IN VIVO EFFECTS OF FIVE SECRETIN ANALOGS ON FLUID AND POTASSIUM SECRETION FROM THE RAT PANCREAS

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(Received in final form February 4, 1980)

Summary

The importance of the N-terminal part of the secretin molecule for inducing fluid and potassium secretion from the pancreas was tested on anesthetized rats by comparing the biological capacity of bolus intravenous injections of secretin, secretin analogs, and the secretin (7-27) fragment. Except in one case, the relative potencies with which these peptides influenced fluid secretion correlated with the potencies on potassium secretion. [Glu³]secretin and [Asn³]secretin were 2-3 and 14 times less potent, respectively, than secretin. [Ala⁴]secretin, [D-Ala⁴]secretin and secretin were almost equipotent. [Val⁵]secretin was as potent as secretin on water secretion but 2-fold less potent on potassium secretion. Secretin (7-27) was at best a very weak agonist of secretin.

TABLE I

Comparison Of The 1-7 N-Terminal Amino Acid Sequence Of Porcine Secretin And Related Peptides.

POSITION :	1	2	3	4	5	6	7	(Symbol)
Secretin	HIS	SER	ASP ⁻	GLY	THR	PHE	THR	X
VIP	HIS	SER	ASP ⁻	ALA	VAL	PHE	THR	
Glucagon	HIS	SER	GLN	GLY	THR	PHE	THR	
[ASN ³]secretin	HIS	SER	ASN	GLY	THR	PHE	THR	□
[GLU ³]secretin	HIS	SER	GLU ⁻	GLY	THR	PHE	THR	○
[ALA ⁴]secretin	HIS	SER	ASP ⁻	ALA	THR	PHE	THR	▲
[D-ALA ⁴]secretin	HIS	SER	ASP ⁻	D-ALA	THR	PHE	THR	■
[VAL ⁵]secretin	HIS	SER	ASP ⁻	GLY	VAL	PHE	THR	●
Secretin (7-27)	-	-	-	-	-	-	THR	▽

Secretin stimulates the in vitro secretion of water, bicarbonate, and potassium (1) and the in vitro secretion of hydrolases from the rat pancreas (2,3). These effects are mediated by specific membrane receptors which are, at least partially, coupled to an adenylate cyclase system present in both

Abbreviation : VIP, vasoactive intestinal peptide.

duct (4) and acinar cells (5,6). The integrity of the secretin molecule is required for optimal efficacy and/or potency: the C-terminal secretin (5-27) fragment acts as an efficient competitive inhibitor of secretin but is at best a very weak partial agonist of secretin on both pancreatic flow rate (7) and amylase secretion (8). This points to the major biological importance of the N-terminal sequence of secretin in implementing the biological response. The present work compares the *in vivo* efficacy and potency of synthetic secretin, of five secretin analogs with a single amino acid substitution in position 3, 4 or 5, and of the secretin (7-27) fragment on fluid and potassium secretion from the rat pancreas.

FIG. 1

Time course of a single typical experiment in which pancreatic fluid (upper panel) and potassium (lower panel) secretions were recorded before and after four intravenous injections of [D-Ala⁴] secretin. Fluid output was expressed in $\mu\text{L}/5\text{ min}$ and potassium secretion was given by the ratio $K^+/Na^+ \times 100$. The amount of peptide injected is indicated above each arrow, which shows the corresponding time of injection. B represents the basal average unstimulated value and is the mean of data collected during the 20-min period preceding the first peptide injection.

Materials and Methods

After an overnight fast, male Wistar rats weighing 250-270 g were anesthetized with urethane (0.5 ml intraperitoneally of a 20% urethane solution per 100 g body weight) and provided with an acute pancreatic fistula as described by Dockray (9). A 30 min period was allowed for recovery. A calibrated capillary tube (total volume of 10 μL) was attached to the free end of the pancreatic duct cannula and the rate of flow of the pancreatic juice was recorded at 5-min intervals. The capillary tube was replaced after each collection period and the sample of juice in each tube was diluted 100-fold with water. After a 20-min basal period, a bolus injection of the tested peptide was administered in the jugular vein at 40-min intervals in a total volume of 0.2 ml saline. Each animal received four injections of increasing amounts of a single peptide.

FIG 2

Dose-response curves of pancreatic fluid secretion from anesthetized rats stimulated by 5 secretin analogs or by secretin (7-27). The volumes secreted before stimulation (Basal) and those secreted during the first 20-min period following peptide administration were plotted against the injected doses of X secretin, ■ [D-Ala⁴] secretin, ● [Val⁵]secretin, ▲ [Ala⁴]secretin, ○ [Glu³]secretin, □ [Asn³]secretin, and ▽ secretin (7-27). The curves are the means $\pm$ SEM of 6 experiments.

Potassium and sodium concentrations were measured by atomic absorption spectrophotometry using a 460 Perkin-Elmer apparatus. Dose-response curves of peptide stimulation were drawn by plotting the amount of peptide administered versus the biological response developing during the first 20 minutes following injection. Statistical analysis of the data was performed using the Student's *t* test.

Secretin, [Asn³]secretin, [Ala⁴]secretin, [D-Ala⁴]secretin, [Val⁵]secretin and the C-terminal secretin fragment (7-27) were synthesized by the fragment condensation method described previously (10,11). The amino acid sequence of the N-terminal portion of the peptides utilized is presented in Table 1 and is compared with the corresponding sequence in glucagon and VIP.

Results

1. Time-course of pancreatic flow rate and potassium secretion.

The bolus intravenous administration of secretin or secretin analogs elicited an immediate and transient hypersecretion of a potassium-enriched fluid. Taken as an example, the injection of increasing amounts of [D-Ala⁴] secretin increased the peak value as well as duration of each response (Fig. 1) but the secretion returned to near basal values before the next peptide injection, administered 40 min after the preceding one, the apparent half-life of peptide action being 5-10 min. The time-courses of the response obtained with secretin, the five secretin analogs, and secretin (7-27) were similar (data not shown).

FIG. 3

Dose-response curves of the K^+/Na^+ ratio in pancreatic juice from anesthetized rats stimulated by 5 secretin analogs or by secretin (7-27). The cation ratio was observed for 20 min before the first peptide injection (basal) and during each 20 min period following the injection of increasing doses of $\times$ secretin, $\bullet$ [D-Ala⁴]secretin, $\blacktriangle$ [Val⁵]secretin, $\triangle$ [Ala⁴]secretin, $\circ$ [Glu³]secretin, $\square$ [Asn³]secretin, and ∇ secretin (7-27). The curves are the means $\pm$ SEM of 6 experiments.

2. Dose-response curves of flow rate and potassium secretion.

In fasted anesthetized adult rats resting flow rates averaged 8 μ l/20 min/animal (Fig. 2). Secretin and the five substituted secretins exhibited various potency on flow rate but the same efficacy i.e. a similar maximal four-fold increase in fluid secretion. [D-Ala⁴]secretin, [Val⁵]secretin and secretin were equipotent. [Ala⁴]secretin [Glu³]secretin and [Asn³]secretin were, respectively, 2, 3, and 14 times less potent than secretin. The difference between the potency of [D-Ala⁴]secretin and [Ala⁴]secretin was at the limit of significance. The secretin (7-27) C-terminal fragment significantly increased fluid secretion at concentrations higher than 4096 pmol per animal but concentrations higher than 16 384 pmol could not be tested, due to the limited availability of this peptide.

Similar responses were observed for potassium secretion (Fig. 3). In unstimulated rats, the mean K^+ concentration in pancreatic juice was 4.5 mM and the average concentration of Na^+ was 133.0 mM so that the $K^+/Na^+ \times 100$ ratio was 3.4 (Fig. 3). These data accord with those previously reported by Sewell and Young (1). Synthetic secretin caused a 56 % increase in the K^+/Na^+ ratio (Fig. 3) due to a rise in K^+ concentration while the Na^+ concentration remained constant (not shown). The potency of [D-Ala⁴]secretin and [Ala⁴]secretin was not significantly different from that of secretin but the potency of [Glu³]secretin and [Val⁵]secretin was 2 times lower than that of secretin, and that of [Asn³]secretin was 14 times less potent. Secretin (7-27) again exerted a significant but weak activity at concentrations higher than 4096 pmol per rat.

Since secretin, [Ala⁴]secretin, [D-Ala⁴]secretin, [Glu³]secretin and [Val⁵]secretin exerted maximal effects at the dose of 2048 pmol/rat and [Asn³]secretin at doses in the 8192 - 16 384 pmol concentration range, the biological responses recorded in Figs. 2 and 3 were plotted as percentage of maximal effect versus the amount of peptide injected (Fig. 4). This clearly shows that both water and potassium secretions were stimulated with the same potency by each peptide, except for [Val⁵]secretin which stimulated water secretion with a greater potency than potassium secretion.

FIG. 4

Effects of increasing concentrations of secretin (X), [D-Ala⁴]secretin (■), [Ala⁴]secretin (▲), [Val⁵]secretin (●), [Glu³]secretin (○), and [Asn³]secretin (▽) on fluid (—) and potassium secretion (---) from anesthetized rat pancreas. The data are derived from Figs. 2 and 3 and are expressed in % of maximal responses in excess of the basal unstimulated values.

Discussion

The present study confirmed the importance of the N-terminal part of the secretin molecule for inducing pancreatic water secretion. The same was true for potassium secretion, a unique effect of secretin on the rat pancreas (1). The secretin (7-27) fragment was found to be a very weak agonist of secretin *in vivo* (Figs. 2 and 3) whereas this fragment acts only as a secretin antagonist on rat pancreatic membrane adenylate cyclase (12). It is not entirely clear whether secretin (7-27) exerted direct effects *in vivo* or whether this secretin fragment exerted a protective effect on endogenous secretin secreted by the anesthetized rat. Similar *in vivo* findings have been described (6,7,8) with the larger secretin fragment (5-27). Thus, secretin fragments lacking the four or six first amino acid residues are able to recognize pancreatic secretin receptors but are at best very poor activators of cellular effector mechanisms. By contrast, shorter secretin fragments such as secretin (14-27) are completely inactive (6).

The systematic investigation of the N-terminal portion of secretin indicated that the complete amino acid sequence, however unnecessary for recognition of secretin receptors, was required for full biological activity at low peptide concentration. Limited changes in the sequence of this part of secretin indeed sufficed to affect the potency of the hormone (Figs. 2 and 3). The substitution of aspartic acid residue in position 3 by the more hydrophobic glutamic acid provoked already a definite decline in biological activity, in line with similar data on fluid secretion from the dog pancreas (13). The neutralization of the acidic function of aspartic acid into asparagine markedly reduced the potency, as was already shown to be the case on fluid secretion from the isolated perfused cat pancreas, when aspartic acid is replaced by glutamine (the amino acid residue present in the 3 position of the parent peptide glucagon) (14).

The critical importance of aspartic acid in position 3 might reflect not only a real contribution to biological activity but also a stabilizing effect on the peptide structure. The lability of secretin in solution results indeed from the conversion of the [Asp³]peptide into a [β -Asp³]peptide (15). Glycine in position 4 favors this rearrangement. This is why analogs with a substitution of glycine in position 4 by alanine or D-alanine were synthesized and tested. Both analogs proved to be as active as secretin on water and electrolyte secretion (Figs. 2 and 3) but the time-study of their effects did not suggest any increase in the resistance of such peptides to *in vivo* degradation (data not shown). The present data on the rat pancreas were at variance with *in vivo* data on dog showing that [Ala⁴]secretin and [D-Ala⁴]secretin exhibit, respectively, 2 and 8 % only of secretin activity (13) and with *in vitro* data on guinea pig acinar cells (16) showing that [Ala⁴, Tyr¹⁰]secretin is 10-fold less potent than [Tyr¹⁰]secretin.

The substitution of threonine in position 5 by valine (17) gave a compound as active as secretin on rat pancreatic water secretion (Fig. 2) but 2-fold less potent than secretin on potassium secretion (Fig. 3). A possible explanation for this discrepancy is that flow rates may be activated through both secretin- and VIP-preferring receptors while K⁺ secretion might be stimulated through secretin-preferring receptors only. [Val⁵]secretin is indeed more VIP-like than secretin (Table 1) and might therefore recognize VIP-preferring receptors in addition to secretin-preferring receptors. In brain and adeno-hypophysis the preponderance of VIP-preferring sites is such that the adenylate cyclase membrane system is activated by [Val⁵]secretin as well as by VIP but not any more by secretin (18,19).

The present *in vivo* data differ qualitatively and quantitatively from those obtained *in vitro* on the adenylate cyclase system from rat pancreatic plasma membranes (12). In the latter system, secretin (7-27) is inactive, [Val⁵]secretin and secretin are equipotent, [Glu³]secretin, [Ala⁴]secretin, and [D-Ala⁴]secretin are 10-times less potent than secretin, and [Asn³]secretin is 100-times less potent. At least two explanations are conceivable: 1/ the *in vivo* effects of secretin and secretin-like peptides observed in the present study were essentially exerted on centroacinar and/or duct cells since secretin exerts inconsistent effects on protein secretion *in vivo* in anesthetized rats. By contrast, the *in vitro* effects previously documented originate mostly from rat plasma membranes derived from acinar cells which represent 80 % of the whole pancreatic cell population; 2/ distinct classes of secretin receptors coexist in a given cell type and, as for catecholamines, each class has its own recognition specificity. To differentiate between those two possibilities will require the use of a series of secretin analogs on homogeneous populations of pancreatic cells.

Acknowledgments

Aided by Grant 20,403 from the Fonds de la Recherche Scientifique Médicale (Belgium) and Grant RO-1AM-17010 from the National Institutes of Health (U.S.A.).

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