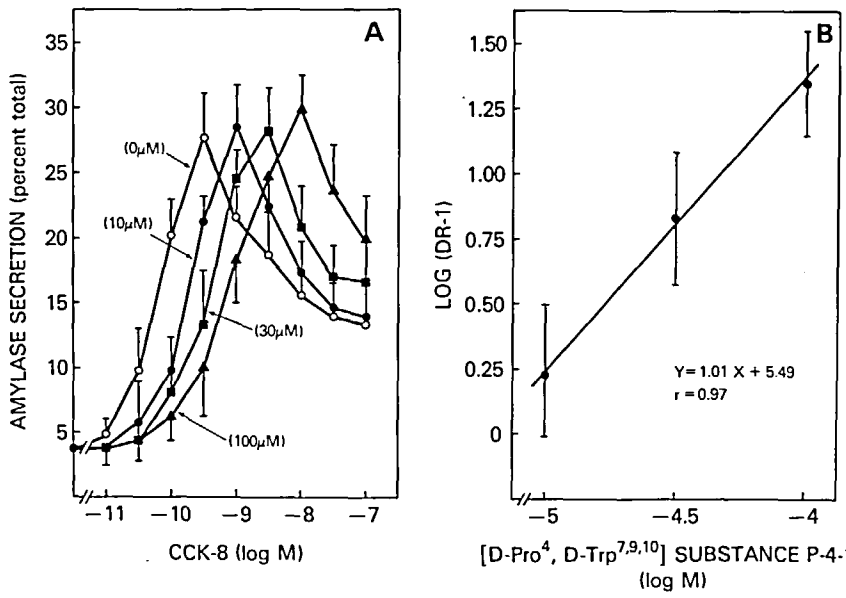

Fig. 2. (A) Effect of various concentrations of [DPro^4,DTrp^{7,9,10}]substance P-4-11 on the dose-response curve for CCK-8-stimulated amylase secretion. Acini were incubated with the concentrations of [DPro^4,DTrp^{7,9,10}]substance P-4-11 given in parentheses plus the indicated concentrations of CCK-8. Amylase secretion was measured during a 30-min incubation at 37 °C and is expressed as the percentage of amylase activity in the acini at the start of the incubation released into the extracellular medium during the incubation. In each experiment, each value was determined in duplicate and results given are means from five separate experiments. Vertical bars represent 1 S.D. (B) Results from (A) plotted in the form described by Schild [18]. DR, dose-ratio, i.e., the ratio of the concentration of CCK-8 required to give half-maximal stimulation of amylase secretion in the presence of a given concentration of [DPro^4,DTrp^{7,9,10}]substance P-4-11 to the concentration of CCK-8 required to give half-maximal stimulation of amylase secretion without [DPro^4,DTrp^{7,9,10}]substance P-4-11. The best fit to the line ($Y = aX + b$) and correlation coefficient (r) were calculated by least-squares analysis. Vertical bars represent ± 1 S.D.

DTrp^{7,9,10}]substance P-4-11 caused a progressive rightward shift in the dose-response curve for substance P-stimulated amylase secretion with no change in the maximal stimulation (Fig. 1A). A Schild plot [18] of these results gave a straight line with a slope that was not significantly different from unity (Fig. 1B). The value of K_i (concentration of antagonist required to occupy 50% of the receptor sites with no agonist present) calculated from the Schild plot was 2.8 ± 1.8 μM (mean ± 1 S.D.; $n = 5$).

With increasing concentrations of CCK-8 amylase secretion increased, became maximal and then decreased (Fig. 2A). Increasing concentrations of [DPro^4,DTrp^{7,9,10}]substance P-4-11 also caused a progressive rightward shift in the dose-response curve for CCK-8-stimulated amylase secretion with no change in the maximal stimulation (Fig. 2A). A Schild plot [18] of these results gave a straight line with a slope that was not significantly different from unity (Fig. 2B). The value of K_i calculated from the Schild plot was 3.7 ± 1.8 μM (mean ± 1 S.D.; $n = 5$). The value of K_i obtained with CCK-8 was not significantly different from that obtained with substance P ($P > 0.05$).

With increasing concentrations of bombesin, amylase secretion increased, became maximal and then decreased slightly (Fig. 3A). Increasing concentrations of [DPro^4,DTrp^{7,9,10}]substance P-4-11 caused a progressive rightward shift in the dose-response curve for bombesin-stimulated amylase secretion with no change in the maximal stimulation (Fig. 3A). A Schild plot [18] of these results gave a straight line with a slope that was not significantly different from unity (Fig. 3B). The value of K_i calculated from the Schild plot was 13.3 ± 2.1 μM (mean ± 1 S.D.; $n = 5$). The value of K_i was significantly greater ($P < 0.05$) than those obtained with substance P or CCK-8.

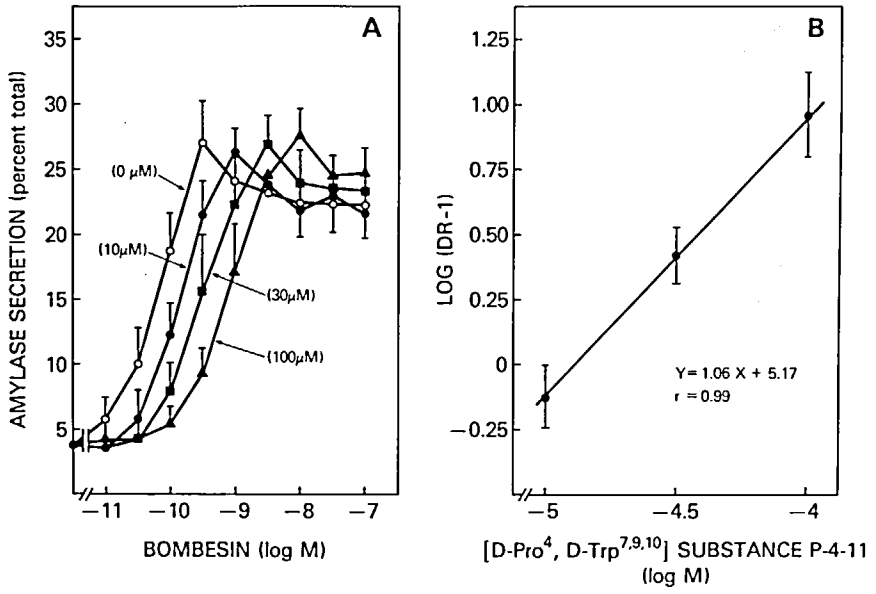

Fig. 3. (A) Effect of various concentrations of [DPro^4,DTrp^{7,9,10}]substance P-4-11 on the dose-response curve for bombesin-stimulated amylase secretion. Acini were incubated with the concentrations of [DPro^4,DTrp^{7,9,10}]substance P-4-11 given parentheses plus the indicated concentrations of bombesin. Amylase secretion was measured during a 30-min incubation at 37 °C and is expressed as the percentage of amylase activity in the acini at the start of the incubation that was released into the extracellular medium during the incubation. In each experiment, each value was determined in duplicate and results given are means from five separate experiments. Vertical bars represent 1 S.D. (B) Results from (A) are plotted in the form described by Schild [18]. DR, dose ratio, i.e., the ratio of the concentration of bombesin required to give half-maximal stimulation of amylase secretion in the presence of a given concentration of [DPro^4,DTrp^{7,9,10}]substance P-4-11 to the concentration of bombesin required to give half-maximal stimulation of amylase secretion without [DPro^4,DTrp^{7,9,10}]substance P-4-11. The best fit to the line ($Y = aX + b$) and the correlation coefficient (r) were calculated by least-squares analysis. Vertical bars represent ± 1 S.D.

The results in Figs. 1–3 indicate that [DPro^4,DTrp^{7,9,10}]substance P-4-11 functions as a receptor antagonist for the interaction of substance P, CCK-8 and bombesin with their respective receptors on pancreatic acini. To examine this possibility directly we measured the ability of [DPro^4,Trp^{7,9,10}]substance P-4-11 to inhibit binding of ^{125}I-substance P, ^{125}I-CCK-8 and ^{125}I-[Tyr^4]bombesin to pancreatic acini (Fig. 4). [DPro^4, DTrp^{7,9,10}]Substance P-4-11 inhibited binding of ^{125}I-substance P (Fig. 4, left), ^{125}I-CCK-8 (Fig. 4, center) and ^{125}I-[Tyr^4]bombesin (Fig. 4, right). When the data in Fig. 4 were analyzed with a digital computer and a nonlinear, least-squares, curve fitting program (LIGAND [19]) the inhibition of binding of each labeled peptide by [DPro^4,DTrp^{7,9,10}]substance P-4-11 was fit by a one-site model. A two-site model did not give a significantly better fit. The K_i value for inhibition of binding of ^{125}I-substance P was 3.8 ± 1.2 μM (mean $\pm$1S.D.; $n = 6$), for inhibition of binding of ^{125}I-CCK-8, it was 5.0 ± 1.3 μM (mean $\pm$S.D.; $n = 6$) and for inhibition of binding of ^{125}I-[Tyr^4]bombesin was 16.7 ± 5.5 μM (mean $\pm$1 S.D.; $n = 6$). The K_i values for inhibition of binding of ^{125}I-substance P and ^{125}I-CCK-8 were not significantly different ($P > 0.20$). The K_i value for inhibition of binding of ^{125}I-[Tyr^4]bombesin was significantly greater ($P < 0.05$) than those for inhibition of binding of ^{125}I-substance P and ^{125}I-CCK-8.

Discussion

The present results indicate that [DPro^4, DTrp^{7,9,10}]substance P-4-11 is a specific, competitive, reversible antagonist of the interaction of substance P, CCK and bombesin with their membrane receptors on pancreatic acini. [DPro^4, DTrp^{7,9,10}]substance P-4-11 inhibits the stimula-

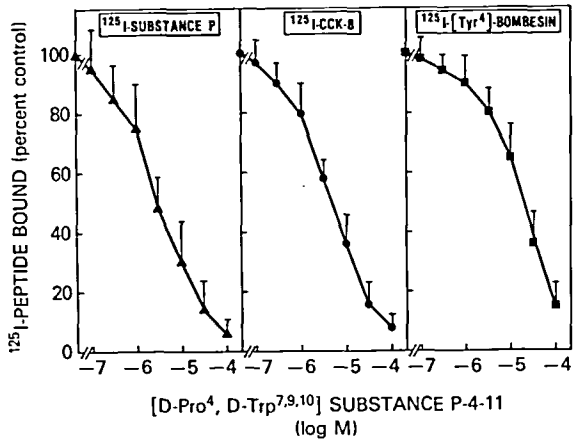

Fig. 4. Ability of [DPro^4,DTrp^{7,9,10}]substance P-4-11 to inhibit binding of ^{125}I-substance P, ^{125}I-CCK-8 and ^{125}I-[Tyr^4]bombesin. Acini were incubated for 30 min at 37°C with 82 pM ^{125}I-substance P, 41 pM ^{125}I-CCK-8 or 41 pM ^{125}I-[Tyr^4]bombesin plus the indicated concentrations of [DPro^4,DTrp^{7,9,10}]substance P-4-11. Saturable binding of the labeled peptide is expressed as the percentage of the saturable binding measured with labeled peptide alone. In each experiment, each value was determined in duplicate and results given are means from six separate experiments. Vertical bars represent 1 S.D.

tion of amylase secretion caused by substance P, CCK-8 and bombesin, but does not alter the stimulation caused by carbachol, VIP, secretin, 8-Br-cAMP or CGRP. This pattern of action of [DPro^4,DTrp^{7,9,10}]substance P-4-11 results from the D-amino acid substitutions, because substance P-4-11 itself does not inhibit the action of substance P, CCK-8 or bombesin (or any of the other agents tested) on amylase secretion. With substance P, CCK-8 and bombesin [DPro^4,DTrp^{7,9,10}]substance P-4-11 cause a progressive rightward shift in the dose-response curve for stimulation of amylase secretion with no change in the maximal response. Schild plots of these data give straight lines with slopes that are not significantly different from unity. When acini are first incubated with [DPro^4, DTrp^{7,9,10}]substance P-4-11 and then washed, there is complete reversal of the antagonistic effects of the peptide. Finally, direct evidence that [DPro^4, DTrp^{7,9,10}]substance P-4-11 is functioning as a receptor antagonist in that the peptide inhibits binding of ^{125}I-substance P, ^{125}I-CCK-8 and ^{125}I-[Tyr^4]bombesin to their specific receptors on pancreatic acini.

For inhibition of the action of a given peptide, there is a close correlation between the K_i value derived from the Schild plot and the K_i value determined from inhibition of binding of radiolabeled peptide. When we compared the K_i values for [DPro^4,DTrp^{7,9,10}]substance P-4-11 for inhibiting the action of each of the various peptides, the apparent affinity of [DPro^4, DTrp^{7,9,10}]substance P-4-11 for substance P receptors was the same as it was for CCK receptors and the apparent affinity of [DPro^4,DTrp^{7,9,10}]substance P-4-11 for substance P receptors and CCK receptors was approx. 6-times greater than the apparent affinity of [DPro^4,DTrp^{7,9,10}]substance P-4-11 for bombesin receptors.

When one examines the amino acid sequences of substance P, CCK-8 and bombesin [20], one can understand how [DPro^4,DTrp^{7,9,10}]substance P-4-11 might function as a substance P receptor antagonist, because it contains four amino acid identities with substance P. However, the ability of [DPro^4,DTrp^{7,9,10}]substance P-4-11 to function as an antagonist of the interaction of bombesin and CCK with their receptors does not have an obvious structural basis that can be appreciated from examining the amino acid sequences of [DPro^4, DTrp^{7,9,10}]substance P-4-11, bombesin and CCK-8.

It appears to be a general property of substance P receptor antagonists that they can also function as bombesin receptor antagonists [21]; however, the converse is not true. That is, the bombesin receptor antagonists described to date do not function as substance P receptor antagonists [21]. [DPro^4,DTrp^{7,9,10}]substance P-4-11, a substance P receptor antagonist, is also a bombesin receptor antagonist; however, this analogue of substance P-4-11, in contrast to other substance P receptor antagonists [21], also functions as a CCK receptor antagonist.

Previous studies [21] have demonstrated that there are four classes of CCK receptor antagonist: (1) derivatives of cyclic nucleotides, (2) derivatives of amino acids, (3) partial sequences and analogues of the C-terminal region of CCK and (4) derivatives of benzodiazepine. The present results

indicate that there is a fifth class of CCK receptor antagonist; namely, analogues of substance P-4-11.

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