Pancreatic secretory factor (PSF), a protein from Gila monster venom stimulating enzyme secretion from rat pancreatic acini

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Pancreatic secretory factor (PSF), a 17.5-kDa protein purified from the venom of Gila monster (*Heloderma suspectum*), stimulated amylase secretion from dispersed rat pancreatic acini more efficiently than CCK-8, bombesin, carbachol and secretin, and without increasing $^{45}Ca^{2+}$ efflux and cyclic AMP levels. The secretory action was dependent on the presence of extracellular calcium and was additive to the secretion induced by agents acting via cyclic AMP or via Ca^{2+} efflux.

Gila monster venom *Pancreatic secretory factor* *Secretin* *Vasoactive intestinal peptide* *Cholecystokinin* *Bombesin* *Carbamylcholine* *Cyclic AMP* *Calcium efflux* *Stimulus–secretion coupling*

1. INTRODUCTION

The secretory action of muscarinic agents, CCK-8 and bombesin on rat pancreas is accompanied by net calcium efflux during the first minutes following addition of the secretagogue [1]. Secretin is also a good secretagogue and increases cyclic AMP levels while VIP, which is closely related to secretin, is only active on the second parameter. It has been reported that the venom of *Heloderma suspectum* contains a VIP-like component that increases amylase secretion as well as cyclic AMP levels in guinea pig pancreatic acini [2]. More recently, we tested the high secretory efficacy of the venom on dispersed rat pancreatic acini and came to the conclusion that, besides a secretin/VIP-like component increasing cyclic AMP, the venom also contained a factor implementing secretion [3]. This has now been confirmed by the purification of two components: helodermin and PFS [4]. Here, the secretory capacity of PSF was compared with its effects on cyclic AMP levels, calcium efflux, and its dependence on extracellular calcium.

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Abbreviations: PSF, pancreatic secretory factor; CCK-8, C-terminal octapeptide of cholecystokinin-pancreozymin

2. MATERIALS AND METHODS

2.1. *Materials*

PSF was purified from Gila monster venom, lot 42F-0747 from Sigma (St. Louis) as in [4]. The molar concentration of this protein was estimated with the Folin reagent [5] considering an M_r value of 17500 [4].

We acknowledge the generous gift of synthetic secretin from Dr W. König (Hoechst Aktiengesellschaft, Frankfurt/M.), synthetic VIP from Dr D.H. Coy (Tulane University, New Orleans, LA), synthetic C-terminal octapeptide of cholecystokinin-pancreozymin (CCK-8) from Dr J. Lucania (Squibb, Princeton, NJ), and bombesin from Dr R. de Castiglione (Farmitalia, Milano). Collagenase CLSPA was from Worthington (Freehold, NJ).

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2.2. *Methods*

The procedures used for preparing and incubating rat pancreatic acini, and for measuring amylase secretion have been described in [6]. The basal medium used in all experiments consisted of 24.5 mM Hepes (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM NaH_2PO_4, 1.0 mM $MgCl_2$, 0.5 mM $CaCl_2$, 11.5 mM glucose, 5 mM sodium fumarate, 5 mM sodium glutamate, 5 mM sodium pyruvate, 2 mM glutamine, 1% (w/v) amino acid mixture BME without L-glutamine (Gibco Europe, Uxbridge), 0.5 mM isobutylmethylxanthine, 1% (w/v) bovine serum albumin, and 0.01% Trasylol (500 kallikrein inhibitor units/ml).

^{45}Ca efflux was determined as follows: ^{45}Ca (2.5 μCi/ml) was included in the media used for preparing and washing the acini [6], i.e., for a 75 min period. The acini, preloaded with ^{45}Ca, were preincubated in an unlabelled incubation medium, containing 0.1 mM EGTA and no calcium for 45 min. The acini were then briefly washed and incubated in the same medium for 5 min, in the presence of the tested agents. An aliquot of the suspension was centrifuged for 15 s in a Beckman 152 microfuge. The radioactivity in the supernatant was counted. Controls taken at the beginning of each 5-min incubation period for determining the ^{45}Ca released into the medium at time zero, allowed appropriate corrections to be made.

Cyclic AMP levels were determined after a 30-min incubation of rat pancreatic acini at 37°C (under all conditions tested, cyclic AMP levels plateaued before 15 min: not shown). One ml cold ethanol was then added, and the suspension was centrifuged for 30 min at 2000 × *g*. The supernatant was evaporated and the residue was dissolved in 0.05 M acetate buffer (pH 6.2). Cyclic AMP was assayed using a cyclic AMP-^{125}I RIA Kit (New England Nuclear, Dreieich).

Lactate dehydrogenase released into the incubation medium was assayed after a 30-min incubation period as in [7]. Protein determination was performed using bovine serum albumin as a standard [5].

3. RESULTS

3.1. *Dose–response curves of amylase secretion*

CCK-8, bombesin and secretin, used as control peptides, dose-dependently stimulated amylase secretion from a basal rate of 2% of the total amylase content released in 30 min, to, respectively, 15, 14, and 12% (fig.1). As reported in [3], crude Gila monster venom, used at a 1 mg/ml concentration was a much better secretagogue since it increased secretion to a rate of 30%/30 min. The purified PSF also stimulated amylase secretion dose-dependently, the maximal concentration tested (0.4 μM) releasing 23%/30 min of the amylase content of acini. The secretory effect of PSF was observed within 5 min (the shortest time interval tested) and remained linear for at least 45 min (not shown). This secretory effect was also additive to that of helodermin, secretin, VIP, carbamylcholine, bombesin, and CCK-8 (fig.2).

The secretory effects of PSF and Gila monster venom did not merely reflect a deleterious influence, being, like those of other secretagogues tested, without consequence on LDH release (table 1).

3.2. *Dose–response curves on cyclic AMP levels*

Maximal concentrations of secretin (10 μM), VIP (10 μM), and crude Gila monster venom (1 mg/ml) increased cyclic AMP levels 27-fold, 20-fold and 14-fold, respectively. CCK-8 at maximal secretory concentration (3.2 nM) increased cyclic AMP levels by 150% only. PSF and

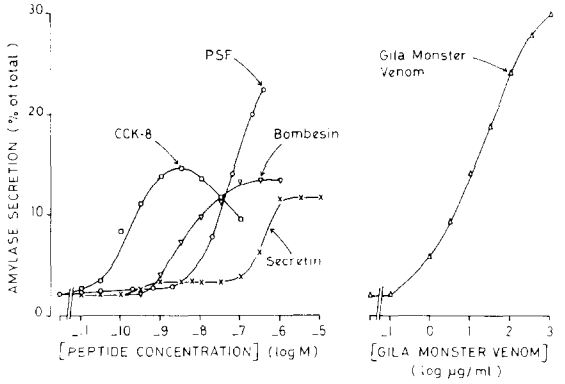

Fig.1. Dose–effect curves of stimulation of amylase release from rat pancreatic acini by PSF (○), CCK-8 (□), bombesin (▽) and secretin (×) (left panel) and by crude Gila monster venom (right panel). Acini were incubated for 30 min at 37°C. Each value was determined in duplicate. This experiment is representative of 3 others.

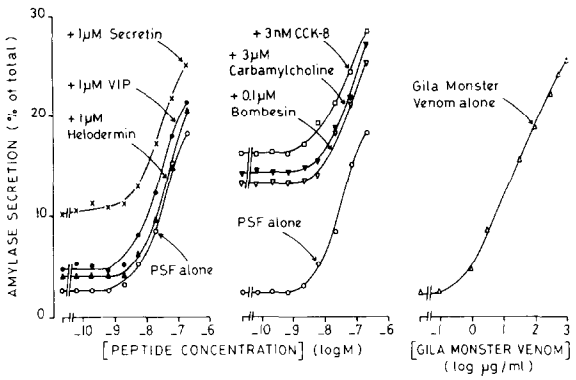

Fig.2. Dose–effect curves of stimulation of amylase release from rat pancreatic acini by PSF (○) alone or in the combined presence of 1 μM secretin (×), 1 μM VIP (●) or 1 μM helodermin [4,8] (▲) (left panel), 3 nM CCK-8 (□), 3 μM carbamylcholine (▼), 0.1 μM bombesin (▽) (middle panel) and by Gila monster venom (△) (right panel). Acini were incubated for 30 min at 37°C. Each value was determined in duplicate. This experiment is representative of 2 others.

bombesin were without effect on this parameter (table 1).

3.3. *Role of calcium in PSF action*

Rat pancreatic acini were preincubated for 45 min in the absence of calcium and with 0.1 M EGTA in order to deplete the intracellular stores of calcium. They were then incubated for 30 min in a similar medium with or without secretagogue. As shown in table 2, calcium depletion reduced, but did not abolish, the secretory response to CCK-8 and bombesin. These two peptides were also able to mobilize residual calcium (see section 2 and table 1). Markedly decreased secretion was also observed with secretin and VIP, two agents acting via cyclic AMP. Adding calcium during the incubation period did not restore a normal response to these secretagogues.

By contrast, the secretory response to PSF was totally suppressed after intracellular calcium depletion (table 2) and this protein was unable to mobilize residual calcium (table 1). The dependency of PSF action towards extracellular calcium was obvious when considering that calcium reintroduction in the incubation media fully restored the secretory response to PSF (table 2).

4. DISCUSSION

Previous studies showed that crude Gila monster venom increases amylase secretion and cyclic levels in guinea pig pancreatic acini. It also inhibits VIP and secretin binding, showing a higher affinity for VIP receptors. It was thus concluded that the venom contains a VIP-like component [2]. This hypothesis did not fit well with our preliminary results on rat pancreatic acini [3]. Indeed, the crude venom and VIP both increased cyclic AMP levels but the venom proved to be a most effective secretagogue while VIP only increased amylase secretion by a mere 40%. Besides, the venom induced a refractoriness period with respect to amylase secretion but not to cyclic AMP levels. We

Table 1

Effects of PSF, secretin, VIP, CCK-8, bombesin, and Gila monster venom on ^{45}Ca efflux (measured after 5-min incubations as described in section 2), cyclic AMP levels (measured after 30-min incubations), and lactate dehydrogenase release (measured after 30-min incubations) from rat pancreatic acini

	^{45}Ca efflux (% of total)	Cyclic AMP levels (pmol/mg protein)	LDH released (% of total)
Control	4.1 ± 0.6	6 ± 1	1.5 ± 0.2
0.2 μM PSF	4.2 ± 0.4	7 ± 2	1.1 ± 0.3
10 μM secretin	6.7 ± 1.0	164 ± 18	1.8 ± 0.4
10 μM VIP	9.0 ± 2.2	121 ± 12	1.2 ± 0.6
3.2 nM CCK-8	13.2 ± 1.3	16 ± 4	1.3 ± 0.3
0.1 μM bombesin	14.6 ± 0.3	6 ± 2	1.9 ± 0.2
Gila monster venom (1 mg/ml)	5.0 ± 1.1	85 ± 10	2.5 ± 0.3

Results are the means ± SE of at least 4 experiments

Table 2

Effects of extracellular calcium on the stimulation of amylase release from rat pancreatic acini

	A	B	C
Preincubation:	0.5 mM Ca^{2+}	0.1 mM EGTA	0.1 mM EGTA
Incubation:	0.5 mM Ca^{2+}	0.1 mM EGTA	0.5 mM Ca^{2+}
Control	1.0 ± 0.4	0.8 ± 0.2	0.6 ± 0.2
0.2 μM PSF	19.3 ± 1.3	0.9 ± 0.3	19.7 ± 0.8
10 μM secretin	11.9 ± 0.7	2.1 ± 0.6	4.2 ± 1.0
10 μM VIP	4.1 ± 1.0	1.1 ± 0.2	1.2 ± 0.2
3.2 nM CCK-8	15.4 ± 2.4	5.6 ± 1.0	5.3 ± 0.7
0.1 μM bombesin	13.3 ± 1.4	3.7 ± 0.8	3.3 ± 0.6
Gila monster venom (1 mg/ml)	30.2 ± 2.8	12.8 ± 1.7	30.8 ± 1.3

Acini were: (A) preincubated for 45 min at 37°C in standard medium containing 0.5 mM Ca^{2+}, then incubated in the same medium; (B) preincubated for 45 min without added Ca^{2+} and with 0.1 mM EGTA, then washed and incubated for a further 30 min period in the same medium; (C) preincubated for 45 min without added Ca^{2+} and with 0.1 mM EGTA, then incubated with 0.5 mM Ca^{2+}. Results are expressed as % of total amylase present in the acini released in 30 min and are the means ± SE of 4 experiments

therefore postulated that the agent stimulating amylase secretion from rat pancreatic acini was probably distinct from the VIP/secretin-like component [3].

This has not been confirmed by separating PSF from 'helodermin', the newly isolated VIP/secretin-like component [4,8] from crude Gila monster venom. Here, PSF is indeed shown to stimulate amylase secretion without affecting cyclic AMP levels. Furthermore, PSF, at variance with crude venom, does not inhibit [^{125}I]VIP binding to rat acini (not shown).

Having ruled out cyclic AMP as a second messenger of PSF in stimulus–secretion coupling, we explored whether calcium mobilization from intracellular pool(s) and/or the uptake of extracellular calcium were involved [9].

After a 45 min preincubation period, without added calcium and in the presence of 0.1 mM EGTA (a treatment aimed at decreasing intracellular calcium [10]), CCK-8 and bombesin still stimulated the release of amylase probably because the peptides were able to mobilize enough residual calcium from intracellular pool(s) resisting depletion. In agreement with data in [11], the maximal effects of these peptides were, however, reduced. A marked reduction in the secretory efficacy of secretin and VIP, two agents capable of elevating cyclic AMP levels without increasing $^{45}Ca^{2+}$ efflux, was observed, indicating that exogenous calcium was required to support distal step(s) in stimulus–secretion coupling [12]. The fact that calcium addition, during the incubation period, could not restore a normal response to these various secretagogues suggests that the hormone-stimulated discharge depended, initially, on the release of intracellular Ca^{2+}, with a shift to extracellular Ca^{2+} dependence, once appropriate intracellular calcium store(s) were depleted [12]. When compared to these classical secretagogues, PSF exerted distinct effects on calcium movements. The preincubation and incubation of acini in a calcium-free medium totally blocked the secretory response to PSF. This protein was also unable to mobilize residual calcium. Indeed, PSF was without effect on ^{45}Ca efflux. The dependency towards extracellular calcium was established by the fact that a full response to PSF was observed when a preincubation period, in a calcium-free medium, was followed by an incubation in the presence of calcium.

Up to now, the role of extracellular calcium in PSF action is only conjectural:

(i) PSF might act as an ionophore and facilitate calcium translocation from the extracellular medium to the cytosol [14]. This is unlikely, however, when considering the high M_r of PSF;

(ii) PSF might stimulate the uptake of extracellular Ca^{2+} by activating calcium channels. Such channels have not yet been demonstrated in rat exocrine pancreas by patch clamp experiments [15];

(iii) Extracellular Ca^{2+} might be solely necessary for PSF binding to plasma membranes. This hypothesis implies that PSF could stimulate amylase secretion independently of intracellular increases of either cyclic AMP or calcium. Such an hypothesis has already been formulated with respect to the secretory effect of high concentrations of secretin (in preparation) and of phorbol esters [13]. The fact that the secretory action of PSF was additive to the effects of either secretin and VIP (which increase cyclic AMP) or bombesin and CCK-8 (which increase calcium movements) (fig.2) favors this hypothesis.

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