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The islet-acinar axis of the pancreas: Is there a role for glucagon or a glucagon-like peptide?

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Abstract. Intravenous glucagon inhibits exocrine pancreatic secretion in vivo, but exogenous glucagon does not affect exocrine secretion in vitro. Recent work, however, suggested that endogenous glucagon may be involved in the regulation of exocrine secretion even in vitro. We therefore investigated the effects of exogenous and endogenous glucagon on exocrine secretion by the isolated perfused rat pancreas in the presence of 1.8 mM glucose. Exogenous glucagon did not affect CCK-stimulated amylase output. 20 mM arginine stimulated glucagon release, but did not affect basal enzyme secretion. CCK-stimulated amylase output, however, was significantly inhibited in the presence of arginine. This inhibitory effect of arginine on exocrine pancreatic secretion could be blocked by glucagon antibodies, but not by nonspecific gammaglobulins. Thus exogenous glucagon failed to affect exocrine pancreatic secretion in vitro, but endogenously released glucagon or a glucagon-like peptide inhibited amylase release in the isolated perfused pancreas. We conclude that glucagon or a glucagon-like peptide may be a mediator in the islet-acinar axis.
Key words. Glucagon; endocrine pancreas; exocrine pancreas; islet-acinar axis.

In many species a portal venous system has been demonstrated, which directly links pancreatic islets with exocrine pancreatic tissue[1–3]. Due to this specific angioarchitecture, pancreatic acinar cells are exposed to high concentrations of pancreatic hormones. Different groups have estimated that periacinar concentrations of pancreatic hormones are at least 20-fold that of the systemic circulation and this is probably of functional relevance[4,5]. Research into an islet-acinar axis has focussed on the role of insulin in the regulation of the exocrine pancreas. Thus insulin has been shown to stimulate various functions of pancreatic acinar cells[4,5]. The role of other islet hormones in the regulation of the exocrine pancreas, however, is incompletely understood[4–6].

In vivo, intravenous glucagon has been shown to inhibit exocrine pancreatic secretion stimulated by cholecystokinin, secretion or food in cat, dog and humans[7–10]. These studies obviously cannot distinguish between local and systemic effects of exogenous glucagon, but Tseng et al. also demonstrated inhibition of stimulated amylase output after injection of glucagon into the coeliac artery of anaesthetized cats[11]. Yet in vitro preparations have failed to show an inhibitory effect of exogenous glucagon[4,12]. This suggests that the effect of glucagon on acinar cells is an indirect one.

All these studies share a common experimental approach in that glucagon was administered exogenously, although physiologically it may be more relevant to study the effect of the endogenous hormone. When we studied the effects of pancreastatin or cysteamine on endocrine and exocrine pancreatic secretion, results suggested that endogenous glucagon may affect exocrine secretion even in vitro[13,14].

We therefore used the isolated perfused rat pancreas to investigate the effect both of exogenous glucagon and arginine-released endogenous glucagon on exocrine pancreatic secretion.

Methods

Animal preparation and experimental design. Male Wistar rats (Versuchstieranstalt Lippe, Lippe, Germany), weighing 200–250 g, were used in all experiments after an overnight fast with free access to water. The pancreas was dissected as previously described[15]. Briefly, the animals were anaesthetized with 60 mg pentobarbital per kg bodyweight (Nembutal) applied intraperitoneally. The preparation consisted of pancreas with a small residue of duodenum. The proximal end of the bile duct was ligated and every ten minutes a calibrated polyethylene tube was inserted into the distal end of the common duct to collect pancreatic juice. The preparation was perfused via the superior mesenteric artery and the coeliac trunk at a constant rate of 4 ml/min without recirculation. The perfusate was a modified Krebs-Ringer bicarbonate solution with 0.2% bovine serum albumin (RIA-grade), 3% dextran (T 70) and 1.8 mM glucose gassed with 95% O_2 and 5% CO_2 to give a pH of 7.4.

Experimental design. After an equilibration period of 15 min, three subsequent 10-min periods were analysed. During the first 10 min basal values were determined

(minutes 1–10). During the second (minutes 11–20) and third (minutes 21–30) periods CCK-8 (Sigma), porcine glucagon (Sigma), arginine (Sigma), glucagon antibodies (TR 211) and/or nonspecific gammaglobulins (Behring) were infused via a side-arm injection using an infusion pump (Braun Melsungen). The compounds were infused at a concentration of 20 pg/ml for CCK-8, 60 and 600 ng/ml for glucagon and 20 mM for arginine. Glucagon antibodies were infused to result in a final dilution of 1:512. Nonspecific gammaglobulins were infused at a rate calculated to give the same protein concentration as the specific glucagon antibodies. CCK, at the concentration tested, has been shown in our laboratory to result in submaximal stimulation of exocrine pancreatic secretion. 20 mM arginine has previously been shown to stimulate glucagon secretion[16]. Total portal vein effluent was collected at 60 s intervals, aliquoted and stored at −20 °C prior to determination of insulin- and glucagon-like immunoreactivities.

Amylase activity in pancreatic juice was determined using a standard laboratory method[17]. Insulin- and glucagon-like immunoreactivities were determined as previously described[14].

Statistical analysis and data presentation. Secretion of glucagon-like immunoreactivity is given as output/min as well as total output during the 20-min period between minutes 11 and 30 (mean ± SEM). Amylase secretion is expressed as U/min (mean ± SEM). Each experiment was performed on 8 animals. Statistical analysis was performed using one-way analysis of variance, (ANOVA) followed by Scheffés multiple comparison procedure. P values below 0.05 and *F* values at 95% were considered significant.

Results

Effect of exogenous glucagon. There were statistically significant differences in amylase secretion between the four groups (NaCl, CCK, CCK + 60 ng glucagon/ml and CCK + 600 ng glucagon/ml). CCK stimulated amylase secretion, whereas exogenous glucagon did not affect stimulated amylase secretion at the two concentrations tested (fig 1). Insulin-like immunoreactivity could not be detected by our assay in any of the four groups.

Effect of endogenous glucagon. There were statistically significant differences in glucagon secretion between the four groups (NaCl, arginine, CCK and arginine + CCK). Arginine stimulated glucagon secretion (23227 ± 1750 vs. 2840 ± 330 pg/20 min), whereas CCK did not modulate basal or arginine-stimulated total output of glucagon (21175 ± 1660 pg/20 min) (fig. 2). Insulin-like immunoreactivity could not be detected by our assay in any of the four groups.

There was no statistically significant difference in basal amylase secretion between the two groups (NaCl and arginine), whereas CCK-stimulated amylase secretion was significantly different between the two groups (CCK and CCK + arginine). This was true both for the first (4.1 ± 0.4 vs. 10.9 ± 1.2 U/min) and the second (4.0 ± 0.3 vs. 14.3 ± 0.9 U/min) 10-min period of CCK-stimulation (fig. 2).

Effect of glucagon antibodies. There were statistically significant differences in the output of immunoreactive glucagon measured between the three groups (arginine + CCK, arginine + CCK + glucagon antibodies and arginine + CCK + nonspecific gamma globulins). Glucagon antibodies immunoneutralized arginine-induced glucagon secretion (7066 ± 685 pg/20 min), whereas nonspecific gammaglobulins had no effect on arginine-induced glucagon secretion (23756 ± 1038 pg/20 min).

There were significant differences in the CCK-stimulated amylase release between the three groups (arginine + CCK, argine + CCK + glucagon antibodies and arginine + CCK + nonspecific gammaglobulins). Amylase response to CCK in the presence of arginine was significantly increased by glucagon antibodies in the first (6.4 ± 0.6 vs. 4.1 ± 0.4 U/min) and second

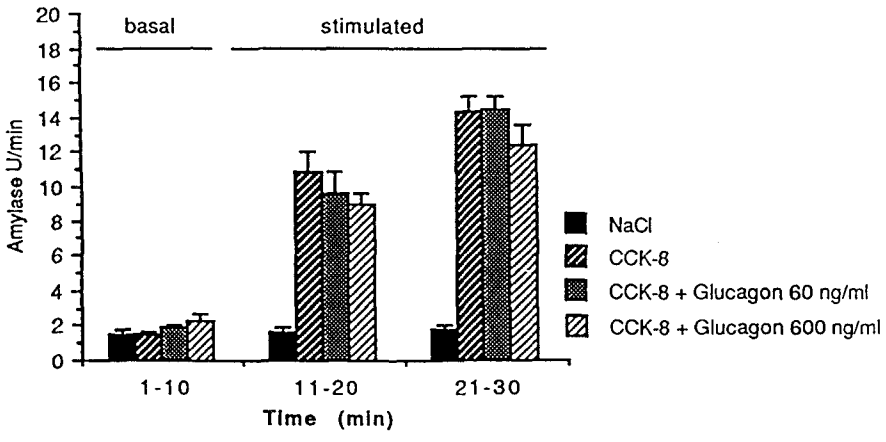

Figure 1. Effect of exogenous glucagon on CCK-stimulated amylase release from the isolated perfused rat pancreas.

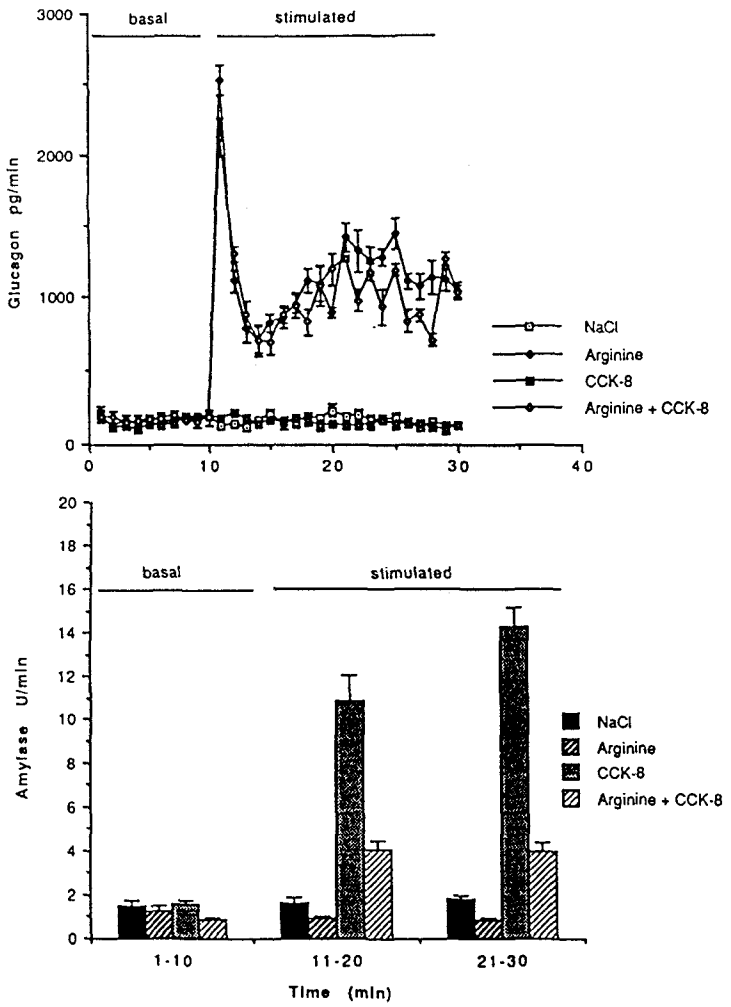

Figure 2. Effect of arginine and CCK on glucagon and amylase release from the isolated perfused rat pancreas.

(12.8 ± 1.2 vs. 4.0 ± 0.3 U/min) test period, whereas nonspecific gammaglobulins failed to affect amylase response to CCK in the presence of arginine in both test periods (fig. 3). In fact, amylase secretion during the second 10-min period in the presence of CCK, arginine and glucagon antibodies was no different from that in the presence of CCK alone (14.3 ± 0.9 U/min).

Discussion

It is well established that insulin affects different functions of pancreatic acinar cells. Thus insulin increases acinar amylase mRNA and protein synthesis and it also potentiates stimulated exocrine pancreatic secretion[2,4,5,18,19]. The role of glucagon in the regulation of acinar cell functions, however, is only incompletely understood.

Treatment with high doses of glucagon decreased enzyme activities in rat and rabbit pancreas[20,21]. Furthermore different groups have consistently shown that glucagon inhibits exocrine pancreatic secretion in man, cat and dog in vivo[7–11]. Early in vitro experiments, however, have given inconsistent results[22,23], because of a contaminant then present in natural glucagon[12]. This renders interpretation of some of the available data difficult, but it seems that exogenous glucagon does not inhibit exocrine pancreatic secretion in vitro[4,6,12]. This is confirmed in the present study.

In agreement with previous reports[16] arginine stimulated glucagon secretion in this study. This did not affect basal amylase release, but with the low flow rates of unstimulated exocrine secretion, any inhibitory effect would obviously be diffcult to detect. CCK-stimulated exocrine secretion, however, was inhibited in the presence of arginine. The same phenomenon has previously been described by Harada et al.[24] They demonstrated that arginine dose–dependently inhibited stimulated protein release from the isolated perfused rat pancreas. Since exogenous hormones did not produce the same effect, Harada et al. considered it unlikely that this inhibitory effect was mediated by pancreatic hormones, but they did not measure hormone secretion in their study[24]. While we were able to confirm their finding that

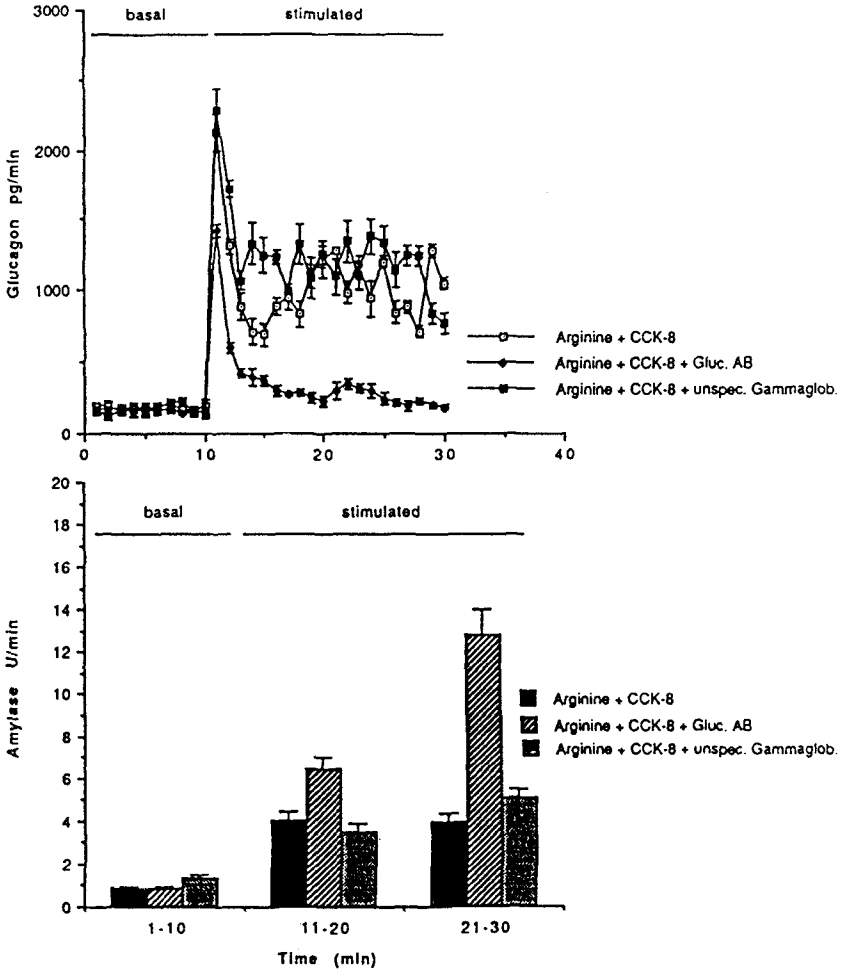

Figure 3. Effect of glucagon antibodies and nonspecific gammaglobulins on glucagon and amylase release from the isolated perfused rat pancreas in the presence of arginine and CCK.

exogenous glucagon does not inhibit exocrine secretion, our results point to a different conclusion. Not only does the amylase output closely reflect the arginine-induced changes in glucagon secretion, but we were also able to reverse arginine-induced inhibition of exocrine secretion by glucagon antibodies. This strongly suggests that the two phenomena are linked, in that endogenous glucagon inhibited exocrine secretion.

Since glucagon is derived from precursor molecules which also give rise to other glucagon-like peptides[25], we cannot exclude that a different peptide may have mediated the inhibitory effect, at least in part. It is also possible that the antiglucagon serum itself had a stimulatory effect on exocrine pancreatic secretion. This in itself, however, would also point to a role of glucagon in the regulation of exocrine pancreatic secretion. In addition, the glucagon antibodies exactly reversed the inhibitory effect of arginine-released glucagon.

In vivo, exogenous glucagon inhibits exocrine pancreatic secretion[7–11]. It seems likely that this is also true for endogenous glucagon, since DiMagno et al. were able to show that the intravenous infusion of protein hydrolysate increases plasma glucagon levels and decreases exocrine pancreatic secretion in healthy volunteers[7]. Yet we cannot explain why only endogenous but not exogenous glucagon inhibited amylase secretion in our in vitro experiment. Although it is not possible to measure periacinar hormone concentrations, we expect that in our experiment the glucagon concentration at the acinar cell was not lower after exogenous administration than during stimulated release of endogenous glucagon.

Other inhibitory peptides show similar characteristics. Thus exogenous somatostatin inhibits exocrine pancreatic secretion only in vivo, but not in vitro. Possibly there is some common underlying principle which accounts for the discrepancies. The extrinsic denervation during the preparation of in vitro models may be of relevance in this respect[6].

Since endogenous glucagon or a glucagon-like peptide inhibited exocrine pancreatic secretion in this in vitro study, we conclude that glucagon or a glucagon-like peptide may be a regulator in the islet-acinar axis. Then

glucagon and insulin would be an antagonistic pair of hormones not only in the regulation of blood glucose, but also in the regulation of exocrine pancreatic secretion.

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