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Effects of Pancreozymin and Secretin on Insulin Release and the Role of the Exocrine Pancreas

Å. Danielsson and Å. Lernmark

Department of Histology, University of Umeå, Umeå, Sweden

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Summary. Mouse pancreatic islets microdissected free from, or surrounded by, exocrine cells were used to study the effects of secretin and cholecystokinin-pancreozymin on insulin release. Both secretin and cholecystokinin-pancreozymin potentiated glucose-stimulated insulin release regardless of whether exocrine cells were present. The results fail to support the idea that the presence of the exocrine parenchyma is essential for elicitation of insulin release by these gastrointestinal hormones.

Key words: Insulin release, mouse islets, secretin, pancreozymin, exocrine cells.

It is not clear whether the intestinal hormones secretin and cholecystokinin-pancreozymin (CCK-PZ) are able to elicit insulin release by a specific interaction with the pancreatic B-cells. Although these hormones are generally regarded as insulinotropic *in vivo* and *in vitro*, it has been suggested that the stimulatory action of secretin is mediated by the exocrine pancreatic cells. Thus it was reported [1, 2] that secretin stimulated insulin release only from pieces of pancreas and not from isolated islets or from pieces of duct-ligated pancreas. In a similar study with isolated rat islets [3], in rats with exocrine insufficiency [4] and in patients suffering from diseases of the exocrine pancreas [5] some support was obtained for the hypothesis that secretin stimulates insulin release via the surrounding exocrine pancreatic cells.

In the present study an attempt was made to test this hypothesis by studying the effects of CCK-PZ and secretin on the release of insulin from mouse islets microdissected free from, or surrounded by, exocrine cells.

Materials and Methods

Cholecystokinin-pancreozymin (CCK-PZ) (3 Ivy dog units/μg) and secretin (3.5 clinical units/μg) were gifts from Professor Viktor Mutt, Karolinska institutet, Stockholm, Sweden. Both preparations were essentially pure and did not contain gastric inhibitory polypeptide (Mutt, personal communication). Farbwerke Hoechst AG, Frankfurt/Main, Germany, supplied ^{125}I-insulin and Wellcome Reagents Ltd., Beckenham, Kent, England supplied insulin antiserum. Mouse insulin was prepared by Novo industry A/S, Copenhagen, Denmark. Human serum albumin, grade A (HSA) was obtained from AB Kabi, Stockholm, Sweden, and the kallikrein inhibitor Trasylol® was donated by Bayer AG., Leverkusen, Germany.

Islets of similar size were microdissected [6] from the pancreases of starved ob/ob-mice, taken from a local colony. The islets were either dissected free of the exocrine parenchyma or a narrow zone of exocrine cells was allowed to remain around them. The basal medium used was Krebs-Ringer bicarbonate buffer [7], equilibrated with O_2-CO_2 (95:5) and supplemented with 3 mM glucose, 1 mg/ml HSA and 500 KIU/ml Trasylol. All islets were subjected to a prestimulatory period of 30 min at 37° in the basal medium. Two islets per vessel were then incubated in 300 μl medium for 30 min (period 1) and, after transfer to a new vessel containing 300 μl fresh medium, for a further 30 min period (period 2). This procedure permitted the use of each pair of islets as its own control. The detailed composition of the incubation media is given in the legends to the tables. The islets were freed of contaminating fluid and frozen in isopentane cooled to its melting point (−160°). After freeze-drying overnight, the islets were weighed on a quartz fiber balance. Samples of incubation media were assayed radioimmunologically for insulin. Ethanol was used to separate free and antibody-bound insulin [8]. Crystalline mouse insulin was used as standard.

The amounts of insulin released have been expressed as ng insulin released per μg tissue dry weight. Statistical significance was judged from the mean differences between periods 1 and 2 with islets from the same animals in a series of repeated experiments.

Results

The release of insulin from islets both with and without surrounding exocrine cells was stimulated by 10 mM glucose (Table 1 and 2). It was ascertained that there was no difference in the amounts of insulin released when islets were incubated in the presence of the same glucose concentration during periods 1 and 2. Islets without exocrine cells incubated with 10 mM glucose were found to release 1.51 ± 0.44 and 1.47 ± 0.34 ng insulin per 30 min and μg dry weight during periods 1 and 2 respectively (mean values $\pm$ SEM

for six different experiments). Lower insulin values were encountered in the media containing islets with remaining exocrine cells. The mean dry weight of these specimens was 2.2±0.1 times (mean values ± SEM for 31 observations) that of islets devoid of exocrine tissue. Even after correction for this weight difference the insulin release from islets with exocrine cells during the first incubation period was about 60% lower than that recorded for islets devoid of exocrine cells. It is obvious that although 500 KIU/ml Trasylol was added to all incubation media the mere presence of exocrine cells led to a considerable loss of released insulin. Since corrections for weight increase due to exocrine tissue or for insulin loss did not change the relative effect of 10 mM glucose, uncorrected values have been presented in the Tables.

Table 1 shows the effects of secretin on the insulin release from islets with or without surrounding exocrine tissue. Secretin had no effect at a low glucose concentration, but potentiated insulin release induced by 10 mM glucose. The effect was significant for islets devoid of exocrine tissue, but was less marked when this tissue was present.

Table 2 presents a similar experiment with CCK-PZ. The insulin release at 3 mM glucose from islets with or without surrounding exocrine cells was unaffected by CCK-PZ. A significant potentiation of the glucose-induced insulin release was recorded from the islets regardless of whether exocrine cells were present.

Table 1. *Effects of secretin on insulin release from isolated islets with or without exocrine cells*

Isolated islets	Glucose concn.	Dry weight (µg)	Insulin release (ng/µg dry weight, 30 min) Period 1	Period 2 (0.1 U/ml Secretin)	Difference
Without exocrine cells	3 mM	9.5±0.3 (8)	0.42±0.09 (8)	0.59±0.16 (8)	0.17±0.12 (8)
With exocrine cells	3 mM	18.7±1.5 (7)	0.06±0.01 (7)	0.08±0.02 (7)	0.02±0.01 (7)
Without exocrine cells	10 mM	9.7±0.6 (8)	1.62±0.34 (8)	2.93±0.35 (8)	1.31±0.20[a] (8)
With exocrine cells	10 mM	18.5±1.2 (8)	0.15±0.02 (8)	0.27±0.08 (8)	0.12±0.07 (8)

Following the prestimulatory period, islets with or without exocrine cells were first incubated for 30 min (period 1) and then transferred to a new vessel for a further 30 min incubation (period 2). The media in periods 1 and 2 were supplemented with glucose and secretin as indicated. Mean values ± SEM for the number of experiments shown within parentheses. [a] Effect of secretin: $P < 0.001$

Table 2. *Effects of CCK-PZ on insulin release from isolated islets with or without exocrine cells*

Isolated islets	Glucose concn.	Dry weight (µg)	Insulin release (ng/µg dry weight, 30 min) Period 1	Period 2 (0.75 U/ml CC K-PZ	Difference
Without exocrine cells	3 mM	9.3±0.4 (10)	0.52±0.11 (10)	0.40±0.07 (10)	0.12±0.07 (10)
With exocrine cells	3 mM	24.0±3.8 (10)	0.11±0.05 (10)	0.22±0.11 (10)	0.11±0.06 (10)
Without exocrine cells	10 mM	8.6±0.5 (6)	1.81±0.25 (6)	3.25±0.59 (6)	1.44±0.39[a] (6)
With exocrine cells	10 mM	17.1±0.5 (6)	0.30±0.06 (6)	0.68±0.10 (6)	0.38±0.11[a] (6)

Following the prestimulatory period, islets with or without exocrine cells were first incubated for 30 min (period1) and then transferred to a new vessel for a further 30 min incubation (period 2). The media in periods 1 and 2 were supplemented with glucose and CCK-PZ as indicated. Mean values ± SEM for the number of experiments shown within parentheses. [a] Effect of pancreozymin: $P < 0.02$

Discussion

The dispersal of the islets of Langerhans in the exocrine pancreas has raised the question whether there are functional relationships between these two parts of the pancreas. Insulin has been shown to inhibit amylase secretion from incubated mouse pancreas [9], and to increase the total amylase content *in vivo* [10]. Administration of insulin *in vivo* stimulates incorporation of labelled amino acids into pancreatic amylase [11]. It is interesting to note that the exocrine cells surrounding the islets display special morphological characteristics, with more prominent granulation [12, 13] than exocrine cells located farther away from the islets. These characteristics have been attributed to a local high concentration of insulin [13, 14]. In the present study advantage was taken of the fact that the relatively large islets of obese-hyperglycemic mice can be microdissected free from, or surrounded by a zone of exocrine cells [15].

Hormones from the gastrointestinal mucosa are the main physiological stimulus for the exocrine pancreatic secretion. However, these hormones have

also been reported to cause insulin secretion [16]. In the present study both secretin and CCK-PZ were found to potentiate glucose-stimulated insulin release regardless of whether exocrine cells were present. The presence of exocrine cells decreased the amount of insulin in the incubation media. In spite of this, the relative effects of the hormones and glucose were found to be the same for the two groups. It therefore seems reasonable to assume that CCK-PZ and secretin directly affect the islet cells.

The present results are at variance with previous findings concerning the effects of gastrointestinal hormones on insulin release. Hinz *et al.* [3] and Goberna *et al.* [4] found a difference between CCK-PZ and secretin in their ability to elicit insulin release. CCK-PZ stimulated insulin release both *in vivo* in duct-ligated rats and from isolated rat islets, but secretin had no effect on insulin release in rats with pancreatic insufficiency or from isolated islets; in rats with intact exocrine pancreas secretin stimulated insulin release. In contrast to the findings of Raptis *et al.* [5] both CCK-PZ and secretin stimulated insulin release in patients with chronic pancreatitis, although the same patients exhibited an impaired secretory response to glucose [17]. Our data are consonant with the latter observations and fail to support the idea that an intact exocrine parenchyma is a prerequisite for stimulation of insulin release by secretin. The potentiating effects of CCK-PZ and secretin on glucose-stimulated insulin release in our experiments are similar to previous observations with these gastrointestinal hormones [16, 18] as well as with incretin [19] and the intestinal insulin-releasing polypeptide [20]. However, other authors have found that CCK-PZ and secretin are without effects on glucose-induced insulin release [21].

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Dr. Å. Lernmark
Dept. of Histology
University of Umeå
S-901 87 Umeå
Sweden