CHARACTERIZATION OF IMMUNOREACTIVE COMPONENTS OF INSULIN AND SOMATOSTATIN IN CANINE PANCREATIC JUICE

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Received 21 May 1979

1. Introduction

Recent reports [1–4] of the detection by radioimmunoassay of the polypeptide hormones gastrin and somatostatin in gastric and duodenal juice suggested the possibility of the release of pancreatic hormones into the lumen of the pancreatic duct. Indeed, preliminary studies have demonstrated the presence of immunoreactive pancreatic polypeptide in human pancreatic juice [5] and immunoreactive insulin and glucagon in canine pancreatic juice [8]. Pancreatic juice, however, contains high concentrations of proteolytic enzymes and their zymogens, that can damage the labeled antigen or antibody used in radioimmunoassays [7] and result in artifactual hormone immunoreactivity. This study was, therefore, designed to isolate and characterize the immunoreactive components of the islet hormones, insulin, glucagon, somatostatin and pancreatic polypeptide in canine pancreatic juice.

2. Materials and methods

Secretin was obtained from the GIH Laboratory (Karolinska Institute, Stockholm), the synthetic C-terminal octapeptide of cholecystokinin (OP-CCK) (Kinevac®) from E. R. Squibb and Sons (Princeton, NJ) and phenylmethylsulphonyl fluoride from Sigma Chemical Co. (St Louis, MO).

Pancreatic juice was collected from fasted laparotomized dogs ($N = 5$) through a polyethylene cannula inserted in the pancreatic duct ~1 cm from the duodenum. Flow of juice was stimulated by a 30 min intravenous infusion of secretin at a dose (2 CU $.kg^{-1}$ $.h^{-1}$) shown [8] to produce maximal secretion of bicarbonate. This was followed by a 30 min infusion of the same dose of secretin supplemented with OP-CCK at a dose (0.5 μg $.kg^{-1}$ $.h^{-1}$) shown [8] to produce near maximal secretion of pancreatic enzymes. The juice from both periods of secretion was collected into chilled tubes containing acetic acid and an inhibitor of active site serine proteases, phenylmethylsulphonyl fluoride (0.1 ml of a 1% w/v solution/ml juice). The samples of juice were lyophilized and the residue reconstituted in 0.1 M ammonium bicarbonate buffer (pH 8.8) containing Trasylol (1000 KIU/ml) and EDTA (1.2 mg/ml). After centrifugation at 3000 rev./min for 15 min, the supernatant was subjected to gel filtration on columns (60 × 2.5 cm) of Biogel P-10 using 0.1 M ammonium bicarbonate buffer (pH 8.8) for elution. Eluant fractions containing immunoreactive components were again lyophilized and subjected to isoelectric focusing at 4°C in ampholine containing sucrose gradients using an LKB Model 8100 column as in [9]. The pH of the effluent fractions was measured at 25°C using a Beckman Model 3550 pH meter. pI values at 4°C of immunoreactive components in the fractions were calculated according to [10]. Immunoreactive insulin (IRI) in the chromatographic effluent fractions was measured by the method in [11] using

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an antibody which reacts with both insulin and proinsulin. Immunoreactive somatostatin (IRSS) was measured using Arimura antiserum R101, which is directed against the central residues of somatostatin [12]. Immunoreactive glucagon (IRG) was measured using antiserum 30K by the method in [13]. Immunoreactive pancreatic polypeptide (IRPP) was measured using an antiserum raised against human pancreatic polypeptide [14].

3. Results and discussion

Infusion of secretin resulted in an increase in flow of pancreatic juice from < 0.1 ml/min in the basal state to 0.5 ± 0.2 ml/min and the flow was further increased to 0.8 ± 0.2 ml/min when this was supplemented with OP-CCK. Infusion of OP-CCK resulted in a marked increase in the protein content of the juice in all experiments. The elution profile of the IRI in the juice from a representative gel filtration experiment is shown in fig.1A. Low levels of IRI were observed in the chromatograms of secretin-stimulated juice and this material was eluted from the column as a single peak with the same elution volume as a porcine insulin marker (shaded area). Infusion of OP-CCK resulted in an increase in the IRI content of the juice in 4 out of 5 experiments and this material was eluted as a broad peak with maximum immunoreactivity at the elution volume of porcine insulin (open area). The IRI in the OP-CCK-stimulated juice was isoelectrically focused (fig.1B) into a major peak in the same pH region as insulin (pI 5.7) and into a minor peak in the same pH region as proinsulin (pI 4.8) [15].

IRSS was not observed in any sample of secretin-stimulated juice but was detected in OP-CCK-stimulated juice in 4 out of 5 experiments. The elution

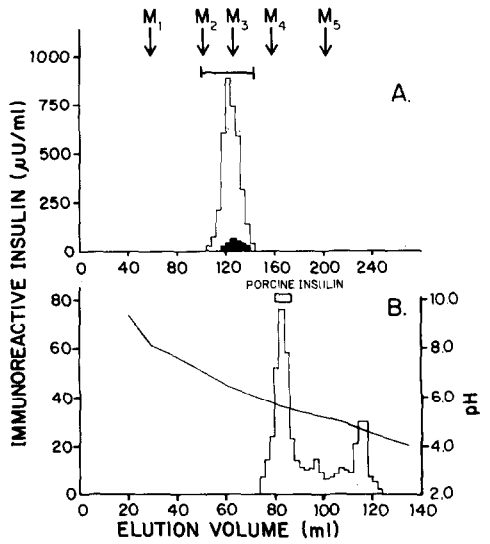

Fig.1. (A) Gel filtration on Biogel P-10 columns at pH 8.8 of IRI from pancreatic juice after stimulation with secretin (shaded area) and with secretin + OP-CCK (unshaded area). The arrows indicate the elution volumes of blue dextran (M_1), cytochrome *c* (M_2), porcine insulin (M_3), porcine glucagon (M_4) and synthetic somatostatin (M_5). Fractions indicated by (⊢⊣) were pooled, lyophilized and subjected to isoelectric focusing. (B) Isoelectric focusing in ampholine-containing sucrose gradients of IRI isolated from secretin + OP-CCK-stimulated pancreatic juice by gel filtration. (—) indicates the pH of the fractions at 25°C and (□) indicates the fractions in which porcine insulin was focused.

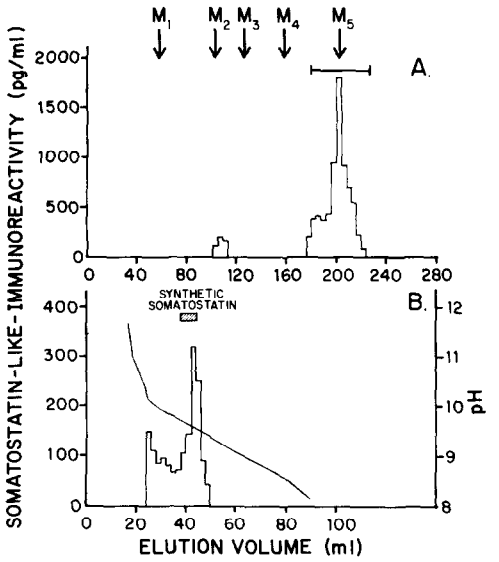

Fig.2. (A) Gel filtration on Biogel P-10 columns at pH 8.8 of IRSS from pancreatic juice after stimulation with secretin + OP-CCK. The molecular weight markers are as in fig.1A and the fractions indicated by (⊢⊣) were pooled, lyophilized and subjected to isoelectric focusing. (B) Isoelectric focusing in ampholine-containing sucrose gradients of IRSS isolated from secretin + OP-CCK-stimulated pancreatic juice by gel filtration. (—) indicates the pH of the fractions at 25°C and (▨) indicates the fractions in which synthetic somatostatin was focused.

profile of IRSS in the juice from a representative experiment is shown in fig.2A. IRSS was eluted as a broad, incompletely resolved peak with maximum immunoreactivity at the elution volume of synthetic somatostatin, together with a minor peak in the $10-12 \times 10^3$ mol. wt zone. The major peak of immunoreactivity was isoelectrically focused (fig.2B) into a component in the same pH range as synthetic somatostatin (pI 10.3–10.5) together with a minor component at higher pH (pI 10.6–10.8). While the predominant molecular form of IRSS in canine pancreatic tissue [9] and in plasma-free pancreatic venous effluent of dogs [16] is indistinguishable from synthetic somatostatin, the existence of larger and more basic components of IRSS in canine pancreas [9] and porcine gut [17] and hypothalamus [18] has been reported.

Serial dilutions of the components of IRSS and IRI isolated from the pancreatic juice were proportional and their dilution slopes were parallel to the radioimmunoassay standards when incubated with antisera under conditions of radioimmunoassay (fig.3). It is concluded, therefore, that the predominant molecular forms of IRI and IRSS in pancreatic juice are indistinguishable from the corresponding components in pancreatic tissue with respect to molecular size, charge and immunometric properties.

The rate of release of IRI into the juice was estimated from the area of the peaks. It rose from a basal level of 4.8 ± 2.8 μU/min (mean ± SEM) (range 0.8–16 μU/min) during stimulation with secretin and to 103 ± 91 μU/min (mean ± SEM) (range

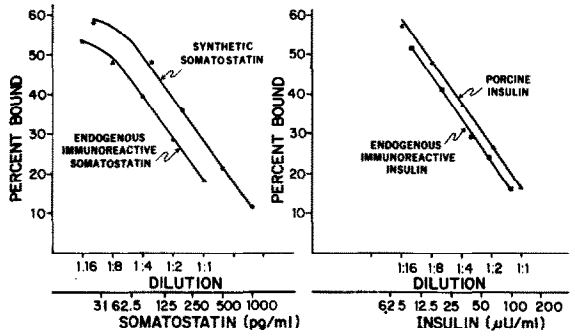

Fig.3. Dilution slopes in the radioimmunoassay of components of IRI and IRSS isolated from secretin + OP-CCK-stimulated pancreatic juice by gel filtration and isoelectric focusing.

1.4–470 μU/min) during stimulation with OP-CCK. The rate of release of IRSS into the OP-CCK-stimulated juice was estimated at 291 ± 184 pg/min (mean ± SEM) (range 0–973 pg/min). The variation in individual secretory rate of the 5 dogs was very large and the mean rate of release in response to a pharmacological dose of OP-CCK represents < 5% of the corresponding secretion rate of the hormones into the pancreatic venous effluent [19].

Although radioimmunoassay of unfractionated pancreatic juice from both periods of secretion suggested the presence of IRG and IRPP, examination of the eluant fractions from the gel filtration experiments failed to reveal immunoreactive material with the same molecular size as glucagon or pancreatic polypeptide in either secretin-stimulated or OP-CCK-stimulated juice. Serial dilutions, under radioimmunoassay conditions, of the apparent IRG in the juice were not proportional and the IRG associated with the juice could not be removed by affinity chromatography on columns of immobilized antibodies specific for glucagon, prepared according to [20]. It is possible, therefore, that the apparent glucagon-like and pancreatic polypeptide-like immunoreactivity in the juice results, at least in part, from non-specific interference in the radioimmunoassay by components in the juice.

The source of the pancreatic hormones present in the juice and their physiologic significance, if any, is not apparent from these results. OP-CCK is a powerful stimulus for the release of IRI and IRSS [21] into the circulation and it is possible that the hormones in the juice are derived from direct flow from the islets to the ductules and/or derived from the endocrine or 'intermediate cells' [22] found throughout the exocrine parenchyma and in the lining of the pancreatic duct of a variety of species including the dog [23]. A recent report [24], in which intraduodenally instilled endogenous and exogenous secretin resulted in an increase in pancreatic bicarbonate secretion in dogs, raises the possibility the hormones in the pancreatic juice may fulfill a physiological role.

Acknowledgements

This work was supported by VA Institutional Research Support Grant 549-8000-01; National Institutes of Health Grants AM 02700-16, 1-R01-AM

18179 and 1-M01-RR 0063; and contract N01-AM-62219; CIBA-Geigy Corp., Ardsley, NY; Eli Lilly and Company, Indianapolis, IN; Dr Karl Thomae GmbH, FRG; Bristol Myers, New York, NY; and the Salk Institute-Texas Research Foundation, Houston, TX. J. Michael Conlon is the recipient of a Medical Research Council (London) Traveling Fellowship. The authors wish to acknowledge the excellent technical help of Loretta Clendenen, John Diffie, Helen Gibson, Virginia Harris, Kay McCorkle, Lovie Peace and Danny Sandlin. For secretarial assistance the authors express their thanks to Susan Freeman and Jessie Reese.

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