Hyperglycemic Effect of Neurotensin

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YAWATA, Y., YAMATANI, K., TOMINAGA, M., EBITANI, I., HARA, M. and SASAKI, H. Hyperglycemic Effect of Neurotensin. Tohoku J. exp. Med., 1984, 143(2), 185-196 — Mechanisms of hyperglycemic action of neurotensin were investigated in anesthetized dogs. The intravenous administration of neurotensin 1.0 μg/kg for 5 min induced an immediate decrease in arterial blood pressure and increases in levels of blood glucose, glucagon and insulin. Although blood levels of glucagon and insulin were greatly reduced and not elevated by neurotensin in the presence of somatostatin, the response of blood glucose to neurotensin was similar to that in the absence of somatostatin. The rise in blood glucose produced by intraportal injection of neurotensin was not greater than that produced by injection of the same dose of neurotensin into the femoral vein. The increments of glucagon and insulin secretion caused by the intraportal injection were also the same as those produced by the peripheral injection. Participation of antihypotensive mechanisms in the neurotensin-induced hyperglycemia was investigated by use of α-adrenoceptor blockade and baroceptor denervation. Only the combination of somatostatin and α-adrenoceptor blockade or the denervation of baroceptors could suppress the hyperglycemic response to neurotensin. Stimulation of the secretion of anterior pituitary hormones by neurotensin infusion could not be recognized in the present experiments. These results suggested the following: 1) both glucagon and catecholamines may contribute to neurotensin-induced hyperglycemia, 2) neurotensin does not directly act on the liver, 3) catecholamine response could be mediated by baroceptor stimulation through hypotension, and 4) the hyperglycemic effect of anterior pituitary hormones does not participate in neurotensin-induced hyperglycemia. ——— neurotensin; hyperglycemia; hypotension; glucagon secretion; baroceptor-mediated catecholamine response

Neurotensin is a tridecapeptide originally isolated from extract of the bovine hypothalamus (Carraway and Leeman 1971, 1973) and recently from that of the human, rat, rabbit and dog gut (Carraway and Leeman 1975a, b, 1976; Kitabgi and Freychet 1978) and the human hypothalamus (Buchan et al. 1978). The structure of this peptide has been determined to be pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Try-Ile-Leu-OH (Carraway and Leeman 1975a).

It has been reported that neurotensin has many biological effects such as hypotension, vasodilatation, cyanosis, increased vascular permeability, hypother-
mia, effects on various smooth muscle preparations (Carraway and Leeman 1973, 1976; Bissete et al. 1976; Kitabgi et al. 1976; Segawa et al. 1977; Kitabgi and Freychet 1978; Loosen et al. 1978) and increases in plasma levels of anterior pituitary hormones and cortisol (Carraway 1972; Carraway and Leeman 1976; Carraway et al. 1976; Rivier et al. 1977; Kamanishi et al. 1978). In addition, neurtensin has been found to induce hyperglycemia in the rat and dog (Brown and Vale 1976; Brown et al. 1976; Nagai and Frohman 1976; Rosell et al. 1976; Ukai et al. 1977). This hyperglycemic effect seemed to be due to stimulation of hepatic glycogenolysis by its direct or indirect action. Carraway et al. (1976) suggested a direct action on the liver based on observations that neurtensin activates hepatic glycogen phosphorylase and reduces hepatic glycogen content. On the other hand, Patton et al. (1976) suggested that the hyperglycemic effect might be mediated by such a decrease in insulin, and/or an increase in glucagon. Hyperglucagonemia and hypoinsulinemia were observed in anesthetized rats (Brown and Vale 1976; Brown et al. 1976), and moderate hyperinsulinemia and exaggerated hyperglucagonemia were also seen in dogs (Ukai et al. 1977).

In short, neurtensin induces increased levels of both pancreatic hormones in portal venous and systemic venous blood, and overwhelming secretion of glucagon, compared to insulin. The present study was performed to determine whether or not neurtensin-induced hyperglycemia is due to direct action.

**Materials and Methods**

Both femoral veins and the external jugular vein on one side of adult mongrel dogs weighing 10–15 kg were catheterized, under pentobarbital anesthesia (25 mg/kg i.v., supplemented when necessary). About 1 hr after operation separate doses of neurtensin, dissolved in 2 ml of 0.9% saline, were infused into the femoral vein on one side for 5 min using an infusion pump (Harvard Apparatus, Model 2681). Blood samples were obtained from the other femoral vein at the times indicated in the results.

Somatostatin (Protein Research Foundation, Osaka) dissolved in 70 ml of 0.9% saline was infused into the external jugular vein from 10 min before through 60 min after the start of neurtensin infusion at a constant rate of 0.5 μg/kg/min.

Five dogs were laparotomized and the portal vein was catheterized with a Medicut tube (16 gauge) via the splenic vein. The outer end of the catheter was brought outside the abdominal wall so that neurtensin could be infused externally. The common carotid artery was exposed in the neck and an electromagnetic flow probe (Nihon Kohden, FB-050) of appropriate size was placed on the artery.

The α-adrenoceptor blocker, phentolamine (Ciba), dissolved in 60 ml of 0.9% saline was infused alone or concomitantly with somatostatin at a constant rate of 0.1 mg/min in the same manner as previously described for somatostatin infusion.

Concerning the baroceptor experiments, the anterior neck was opened by a midline incision, and both carotid arteries were exposed up to the point of bifurcation. The vagosympatic nerves which descended alongside the carotid arteries were carefully freed, and then initially crushed and finally severed. Similarly, both sinus nerves (the glossopharyngeal nerves), being merely fine fibers entering the arterial wall, were cut and pinched around the nerve tissue sufficiently. After those manipulations the carotids were returned gently and the anterior neck was completely closed by suture.

The blood pressure in the carotid artery was measured through a teflon catheter
The plasma glucose level was determined by the glucose oxidase method (Beckmann glucose analyzer, ERA-2001). The plasma glucagon, insulin, GH, ACTH and cortisol levels were measured by radioimmunoassay using Unger's 30K antibody, insulin immunoassay kit (Dinabot), canine GH radioimmunoassay (Tsushima's methods, Tsushima et al. 1971) ACTH kit (CIS) and a cortisol radioimmunoassay kit (Eiken, Tokyo), respectively.

Data were analyzed by the Student's t-test.

RESULTS

Arterial blood pressure

With infusion of neurotensin at a rate of 1.0 μg/kg/min both systolic and diastolic arterial pressures decreased immediately, reaching their nadir about 3 min after the start of infusion (Fig. 1) and returning to the control level gradually within 45 min after the cessation of infusion. Infusion of neurotensin at rates of 0.1-10.0 μg/kg/min for 5 min caused a dose-dependent decrease in mean arterial blood pressure and had no effect at a rate of 0.01 μg/kg/min, as shown in Fig. 2. At the peak effect mean arterial blood pressure was decreased to 86±6% (mean ± s.e.) of the control at 0.1 μg/kg/min and 70±7% at 1.0 and 10.0 μg/kg/min. Despite marked hypotension, neither the heart rate nor the blood flow through the common carotid artery changed significantly during or after infusion of neurotensin at rates of 0.1-10.0 μg/kg/min.

Changes in blood glucose levels

Infusion of neurotensin at 0.1-10.0 μg/kg/min for 5 min caused a dose-dependent rise in the blood glucose levels. Blood glucose levels began to increase 8 min after the start of neurotensin infusion, with a peak at 20 to 30 min, returning to the control level in 60 min (Fig. 3). Increases of blood glucose at its peak values were 7.2±8.6%, 3.8±3.4%, 25.8±7.8% and 33.7±3.9% at infusion rates of 0.01, 0.1, 1.0 and 10.0 μg/kg/min, respectively.
Fig. 2. Changes in mean arterial blood pressure. ▲ ▲, saline, n = 4; ● ●, neurotensin 0.01 μg/kg/min, n = 4; ○ ○, 0.1 μg/kg/min, n = 4; ■ ■, 1.0 μg/kg/min, n = 6; ○ ○ ○, 10.0 μg/kg min, n = 5.

Fig. 3. Changes in blood glucose levels. ▲ ▲, saline, n = 4; ● ●, neurotensin 0.01 μg/kg/min, n = 3; ○ ○, 0.1 μg/kg/min, n = 4; ■ ■, 1.0 μg/kg/min, n = 8; ○ ○ ○, 10.0 μg/kg/min, n = 6.
Changes in plasma levels of glucagon and insulin

With infusion of neurotensin at a rate of 1.0 μg/kg/min for 5 min, a dose high enough to cause hypotension and hyperglycemia, insulin increased to a peak of 43.3 ± 14.6 μU/ml (p < 0.05) at 1 min followed by a rapid decrease for 8 min and then a gradual decrease to the control level at 45 min (Fig. 4). The level of glucagon increased slowly with a peak of 414.8 ± 29.7 pg/ml (p < 0.025) at 5 min, returning to the control level at 60 min. The response of glucagon was more pronounced than that of insulin.

The time course of change in blood glucose was closer to that of glucagon than that of insulin. In this respect, neurotensin-induced hyperglycemia appeared to be due to stimulation of glucagon secretion.

Effect of somatostatin

In order to determine whether or not neurotensin-induced hyperglycemia was produced by stimulation of glucagon secretion, the effects of somatostatin on blood glucose, glucagon and insulin levels were examined. When somatostatin alone was infused at a rate of 0.5 μg/kg/min, plasma insulin and glucagon levels

![Graphs showing changes in blood glucose, glucagon, and insulin levels](image)

**Fig. 4.** The effect of neurotensin (1.0 μg/kg/min) on the levels of blood glucose (BS), plasma glucagon (GI) and insulin (IRI). ○⋯⋯⋯○, saline, n = 4; ●⋯⋯⋯●, neurotensin, n = 8. *p < 0.05, †p < 0.025.
decreased below the sensitivity of radioimmunoassay within 2 min, and the blood glucose level gradually decreased throughout the infusion.

Five min after the start of somatostatin infusion, administration of neurotensin was performed as mentioned above.

Although the levels of glucagon and insulin remained very low, the hyperglycemic effect of neurotensin was still observed (Fig. 5).

The hyperglycemic response was statistically indistinguishable from that without somatostatin. Thus, it seemed that the release of glucagon contributed only partially to hyperglycemia. Consequently, it was suggested that hyperglycemia may be caused not only by stimulation of glucagon secretion but also by other factors such as the direct action on the liver, stimulation of catecholamine or corticosteroid secretion.

Changes in GH, ACTH and cortisol

To examine the effect of neurotensin on the diabetogenic hormones, changes in plasma levels of GH, ACTH and cortisol were investigated.

These levels, although fluctuating, remained at the initial levels, as shown in Fig. 6. Thus, GH, ACTH or cortisol could not be involved in neurotensin-induced hyperglycemia in the present experiments.

Effect of intraportal infusion of neurotensin

To determine the action of neurotensin on the liver, an infusion into the
Fig. 6. Changes in plasma ACTH, cortisol and GH during the infusion of neurotensin.

○-○, saline, n = 3; •-•, neurotensin 1.0 μg/kg/min, n = 3.

Fig. 7. Changes in blood glucose, plasma GI and IRI levels by the infusion of neurotensin 1.0 μg/kg/min into the portal vein. •-•, n = 4; ○-○, saline, n = 4. *p < 0.05, †p < 0.025.
portal vein was performed through a catheter inserted into the splenic vein. The rise in blood glucose caused by intraportal infusion of neurotensin was not greater than that obtained with infusion of the same dose of neurotensin into femoral vein, whether somatostatin was concomitantly infused with the external jugular vein or not. Increases in glucagon and insulin caused by neurotensin and suppression of both peptides by somatostatin were nearly the same, whether neurotensin was infused into the femoral vein or the portal vein. Thus, a direct action of neurotensin on the liver could be ruled out (Fig. 7).

Effect of α-adrenoceptor blockade

Five min after the start of infusion of phentolamine at a rate of 0.1 mg/min, neurotensin was administered as described above.

Plasma glucose response to neurotensin was enhanced by α-adrenoceptor blockade, with a peak of 147.8 ± 7.8% at 20 min, remaining at a high level even 1 hr later. The level of glucagon increased to 161.7 ± 18.5% at 8 min, and the level of insulin to 130.2 ± 52.4 μU/ml at 1 min. With concomitant infusion of somatostatin at 0.5 μg/kg/min and phentolamine at 0.1 mg/min started 5 min prior to the neurotension infusion, there was no elevation of glucose, glucagon or

![Fig. 8. Changes in blood glucose GI and IRI levels by the infusion of neurotensin alone 1.0 μg/kg/min, •—•, n = 6; neurotensin and phentolamine 0.1 mg/min, •—•, n = 3; and neurotensin, phentolamine and somatostatin ○——○, n = 4.](image-url)
Effect of denervation of baroceptors

One hr after the denervation of baroceptors, infusion of neurotensin resulted in increased blood glucose levels with a time course similar to that without denervation, having a peak of 118.6\pm6.3\% at 30 min, and transient elevations of glucagon and insulin at 3 and 5 min (Fig. 9). With concomitant infusion of somatostatin, the response of glucose levels ceased with complete suppression of plasma glucagon and insulin levels, as with phentolamine and somatostatin.

DISCUSSION

In the present study, the intravenous administration of neurotensin into anesthetized adult dogs induced an initial increase of plasma insulin level in less than 1 min to a peak to 40 \(\mu\)U/ml followed by prolonged increase of glucagon to a peak of about 400 pg/ml around 4 min. This marked hyperglucagonemia might induce the hyperglycemia observed at 20 to 30 min.

Brown and Vale (1976) suggested that in ether-anesthetized rats neurotensin-induced hyperglycemia would result from hyperglucagonemia together with hypoinsulinemia. Our results did not, however, agree with theirs partly due to differences in anesthesia and experimental animal species. Although with concomitant infusion of somatostatin plasma levels of glucagon and insulin were suppressed within 1 min and were unchanged by neurotensin, hyperglycemia was
still observed to the same extent without somatostatin.

Therefore, some other factors must be involved in neurotensin-induced hyperglycemia. The extent of hyperglycemia caused by the infusion of neurotensin into the portal vein was not greater than that caused by injection into the femoral vein. These results did not support the idea of a direct action of neurotensin on the liver.

This view was supported by Yamatani et al. (1980) who revealed, using radioreceptor assay and adenylate cyclase assay of the rat liver cell membrane, that neurotensin neither acted on the liver directly nor had an additive effect with glucagon on the liver.

Carraway et al. (1976) directed attention to the condition of increased vascular permeability that resulted in a rapid increase in the hematocrit and hyperglycemia. So it seemed that neurotensin-induced hypotension and hypovolemia would in turn stimulate the antihypotensive mechanisms such as the catecholamine and/or ACTH-cortisol systems. Lorenzen and Ganong (1967) reported that a decrease in blood pressure caused the secretion of some pituitary hormones.

However, there was no evidence of the stimulation of pituitary hormones, such as ACTH and GH, in the present study. Furthermore, many authors have mentioned the relationship between sudden alteration of blood pressure and neuronal reflex. Baroceptors, which were mainly distributed to the carotid sinus and aortic arch, are recognized as being sensitive to the reduced systemic arterial blood pressure. Baroceptors located in the carotid sinus and aortic arch are activated by a decrease in systemic arterial blood pressure and trigger suitable reflexes to maintain it.

Kaindle and von Euler (1951) reported that in their cat experiments the pressor reflex was evoked by lowering systemic arterial blood pressure at the level of the carotid sinus and then catecholamines were liberated from the suprarenals.

Niijima (1976, 1977) reported that an increase in blood glucose level occurred within 10 min after withdrawal of blood in the rabbit, but that the glycemic responses were prevented by cutting the depressor nerve. He also observed that the frequency of the hepatic efferent nerve impulses was increased by blood withdrawal before but not after denervation. Consequently, he postulated that the glycemic response was due to catecholamine secretion through baroceptor excitation during hypotension.

Lindsey et al. (1974, 1975) found that exaggerated hyperglucagonemia and hyperglycemia in dying patients with severe traumatic shock and in the rapidly exsanguinated dog could be prevented by the administration of propranolol. These results also support the idea of the contribution of catecholamines to hyperglycemia. Hypotensive mediators, such as bradykinin, prostaglandins, etc. were not found to have a hyperglycemic effect in themselves.

Thus, it was concluded that 1) neurotensin-induced hyperglycemia is partial-
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1) Hyperglycemia is likely mediated by an increase in plasma glucagon, 2) hyperglycemia is not caused by a direct action of neurotensin on the liver, 3) hyperglycemia is partially caused by hypotension-induced catecholamine response, 4) hypotension-induced catecholamine response is mediated by a baroceptor mechanism and 5) the stimulation of anterior pituitary hormones did not occur.

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References