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Somatostatin Stimulates Glucagon Secretion in Ducks

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Summary. In normal fasting ducks, a somatostatin infusion (800 ng/kg/min for 30 min) elicited a prompt inhibition of insulin secretion, plasma levels falling from 140 ± 20 to 20 ± 6 pg Eq/ml as observed in mammals. – However plasma glucagon-like-immunoreactivity shown to be decreased in mammals by somatostatin was sharply increased from a mean basal level of 1.46 ± 0.13 ng Eq/ml to 6.61 ± 0.77 ng Eq/ml. This effect was not mediated via inhibition of growth hormone secretion since it was also observed in hypophysectomised ducks. Despite the fall in plasma insulin and rise in GLI observed with somatostatin infusion in intact birds, plasma glucose concentrations were lower than with control saline infusion.

Key words: Duck, somatostatin, glucagon-like immunoreactivity, insulin growth hormone, glucose.

Somatostatin (SRIF), characterised as a hypothalamic growth hormone inhibiting peptide [4], has been found to be a potent inhibitor of secretion of other hormones eg. TSH [24], prolactin [24], gastrin [12], secretin [3] and pancreatic hormones. Effects on pancreatic hormone secretion have been widely investigated in many species of mammals [5, 14, 22] and it has been shown that somatostatin simultaneously inhibits insulin and glucagon secretion; moreover somatostatin appears to be one of the most effective A cell suppressants known [22].

In birds, it has been shown that the somatostatin producing D cells are particularly numerous in the islets of the pigeon pancreas [20] and very high somatostatin concentrations have been measured in the chicken pancreas [25]. As the duck appears to be an interesting species for studies on pancreatic hormone regulation [17, 18] the present study was undertaken to explore the response of pancreatic hormones to somatostatin infusion in ducks.

Materials and Methods

Animals

Experiments were performed on male Peking ducks 3 to 6 months old (2 to 2.5 kg), maintained on a normal diet with fowl pellets and tap water ad libitum.

Hypophysectomy

Removal of the pituitary was performed under local anaesthesia (1% xylocaïne, Bellon) according to Benoit's technique [1]. These animals were used for experiments 4 to 10 days after operation. Completeness of hypophysectomy was checked by plasma growth hormone determination.

Infusions

After an overnight fast the conscious ducks, normal or hypophysectomised, were tied to a board.

Somatostatin was dissolved in 0.154 mol/l saline and administered intravenously as a bolus of 800 ng/kg immediately followed by a sustained infusion of 800 ng/kg/min for 30 minutes through a cathether inserted into a leg vein. As controls, normal or hypophysectomised ducks received 0.154 mol/l saline infusions.

Blood Sampling

Blood samples were collected from a catheter inserted into a wing vein immediately before infusion (zero time) and at selected intervals thereafter.

Blood samples kept on ice were centrifuged at 4 °C and plasma stored at −20 °C until the time of assay.

Plasma Determinations

Plasma glucose concentration was measured with a Technicon autoanalyser using a ferricyanide reagent [11] after dialysis of plasma.

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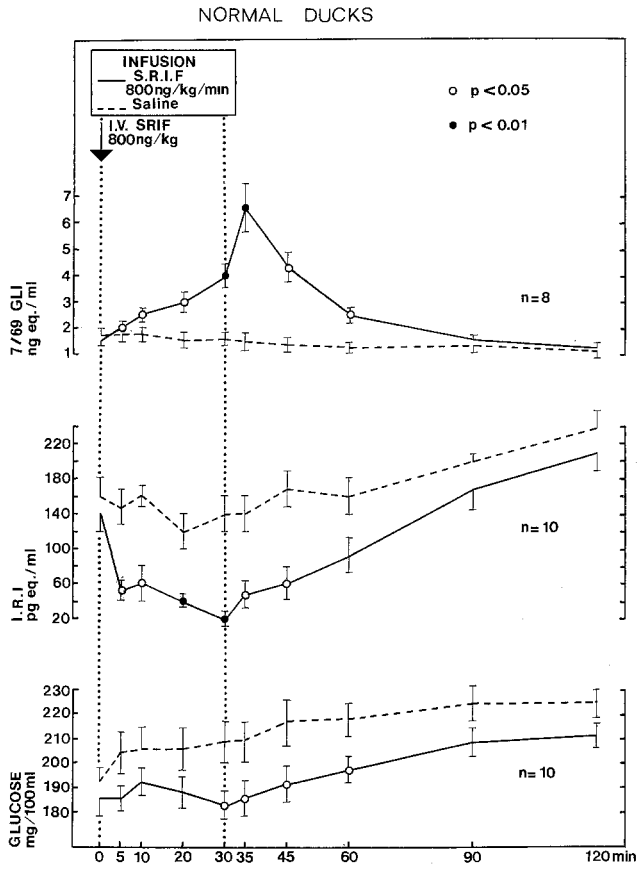

Fig. 1. Effect of somatostatin (SRIF) (——) and saline (-----) infusions on plasma glucose, IRI and 7/69 GLI in normal fasting ducks. SRIF (800 ng/kg) was injected at zero time, followed by a 30 min infusion of 800 ng/kg/min. Vertical bars represent ± SEM. The significant differences from zero time values for IRI and 7/69 GLI, and from saline infused ducks for glucose are shown by: o $p < 0.05$ and • $p < 0.01$

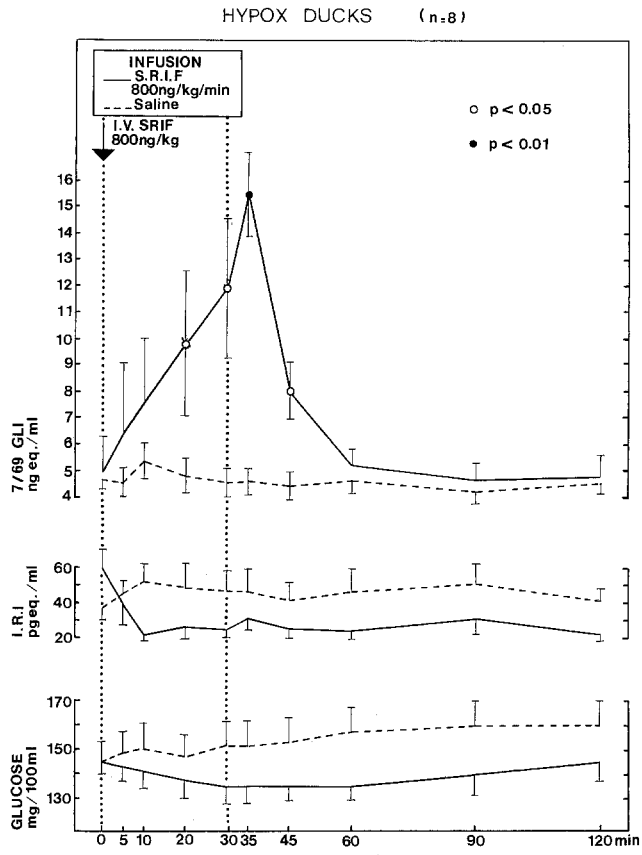

Fig. 2. Effect of somatostatin (SRIF) (——) and saline (-----) infusions on plasma glucose, IRI and 7/69 GLI in hypophysectomised fasting ducks. SRIF (800 ng/kg) was injected at zero time, followed by a 30 min infusion of 800 ng/kg/min. Vertical bars represent ± SEM. The significant differences from zero time values for IRI and 7/69 GLI, and from saline infused ducks for glucose are shown by: o $p < 0.05$ and • $p < 0.01$

Plasma immunoreactive insulin (IRI) was determined by radioimmunoassay with dextran-charcoal separation [8] and beef insulin as standard. At the concentrations used beef and duck insulin crossreact with our antibody [15] and the results are expressed as pg beef IRI equivalents/ml plasma. The sensitivity of the assay was 50 pg/ml and the coefficient of variation between assays at levels between 200 and 300 pg/ml was 19%.

Plasma glucagon-like immunoreactivity (GLI) was determined by the radioimmunoassay of Leclerc-Meyer [16] with porcine glucagon as standard. The results are expressed as ng pig GLI equivalents/ml plasma.

Two antisera were used: 7/69 and 30K, the GLIs measured in plasma are referred to as 7/69 GLI and 30K GLI respectively. In pancreatectomised ducks 7/69 GLI is still detectable but not 30K GLI. Therefore it is assumed that variations in 30K GLI represent modifications of GLI of pancreatic origin [15, 23].

The sensitivity of the assay for 7/69 GLI was 0.1 ng/ml and the coefficient of variation between assays at levels of 0.5 to 1.0 ng/ml was 10.5%.

Plasma immunoreactive growth hormone (GH) concentrations were estimated using a specific radioimmunoassay for avian growth hormone [10] in which duck plasma cross-reacts.

The results of this assay were expressed in terms of a standard preparation of chicken growth hormone, fraction DE 1 [10]. The minimum detectable concentration in the assay was 3.0 ng/ml.

Calculations

All data are represented as mean ± SEM. Statistical differences were sought by analysis of variance and by Student's-t-test.

Results

I. Effect of Somatostatin Infusion in Normal Ducks

1. Plasma Glucose. Somatostatin infusion produced a fall in blood glucose concentration significant at 30 min compared with saline control (208 ± 8 mg/100 ml saline control vs 180 ± 5 mg/100 ml somatostatin).

On cessation of somatostatin infusion blood glucose concentrations remained lower than in saline control for a further 30 min.

2. Plasma Insulin. Somatostatin elicited a prompt (5 min) and significant decrease in plasma IRI concentration from 140 ± 20 pgEq/ml to 50 ± 9 pgEq/ml. A nadir of 20 ± 6 pgEq/ml was reached at the end of the infusion. When infusion was discontinued, plasma IRI levels rose again to preinfusion values.

3. Plasma Glucagon-like Immunoreactivity. Somatostatin infusion resulted in a rapid and significant increase in plasma 7/69 GLI from 1.46 ± 0.13 to 2.06 ± 0.24 ngEq/ml in 5 min. Stimulation was maximal at 35 min : 6.61 ± 0.77 ngEq/ml. Thereafter plasma 7/69 GLI levels progressively decreased to reach preinfusion values at 90 min. When measured with 30K antiserum (2 ducks), GLI secretion increased from an initial level of 1.2 ngEq/ml to 3.6 at 30 min and 5.4 ngEq/ml at 35 min. These results showed an identical profile to those obtained for plasma 7/69 GLI.

II. Effect of Somatostatin Infusion in Hypophysectomised Ducks

Completeness of hypophysectomy was checked by measuring radioimmunoassayable GH concentrations before and after operation. Before hypophysectomy plasma GH levels were 7 ± 0.6 ng/ml whereas after operation the levels were undetectable (< 3 ng/ml).

After hypophysectomy, basal blood glucose levels fell from 249 ± 8 to 145 ± 5 mg/100 ml, plasma IRI from 230 ± 15 to 58 ± 11 pgEq/ml and plasma GLI rose from 1.7 ± 0.2 to 5 ± 1.3 ngEq/ml.

1. Plasma Glucose. No significant change in glucose concentration occurred during somatostatin infusion.

2. Plasma IRI. The basal insulin levels were low in hypophysectomised ducks and close to the sensitivity of our assay. No significant modification in insulin secretion could be detected in this experiment.

3. Plasma GLI. As observed in normal ducks, 7/69 GLI secretion was sharply stimulated during somatostatin infusion. When the infusion was discontinued, plasma 7/69 GLI levels increased further to peak at 35 min (15.4 ± 1.8 ngEq/ml) before returning to basal levels.

Discussion

In all mammalian species so far studied, somatostatin inhibits simultaneously insulin and glucagon secretion [5, 14, 22, 13].

In ducks, insulin secretion is also inhibited but, surprisingly, somatostatin (infused at a rate of 800 ng/kg/min) appears to be a potent stimulus to 7/69 GLI secretion. It seems unlikely that one can attribute this conflicting result solely to the pharmacological dose of somatostatin infused since it is within the range used by other workers in mammals [14]. Measurement with 30K antiserum gives very similar results to those observed with 7/69 antiserum suggesting that the plasma GLI increase during somatostatin infusion is mainly if not totally of pancreatic origin.

Similar effects are observed after hypophysectomy in ducks (inhibition of insulin and stimulation of GLI secretion) and somatostatin could have acted via the suppression of growth hormone secretion. This hypothesis seems unlikely since the basal GH level in ducks of this age is very low (7 ng/ml); moreover, in hypophysectomised ducks, somatostatin also enhanced GLI secretion. Therefore, as in man [9], the effect of somatostatin on pancreatic hormonal secretion is not due to an inhibition of growth hormone secretion.

In ducks, as in mammals, insulin secretion is inhibited by somatostatin. A direct effect of somatostatin on pancreatic B cell secretion has been demonstrated in rats [6] and a similar mechanism is likely in the duck.

The effect of somatostatin on the plasma glucose level seems paradoxical: inhibition of insulin and stimulation of GLI secretion should lead to hyperglycaemia but a small decline in plasma glucose concentration was observed.

In mammals, plasma glucose is lowered [9] or not modified [22] by short-term somatostatin infusions and no direct effect on either basal or insulin stimulated peripheral glucose uptake has been observed [9]. A possible mechanism for the hypoglycaemic effect of somatostatin in ducks may be through a decrease in hepatic glucose production, for which there is some evidence in mammals [14, 2]. In vitro, somatostatin interferes with the effect of glucagon on hepatic glycogenolysis and gluconeogenesis [19, 21].

In conclusion, in ducks, somatostatin inhibits insulin secretion, stimulates GLI secretion and plasma glucose levels are slightly lowered. The role of somatostatin in the regulation of pancreatic hormone secretion in the duck requires further study.

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