Pancreatic hormonal release after glucose and arginine administration in anaesthetized pigs

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Abstract. Somatostatin, pancreatic polypeptide (PP), insulin and glucagon were measured in pancreatic venous effluent and in mixed venous blood of anaesthetized pigs. After stimulation of insulin release with glucose and arginine, respectively, a prompt decrease in PP levels was found in pancreatic venous blood. Somatostatin declined after glucose administration but was unaffected by arginine. The changes in PP and somatostatin levels were not evident in systemic blood. The present results emphasize the advantage of selective sampling of pancreatic venous blood in studies on the possible interactions between pancreatic hormones. The marked decrease in PP seen after stimulation of insulin release in the present study, indicates that PP should be included together with somatostatin in further studies on pancreatic hormonal interplay.

As a contrast to the other known pancreatic hormones, pancreatic polypeptide (PP) has not been demonstrated to be implicated in the regulation of intermediary metabolism. However, PP secretion can be provoked by insulin-induced hypoglycaemia most likely via vagal mechanisms (Adrian et al. 1977; Marco et al. 1978) and substantial amounts of PP is released into the blood as a response to food ingestion (Adrian et al. 1976; Schwartz et al. 1976). Therefore PP has been discussed as a component of the entero-pancreatic endocrine axis.

RIA methods for the determination of plasma levels of somatostatin-like immunoreactivity (SLI) (Arimura et al. 1978; Berelowitz et al. 1978; Lundqvist et al. 1980) and PP (Lin & Chance 1974; Larsson et al. 1976) have been developed. The aim of the present study was to measure plasma levels of all four pancreatic hormonal peptides during basal conditions and after stimulation of the endocrine pancreas by iv glucose and by arginine. Using an experimental model in anaesthetized pigs, blood was sampled both from the pancreatic venous effluent and from the mixed venous blood. Thus the hormone concentrations could be measured also before hepatic transit and dilution in the entire plasma volume.

Preliminary data concerning insulin and somatostatin levels have been reported briefly in an earlier publication (Gustavsson & Lundqvist 1978).

Material and Methods

Animals

Seven pigs of Swedish land-race (21–28 kg body weight) were purchased from the same breeder. The pigs were starved although given free access to water for 18 h before the experiments.

Anaesthesia

Anaesthesia was induced by an im injection of ketamine (Ketalar®) 250–500 and maintained by repeated iv injections of phenoperidin (Lealgin®, 4 mg every 30–45 min) and pancuron bromide (Pavulon®, 4 mg every 30–45 min). After endotracheal intubation positive pressure ventilation was given with a mixture of N₂O and O₂ (4:1) by means of a respirator. Arterial blood pressure was monitored through a catheter (infant feeding tube No. 5) introduced into the common carotid artery of the left side. An infant feeding tube No. 8 was inserted into the right atrium via the internal jugular vein of the left side. This catheter was used for measurement of the central venous pressure and for blood sampling (mixed venous blood).
Surgical procedure

An upper laparotomy was performed using a midline incision. A catheter (infant feeding tube No. 5) was introduced into the superior pancreatico-duodenal vein immediately before its entrance into the portal vein. This catheter was used for blood sampling from the pancreatic venous effluent. The catheter was exteriorized through a stab wound in the abdominal wall and the laparotomy wound was closed.

Experimental protocol

After initial blood sampling at two occasions with 10 min interval glucose (0.350 g/kg body weight) was administered as an iv bolus injection via an ear vein. Blood was then sampled from both catheters at 4, 6, 30, 60 and 90 min after administration. Thirty min after the end of this experiment arginine (0.5 g/kg body weight in 100 ml physiological saline) was infused for 10 min. Blood from the venous catheters was sampled before the arginine infusion at two occasions and at 10, 15, 20, 30 and 40 min after the beginning of the infusion.

In different experiments the drip rate of blood from the catheter in the superior pancreatico-duodenal vein varied between 20–40 per min. Within each experiment the constancy of the drip rate (±10%) was taken as evidence that the venous blood flow did not change.

Blood samples (5 ml each) were collected in chilled tubes with the addition of Heparin (143 USP-units) and Trasylol (400 KIE/ml). After centrifugation at −4°C plasma was decanted and frozen at −20°C until radioimmunoassay.

Radioimmunoassay

Before radioimmunoassay of somatostatin plasma samples were extracted with acetone-petroleum-ether as suggested by Arimura et al. (1978). The radioimmunoassay was performed with a solid phase technique (Lundqvist et al. 1980) with somatostatin antibodies coupled to microcrystalline cellulose. The antisem used, R141, is well characterized and reacts with the sequence 3–12 of the somatostatin molecule with the exception of the lysin residue in position 9 and is also sensitive to the cyclic structure.

Tyr-1-somatostatin (Beckman, Geneva) was used for iodination with the lactoperoxidase method and synthetic somatostatin (Beckman, Geneva) was used for preparation of standards.

The plasma level of PP was determined by a previously described radioimmunoassay (Larsson et al. 1976), modified according to Wide (1969) to a radioimmunosorbent assay with the PP antiserum (Lilly Lot No. 615-1054 B-248-18) coupled to CnBr-activated microcrystalline cellulose. Purified human PP (Lilly Lot No. 615-1054 B-200) was used for preparations of standards and purified bovine PP (Lilly Lot No. 615-D 63-188-8) was used for the preparation of tracer. All reagents were generous gifts from Dr. R. E. Chance, The Lilly Research Laboratories, Indianapolis, USA. The plasma PP level was expressed in pg equivalents of human PP per liter.

Insulin was determined with a solid phase radioimmunoassay (Phadebas, Pharmacia, Sweden).

Plasma immunoreactive glucagon was analysed according to Aguilar-Parada et al. (1969), using a glucagon antiserum 30 K, which is specific for pancreatic glucagon. Pork glucagon (Novo, Copenhagen, Denmark) was used as standard.

Blood sugar was measured with the glucose oxidase method.

Statistical analysis

Mean (± st) was calculated for each sampling occasion. Student's t-test (paired case) was used to determine the significance of differences between hormone levels at different times after stimulation and the initial level. This was defined as the mean of the two initial samples.

Fig. 1.

Mean (± st) levels of immunoreactive insulin, PP, somatostatin and glucagon before and after injection of glucose (0.350 g/kg). * P < 0.05, ** P < 0.01. Pancreatic venous blood.
Results

Plasma levels of the pancreatic hormones in fasting pigs are given in Table 1. It is evident that the hormone concentrations in pancreatic venous blood are increased by a factor 2 as compared to the hormone concentrations in mixed venous blood. This is valid not only for insulin and glucagon but also for somatostatin and PP.

The glucose injection resulted in a peak blood glucose concentration of 8.1 ± 1.0 (mean ± se) mmol/l. Basal levels = 3.9 ± 1.2. The increase in insulin after glucose and the decrease in glucagon were evident both in pancreatic (Fig. 1) and in mixed venous blood (Fig. 2). Simultaneously the PP concentration of the pancreatic venous blood decreased with significant \( P < 0.05 \) changes at 4, 6 and 30 min after injection. The somatostatin concentration also decreased with significant changes at 30, 60 and 90 min after injection. In mixed venous blood no significant \( P > 0.05 \) changes in somatostatin and PP were observed (Fig. 2).

Arginine infusion was followed by an insignificant increase in blood glucose concentration, which was 3.9 ± 0.8 mmol/l before and 4.6 ± 1.6 mmol/l 5 min after the end of the arginine infusion. The level of insulin and glucagon increased significantly (Figs. 3 and 4). The changes in somatostatin and PP levels in pancreatic venous blood were not as evident after arginine infusion as after glucose injection. However, a small decrease in PP was found with a single significant \( P < 0.05 \) change at 10 min after beginning of the arginine infusion. The somatostatin level showed an apparent transient increase although this change was not significant \( P < 0.05 \). In mixed venous blood the hormone changes were less evident (Fig. 4).

Table 1.

Hormone concentrations of 7 fasting, anaesthetized pigs.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Pancreatic venous blood</th>
<th>Mixed venous blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (mU/l)</td>
<td>22.6 ± 5.0</td>
<td>11.3 ± 1.9</td>
</tr>
<tr>
<td>Glucagon (pg/ml)</td>
<td>247 ± 57</td>
<td>95 ± 19</td>
</tr>
<tr>
<td>Somatostatin (pg/ml)</td>
<td>255 ± 44</td>
<td>153 ± 18</td>
</tr>
<tr>
<td>PP (pg/ml)</td>
<td>1495 ± 350</td>
<td>746 ± 137</td>
</tr>
</tbody>
</table>

Fig. 3.

Mean (± se) levels of immunoreactive insulin, PP, somatostatin and glucagon before and after infusion of arginine (0.5 g/kg). \(* P < 0.05 \). Pancreatic venous blood.
Discussion

In studies of the endocrine balance in the gastrointestinal tract the pig has been found to be very suitable as experimental animal (Holst 1977). The main reason for this is the similarities between human and porcine intestinal peptides and also obvious similarities in gastrointestinal physiology. Besides that it was found in the present study to be easy to sample blood from the pancreatic venous effluent. Repeated blood samples could be obtained and the blood flow showed no apparent change during the experimental situation selected.

The statistical spread around the means was greater for all hormones studied in pancreatic venous blood than in systemic blood which may be due to interindividual variation in the location of the catheter tip. This justifies the statistical evaluation of experimental data as percental changes from initial values during the experimental situations studied.

A significant decrease in somatostatin immuno-reactivity in pancreatic venous blood was noted after iv administration of glucose, and this decrease was evident throughout the observation period. Results concerning somatostatin response after glucose is partly contradictory to earlier reports both from studies with isolated perfused pancreas and from in vivo studies. In some studies glucose is considered as a potent somatostatin secretagogue (Ipp et al. 1977; Schauder et al. 1976; Patton et al. 1977). However, recently Schusdziarra et al. (1978) reported a decrease of somatostatin in peripheral blood of normal dogs as a response to an iv infusion of glucose, in accordance with present data.

In contrast to the demonstrated inverse relation between insulin and somatostatin after glucose administration, arginine infusion did not significantly alter the somatostatin levels of either systemic or pancreatic venous blood, although a prompt increase in insulin levels were noted.

As soon as the inhibitory effects by somatostatin upon insulin release have been demonstrated questions have arisen whether endogenous somatostatin is modulating the insulin release within the islets or not. The inverse relation seen in present study after glucose administration would fit with such hypothesis. The unaffected somatostatin levels after arginine-induced insulin release indicate, however, a more complex mechanism for inter-hormononal actions.

Concerning PP data higher basal levels were found in present investigation compared to earlier reported data (Schwartz et al. 1978) from the same species. Although confusing, this discrepancy could possibly be explained by differences in standardisation of the methods used, since similar antiserum have been used in both studies. Concor-dant results of PP levels in uraemia (Hällgren et al. 1977) and after insulin-induced hypoglycaemia compared to other investigators (Schwartz et al. 1978; Collins et al. 1978) further supports the specificity of the PP radioimmunoassay used.

PP levels in pancreatic venous blood has not earlier been reported. There are, however, data from measurements in peripheral blood indicating that intravenous glucose is followed by a diminished PP level (Marco et al. 1978; Sive et al. 1979). This response found in peripheral blood is in good accordance with present finding. However, in vitro studies on isolated perfused pancreas have shown that a high glucose concentration in the perfusate does not affect PP secretion (Adrian et al. 1978; Gingerich et al. 1978; Weir et al. 1979). The reason

![Graph](image-url)

As Fig. 3. Systemic blood.
for this discrepancy between in vivo and in vitro investigations is unclear, but indicates a more complex interplay in the in vivo situation.

In view of its biological effect somatostatin has come into focus in the discussion of the suggested paracrine intrainsular regulation of hormone release. Our findings of a prompt and inverse change in the PP level indicate that also this pancreatic hormone might be involved in the physiological mechanisms for insulin release, although further studies must be awaited before a possible regulatory role of insular peptides upon the synthesis and release of each other is settled.

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References


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