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Abstract

Effects of synthetic neurotensin on the endocrine pancreas were studied in nine normal and six hypophysectomized (10th to 14th day post-hypophysectomy) dogs. Synthetic neurotensin was administered into the superior pancreaticoduodenal artery, and plasma insulin and glucagon concentrations were measured radioimmunologically. In normal dogs, ten microgram/kg neurotensin administration brought about a mild hyperglycemic response and sharp and rapid increase of plasma insulin and glucagon concentrations in the superior pancreaticoduodenal vein. A biphasic insulin response was noted in the pancreatic vein. The results suggest that a large dose of neurotensin acts directly on the endocrine pancreas causing secretion of these hormones. In hypophysectomized dogs, basal levels of plasma insulin and glucagon were decreased and neurotensin had little effect on the endocrine pancreas even with the administration of a large dose.

KEYWORDS: neurotensin, insulin, glucagon, hypophysectomy

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POTENTIAL ENDOCRINE EFFECTS OF HYPOTHALAMIC PEPTIDE "NEUROTENSIN" ON PANCREAS IN DOGS

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Abstract. Effects of synthetic neurotensin on the endocrine pancreas were studied in nine normal and six hypophysectomized (10th to 14th day post-hypophysectomy) dogs. Synthetic neurotensin was administered into the superior pancreaticoduodenal artery, and plasma insulin and glucagon concentrations were measured radioimmunologically. In normal dogs, ten μg/kg neurotensin administration brought about a mild hyperglycemic response and sharp and rapid increase of plasma insulin and glucagon concentrations in the superior pancreaticoduodenal vein. A biphasic insulin response was noted in the pancreatic vein. The results suggest that a large dose of neurotensin acts directly on the endocrine pancreas causing secretion of these hormones. In hypophysectomized dogs, basal levels of plasma insulin and glucagon were decreased and neurotensin had little effect on the endocrine pancreas even with the administration of a large dose.

Key words: neurotensin, insulin, glucagon, hypophysectomy

A hypotensive peptide, neurotensin (1), isolated from bovine hypothalami, has been found to have kinin-like biological activities, including vasodilatation and cyanosis, a local increase in vascular permeability, and contraction of various isolated smooth muscle preparations and relaxation of rat duodenum. Furthermore, neurotensin possesses other interesting biological properties, *e. g.*, intravenous injection of neurotensin in rats brings about an increase in plasma ACTH levels (2), an elevation of plasma luteinizing hormone and follicle-stimulating hormone levels (3), hyperglycemia (3–7) and hyperglucagonemia (5–7). There are controversial reports concerning the effect of neurotensin on insulin secretion. Brown *et al.* (5, 6) reported that neurotensin reduced insulin levels in the peripheral blood, but not in the portal blood in rats. Nagai and Frohman (7) stated that neurotensin stimulated minimal insulin response in normal rats and markedly enhanced insulin response in rats with adrenal autotransplantation. This paper describes the effects of large doses of synthetic neurotensin on the endocrine pancreas in normal and hypophysectomized dogs.

MATERIALS AND METHODS

Nine healthy, adult mongrel dogs weighing 10 to 16 kg were fasted overnight. The animals were anesthetized with sodium pentobarbital at 25 mg/kg BW. A T-shaped tube was implanted in the superior pancreaticoduodenal (pancreatic) artery 1 to 2 cm proximal from the junction of the right gastroepiploic artery for injection of synthetic neurotensin. Synthetic neurotensin was obtained from Protein Research Institute of Osaka University, Osaka, Japan. Another T-shaped tube was implanted in the superior pancreaticoduodenal (pancreatic) vein. One femoral vein was cannulated with polyethylene tubing. In order to observe the effects of neurotensin on the endocrine pancreas of the hypophysectomized animals without any replacement therapy, the same surgical procedure was performed on six hypophysectomized dogs on the 10th to 14th day after hypophysectomy. Hypophysectomy was confirmed by postmortem examination after the experiment in each dog.

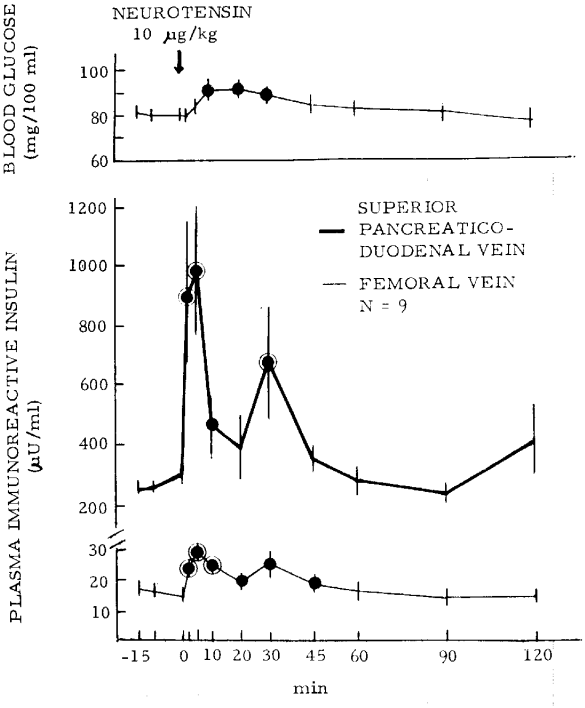

Fig. 1. Mild hyperglycemic response was observed after 10 μg/kg neurotensin injection into the superior pancreaticoduodenal artery in normal dogs. Biphasic insulin response with a rapid and sharp initial elevation and second significant elevation at 30 min post-injection was noted in the superior pancreaticoduodenal vein. Significant differences between basal and neurotensin induced blood glucose and insulin levels are denoted by ◎ $P < 0.01$ and ● $P < 0.05$.

A dose of 10 μg/kg of synthetic neurotensin in 10 μg/ml physiological saline was rapidly administered into the pancreatic artery. Blood pressure of the femoral artery and impedance in the pancreatic tissue were determined by the method of Tasaka and Akagi (8). Impedance change reflects blood flow in the pancreatic tissue; decrease in the impedance indicates increase in the blood flow. Blood samples were collected from the pancreatic vein and the femoral vein. Blood glucose was determined by the glucose oxidase method. Plasma insulin (9) and glucagon (10) were assayed radioimmunologically. Plasma cortisol was measured by radioimmunoassay (11). The mean values and the standard errors of the means were calculated. The Wilcoxon signed-rank test was employed for statistical analysis of the differences of means in paired samples. The Mann-Whitney U test was used to determine statistical differences for non-paired values.

RESULTS

Normal dogs. In a control study of 5 normal dogs, blood glucose, plasma insulin and glucagon in pancreatic and femoral vein showed constant mean value during the experiment after administration of physiological saline (one ml/kg) into the pancreatic artery.

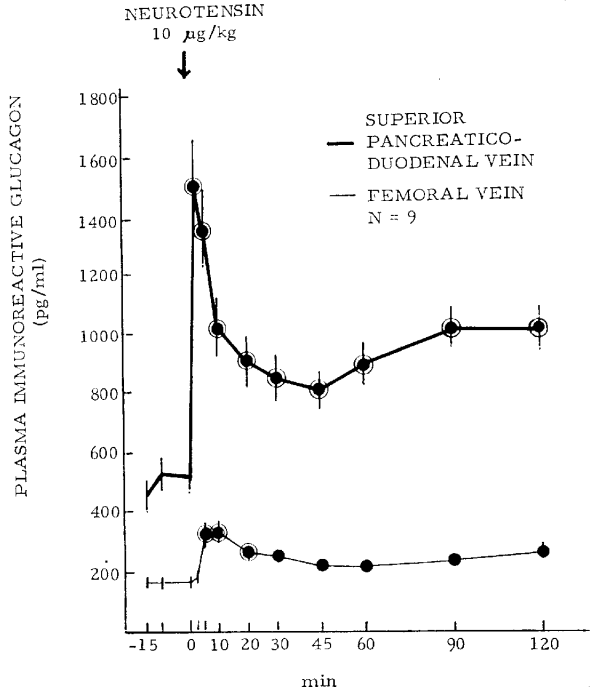

Fig. 2. A sharp and prominent glucagon response was noted in the superior pancreaticoduodenal and femoral vein immediately after neurotensin injection. Plasma glucagon levels remained at significantly high levels during the test. Significant differences between basal and neurotensin induced glucagon levels are denoted by ◉ $P < 0.01$ and ● $P < 0.05$.

Fig. 3. The upper figure shows change of blood pressure of the femoral artery after 10 μg/kg neurotensin administration into the superior pancreaticoduodenal artery in one dog. The blood pressure was lowered to 40% of the initial value at 2 min and returned gradually to the initial level at the end of the experiment. B.P.: blood pressure. The lower figure shows impedance change of the pancreatic tissue after 10 μg/kg neurotensin administration into the superior pancreaticoduodenal artery. Decreasing impedance is recorded as a down ward deflection and indicates increasing blood flow. Calibration of 5Ω is shown on the right-hand side. IMP: impedance.

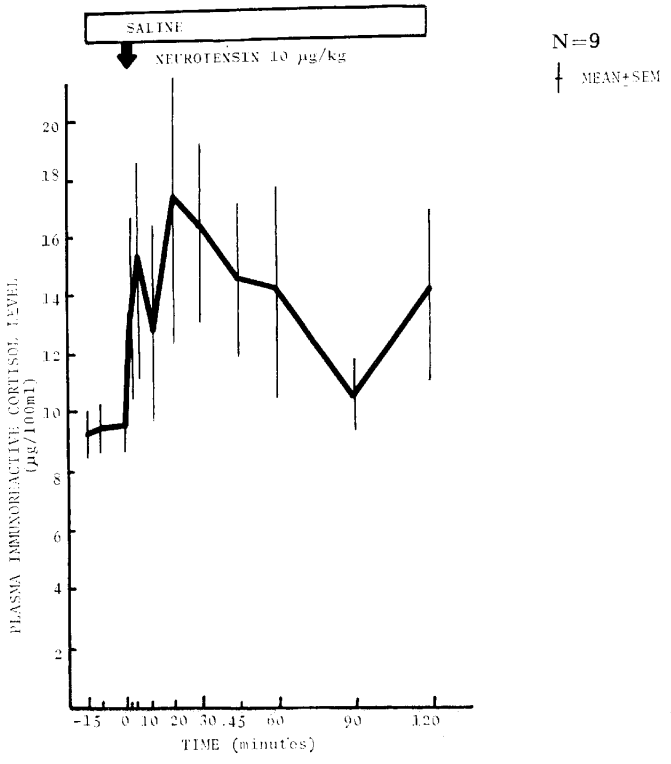

Fig. 4. Plasma cortisol level was rapidly increased after neurotensin administration and reached a peak at 5 min, but the elevation is statistically not significant.

The blood glucose level of the femoral vein was elevated to a peak with an average increase of 114% at 10 min after neurotensin administration (Fig. 1). A rapid and sharp insulin response was observed in pancreatic and femoral veins immediately after neurotensin administration. A biphasic insulin response with the second peak at 30 min was noted in the pancreatic vein. Neurotensin administration provoked a sharp and rapid secretory response of plasma glucagon in the pancreatic vein (Fig. 2). Plasma glucagon levels in the pancreatic vein remained significantly elevated with the nadir at 45 min. Plasma glucagon levels in the femoral vein reached a peak at 10 min and remained at significant high levels with the nadir at 60 min during the test. Blood pressure was lowered to 40% of initial value at 2 min after neurotensin administration and returned gradually to the initial level at the end of the experiment (Fig. 3). After a tiny

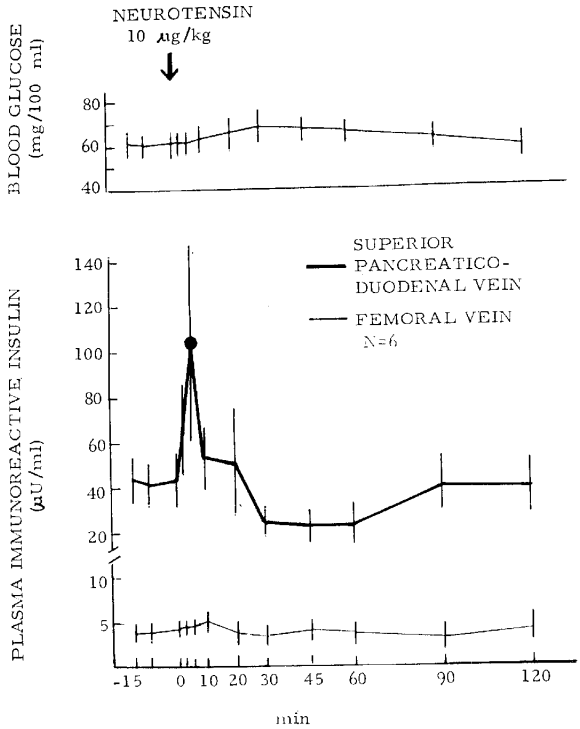

Fig. 5. In hypophysectomized dogs, no significant hyperglycemia was observed after 10 μg/kg BW neurotensin injection. Basal insulin levels were suppressed and a small and transient insulin response to neurotensin was followed by more lowered insulin level in the superior pancreaticoduodenal vein. There was no definite elevation of the femoral insulin level. Significant differences between basal and neurotensin induced blood glucose and insulin levels are denoted by ● $P < 0.05$.

and immediate decrease, impedance in the pancratic tissue transiently and sharply increased at 5 min after injection, and this was followed by a slow decrease for 20 min. The plasma cortisol level was elevated after neurotensin administration but the change was not significant (Fig. 4).

Hypophysectomized dogs. The initial blood glucose level in hypophysectomized dogs was decreased to 74% of the normal level and neurotensin administration resulted in no significant elevation of blood glucose (Fig. 5). Basal levels of plasma insulin in the pancreatic vein and femoral vein were lower than in the normal dogs. A small insulin response was observed in the pancreatic vein at 5 min after injection and was followed by much lower levels at 30 to 60 min. There was no increase of insulin in the femoral vein. The basal plasma glucagon level in the pancreatic vein was lower and its response to neurotensin was blunted (Fig. 6). An insignificant glucagon respone to neurotensin was noted in the femoral vein.

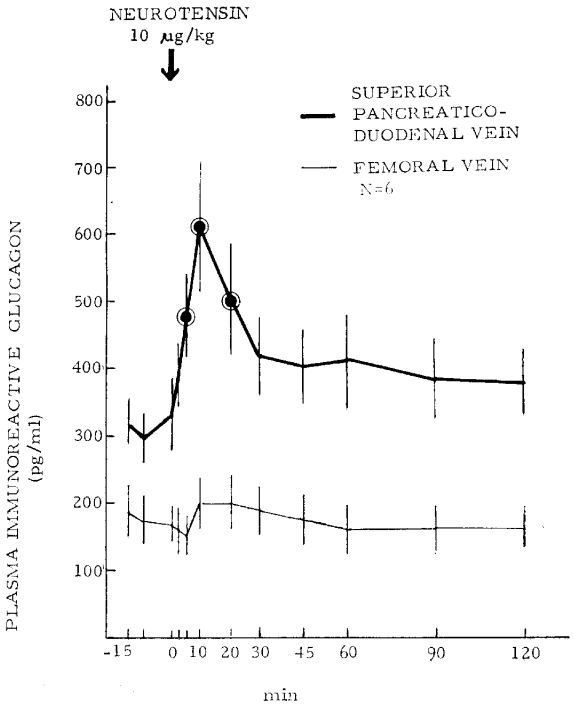

Fig. 6. Basal level of glucagon in the superior pancreaticoduodenal vein was slightly suppressed in hypophysectomized dogs. Delayed and blunted glucagon response was observed in the superior pancreaticoduodenal vein after neurotensin administration. Significant differences between basal and neurotensin induced glucagon levels are denoted by ◉ $P < 0.01$.

DISCUSSION

In the present study, a large amount of neurotensin was administered to observe the pharmacological effect of the peptide on normal and hypophysectomized dogs.

In normal dogs, blood glucose reached a peak at 10 min after injection of neurotensin into the pancreatic artery. Neurotensin has been found to produce a transient hyperglycemia within minutes after intravenous injection in anesthetized rats (3, 4). There was a linear dose response relationship over the range of 20–200 p moles/100 g (glucose 18–145 mg/100 ml) 15 min after injection. The peptide caused a fall in liver glycogen and a 7-fold increase in the activities of glycogen phosphorylase.

The present study showed that a large dose of neurotensin produced rapid increases of plasma insulin and glucagon concentrations after administration into the pancreatic artery. Insulin levels in the pancreatic vein showed a biphasic increase. The mechanism of the biphasic insulin secretion of neurotensin is unknown. In no respect was the increase found in plasma cortisol level statistically significant in this study, but the second peak might be evoked by ACTH or corticosteroids stimulated by neurotensin (2). Blood flow which was reflected by impedance of the pancreatic tissue decreased transiently for the first 10 min. However, the net increase of insulin and glucagon output into the pancreatic vein seem to be significant because of a prominent increase in the concentration of the peptides. Neurotensin appeared more potent to inducing hyperglucagonemia in this study. Our data suggest that neurotensin has a direct action on the endocrine pancreas in anesthetized dogs.

It has been reported that hypophysectomized animals showed decreased blood glucose and serum insulin levels (12–14) and increased serum glucagon levels (14). Carraway and his colleagues (3) demonstrated that the hyperglycemic response in hypophysectomized rats (4 days after operation) was less sensitive to neurotensin, perhaps as a consequence of their diminished liver glycogen levels. The liver glycogen level as well as the hyperglycemic response were partially restored by cortisone replacement indicating that the diminished responses were secondary effects following hypophysectomy but that the presence of the pituitary was not required. Those investigations led to the conclusion that liver glycogen might be subject to regulation by a mechanism that involved this peptide. In the present study, there was no elevation of blood glucose after administration of a large amount of neurotensin in hypophysectomized dogs. Responses of insulin and glucagon secretion to neurotensin were also suppressed as compared with those observed in normal dogs. There was no second peak elevation of insulin in hypophysectomized dogs. This might be due to no secre-

tion of ACTH and decreased response of corticosteroids after hypophysectomy. Hypophysectomy resulted in a greater inhibitory effect upon the endocrine pancreas.

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