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Release of Insulin and Pancreatic Somatostatin in Response to Increased Circulating Growth Hormone (GH) Levels

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ABSTRACT

In an experimental study on 5 anaesthetized pigs the effect of increased circulating levels of growth hormone (GH) on the release of insulin and somatostatin from the pancreas was investigated. Blood was sampled simultaneously from the pancreatic venous effluent and from mixed venous blood. As measured by radioimmunoassay infusion of GH (20 $\mu g \times kg^{-1} \times h^{-1}$) resulted in a significant ($p < 0.01$) increase both in insulin and somatostatin concentration in pancreatic venous blood. In mixed venous blood no significant changes in hormone levels were found. The results illustrate one possible mechanism for the close relation between circulating GH and endocrine pancreas.

INTRODUCTION

Considerable evidence is now available showing that growth hormone (GH) is capable of inhibiting its own secretion (12, 15, 8). Whether this mechanism involves a "short feed-back loop" operating on the hypothalamus /14/ is not finally proven. Indirect evidences have, however, been obtained for such a hypothesis, where hypothalamic somatostatin seems to contribute to the GH release regulation (12). A positive correlation is also demonstrated between GH excess and hypothalamic somatostatin concentration in rats (13), which might indicate an increase in somatostatin secretion during GH treatment.

So far no physiological significance has been attributed neither to the well-known extrapituitary actions of somatostatin, nor to the localization of large amounts of somatostatin in the pancreatic islets and in the gastric mucosa. However, numerous reports on somatostatin as inhibitor of gastrointestinal (GI) hormone release points to a specific function for somatostatin as a modulator of GI hormone response, especially within the pancreatic islets but also in the stomach (7, 3).

The effect of GH on pancreatic hormonal release is controversial. Thus, GH does not appear to have any immediate effect on insulin secretion of the ß-cell

in the in vitro study of Malaisse et al. (10), but the results of Tai & Pek (16) indicate that GH may have a tonic effect both on insulin and glucagon release. Constantly elevated levels of GH in tumour-bearing rats resulted in an increase in the fasting insulin levels as well as in the extractable pancreatic insulin (11). However, judging from clinical studies administration of GH has an inhibitory effect on insulin release (1). Whether circulating levels of GH might induce a change in pancreatic somatostatin secretion or not has not been studied previously.

In the present paper we are evaluating the hypothesis that pancreatic somatostatin release is affected by the circulating levels of GH in the same way as hypothalamic somatostatin is supposed to be regulated. For these studies we have used an experimental model which permits selective blood sampling from the pancreatic veins during and after GH infusion.

MATERIAL AND METHODS

Animals

Five pigs of Swedish land-race weighing 20-28 kg were used.

Anaesthesia

Anaesthesia was induced by an intramuscular injection of ketamine (Ketalar^R) 250-500 mg and maintained by repeated intravenous injections of phenoperidin (Lealgin^R, 4 mg every 30-45 min) and pancuron bromide (Pavulon^R, 4 mg every 30-45 min). After endo-tracheal intubation positive pressure ventilation was given with a mixture of N_2O and O_2 (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 introdued 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 an initial control period of saline infusion via an ear vein and blood sampling from both catheters (-45, -30, -15 and 0) human growth hormone

(Crescormone, KABI AB, Sweden) was infused for 30 min via an ear vein in the dose 20 $\mu g x kg^{-1} x h^{-1}$. The dose of administered GH was chosen according to the results of Adamsson and Efendic (1) to achieve an increase in circulating GH simulating the highest levels seen during a 24 h period in man and well within the range of circulating GH in acromegalic patients. Blood was then sampled at 15, 30, 45, 60, 75 and 90 min after the beginning of the growth hormone administration. In 2 of the pigs a repeated infusion of growth hormone was given in the same dose starting 90 min after the beginning of the first infusion (Fig. 1). Blood was sampled according to an identic experimental protocol.

In different experiments the drip rate of blood from the catheter in the superior pancreatico-duodenal vein varied between 20-60/min. Within each experiment the constancy of the drip rate ($\pm$ 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. (2). The radioimmunoassay was performed with a solid phase technique (9) with somatostatin antibodies coupled to microcrystalline cellulose. The antiserum used, R 141, is well characterized with respect to reactivity against different parts of the somatostatin molecule as well as to the lack of reactivity against other GI peptides (2). Tyr-1-somatostatin (Beckman, Geneva) was used for iodination with the lactoperoxidase method and synthetic somatostatin (Beckman, Geneva) was used for preparation of standards.

Insulin and GH were determined with solid phase radioimmunoassays (Phadebas, Pharmacia, Sweden).

Statistical analysis

The degree of significance for the difference between hormone levels before and after the beginning of the GH infusion was tested by analysis of variance.

RESULTS

The GH infusion in the selected dose resulted in a 10-fold increase in GH levels as measured in one of the pigs (Fig. 1). The insulin concentration in pancreatic venous blood was significantly ($p < 0.01$) increased after the beginning of the GH infusion (Table 1). There was also noted a tendency to an increase in insulin in mixed venous blood although this increase did not reach

Table 1

Levels of immunoreactive insulin (mU/l) in pancreatic venous blood before and after the beginning of the GH infusion. Mean + SEM. Analysis of variance revealed F = 10.08, p = 0.004.

Animal No.	Before GH infusion	After GH infusion
1	10.7 + 2.7	20.5 + 8.6
2	23.6 + 6.0	114 + 13.9
3	121 + 28.0	741 + 188
4	7.8 + 1.8	21 + 15
5	9.3 + 1.8	18.0 + 2.9

Table 2

Levels of immunoreactive insulin (mU/l) in mixed venous blood before and after the beginning of the GH infusion. Mean + SEM. Analysis of variance revealed F = 3.86, p = 0.058.

Animal No.	Before GH infusion	After GH infusion
1	9.6 + 0.7	32.4 + 21
2	3.7 + 0.6	6.2 + 0.5
3	4.2 + 1.9	11.9 + 1.3
4	7.0 + 0.5	9.8 + 0.6
5	7.8 + 1.3	19.4 + 3.0

Table 3

Levels of immunoreactive somatostatin (pg/ml) in pancreatic venous blood before and after the beginning of the GH infusion. Mean + SEM. Analysis of variance revealed F = 10.21, p = 0.003.

Animal No.	Before GH infusion	After GH infusion
1	799 + 47	1755 + 265
2	458 + 44	500 + 62
3	391 + 56	628 + 55
4	331 + 106	487 + 72
5	429 + 56	537 + 87

Table 4

Levels of immunoreactive somatostatin (pg/ml) in mixed venous blood before and after the beginning of the GH infusion. Mean + SEM. Analysis of variance revealed F = 0.73, p = 0.400.

Animal No.	Before GH infusion	After GH infusion
1	567 + 103	611 + 161
2	284 + 64	240 + 33
3	275 + 31	295 + 48
4	244 + 18	250 + 11
5	232 ± 29	329 ± 23

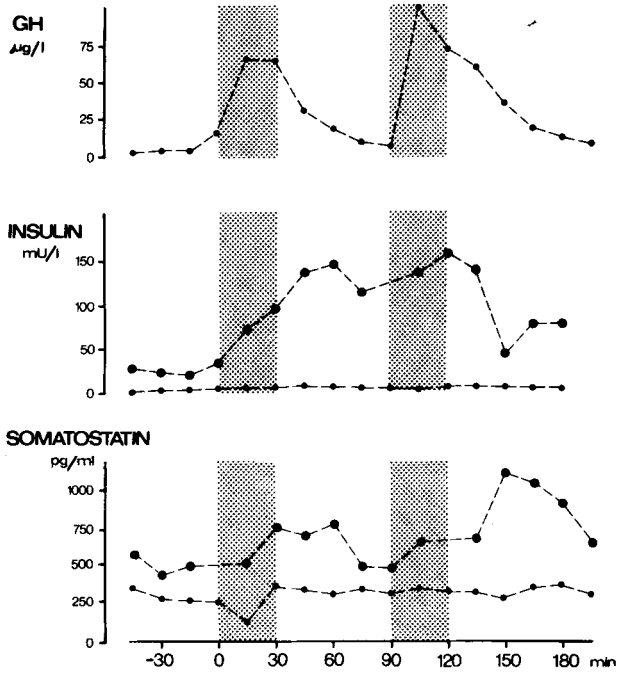

Fig. 1. A representative example of hormone levels in one pig before, during and after GH infusion in the dose 20 $\mu g \times kg^{-1} \times h^{-1}$ (hatched area)

statistical significance (Table 2). According to Table 3 the somatostatin level measured in the pancreatic venous effluent increased significantly ($p < 0.01$). In mixed venous blood no significant change was observed in the somatostatin concentration (Table 4). The increase in insulin and somatostatin in pancreatic venous blood occurred simultaneously. In 2 animals a repeated GH infusion was administered, in both cases followed by a repeated increment in the insulin and somatostatin level in pancreatic venous blood (Fig. 1).

DISCUSSION

The increase in insulin and somatostatin found in the present experiments as a response to GH infusion was statistically significant in pancreatic venous blood but not in mixed venous blood. Thus the present results illustrate the beneficial value of blood sampling also from pancreatic veins in studies of hormonal interplay, as also pointed out before (5, 6). This experimental design is especially necessary in studies of polypeptides with a rapid metabolism or elimination, like somatostatin.

The use of human GH might be erroneous due to the well-known species differences known for GH (4). However, molecular and immunological similarities are noted between human and porcine GH (18) which justifies the present experimental performance. The possibilities of direct measurement of the GH levels reached by GH infusion is another reason for the use of human GH in these experiments. The mechanism whereby GH affects islet function is not known and the effect of GH on insulin secretion is controversial. The present results with an insulin response detected in pancreatic venous blood after GH infusion are in accordance with reports of a stimulant effect of GH on insulin secretion (11, 19). On the other hand a small but significant decrease of insulin release has also been reported after GH infusion in man (1). Whether this discrepancy is due to various doses of GH is unclear.

In the present study the level of immunoreactive somatostatin in the pancreatic venous effluent increased significantly during infusion of GH. This increase was evident in all animals but with considerable inter-individual variation in magnitude, which justifies the present statistical evaluation.

Although little is known about the role of somatostatin-producing cells within the pancreatic islets, it seems to be reasonable to suggest that the pancreatic D cells react in the same manner as hypothalamic somatostatin cells upon blood GH alterations. The unchanged somatostatin level in mixed venous blood, found by us, do not support the hypothesis that pancreatic somatostatin during physiological conditions participates in a feed-back control of GH secretion from the pituitary gland.

It is difficult to further speculate on the complex interplay between GH on one hand and insulin and pancreatic somatostatin on the other. An increase in

somatostatin release would be followed by a diminished release of insulin in view of the well-known suppressive effect of this hormonal peptide on basal and stimulated insulin secretion. On the other hand, in this particular experimental model at least, stimulation of insulin by glucose is coupled to a decrease of somatostatin release into pancreatic veins (5, 6). Hypothetically the release of insulin may be the first event and the increase in somatostatin should then modulate the insulin response. However, such an hypothesis has to be tested by hormone determinations at closer intervals than was made in the present study.

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