The Histaminergic Mechanism of Neurotensin-induced Glucagon Release from Isolated Rat Pancreatic Islets

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Abstract

Isolated rat pancreatic islets were preincubated in a medium with 16.7 mM glucose and incubated with 5.5 mM glucose. Both histamine (100 μ M) and neurotensin (100 nM) stimulated glucagon release from the isolated islets, but not insulin release. The stimulation of glucagon release occurred in the presence of 10 and 100 nM neurotensin, while the release of insulin was inhibited in the presence of 1 and 10 nM neurotensin. The neurotensin-induced glucagon release was completely inhibited by 1 mM metiamide, an histamine H₂-receptor antagonist, added to the incubation medium and not inhibited by 1 mM diphenhydramine, an histamine H₁-receptor antagonist.

The results indicate that the histaminergic mechanism, including the H₂-receptor system, may be involved in neurotensin-induced glucagon release from the endocrine pancreas.

Neurotensin is a tridecapeptide originally isolated from bovine hypothalamus (Carraway *et al.*, 1973) and from bovine small intestine (Carraway *et al.*, 1976; Kitabgi *et al.*, 1976). This peptide has been shown to induce hyperglycemia (Brown *et al.*, 1976; Carraway *et al.*, 1976; Ukai *et al.*, 1976; Nagai *et al.*, 1976; Sasaki *et al.*, 1980), in association with low insulin and high glucagon levels in systemic plasma of rats (Brown *et al.*, 1976) and with high insulin and high glucagon levels in the portal vein plasma (Ukai *et al.*, 1977) or in the pancreatic vein plasma (Kaneto *et al.*, 1978) of dogs.

There are *in vitro* studies indicating the direct action of neurotensin on isolated rat pancreatic islets to release glucagon (Moltz *et al.*, 1977; Shibata *et al.*, 1978; Dolais-Kitabgi *et al.*, 1979; Shibata *et al.*, 1980) and somatostatin (Dolais-Kitabgi *et*

al., 1979). Its effect on insulin release has been variously reported as inhibitory (Moltz *et al.*, 1977) and stimulatory (Dolais-Kitabgi *et al.*, 1979). Although the mechanism of the neurotensin-induced release of pancreatic hormones is not fully understood, the histaminergic mechanism has been thought to be involved in neurotensin-induced hyperglucagonemia, based on *in vivo* studies in rats (Brown *et al.*, 1976; Nagai *et al.*, 1978).

In the present study, we investigated such an histaminergic mechanism of neurotensin-induced glucagon release, using the isolated pancreatic islet system of rat. A part of this study has been reported before (Ukai *et al.* 1980).

Materials and Methods

Isolation and incubation of pancreatic islets

Female Sprague-Dawley rats ranging in weight

Table 1. Effect of Arginine, Neurotensin and Histamine on Glucagon and Insulin Release from Isolated Pancreatic Islets of Rats.

Group	Glucagon Release (pg/islet/30 min)	Insulin Release (μ U/islet/30 min)
Control (20)	66.1 $\pm$ 4.8	13.2 $\pm$ 0.7
Arginine 20 mM (4)	247.9 $\pm$ 43.0*	25.9 $\pm$ 1.4*
Neurotensin 100 nM (9)	127.5 $\pm$ 12.7*	16.4 $\pm$ 1.0
Histamine 100 μ M (4)	119.5 $\pm$ 10.0*	10.7 $\pm$ 0.7

Values are shown as the mean $\pm$ SE. Numbers of experiments are shown in parentheses. Significant difference vs. controls is indicated by * ($p < 0.01$).

from 150 to 250 g were given free access to food and water before the experiments. Under intraperitoneal anesthesia with Nembutal, laparotomy was performed. Pancreatic islets were isolated using the collagenase technique of Lacy and Kostianovsky (1967). Ten islets in each plastic microbeaker were preincubated for 60 min in 3 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 16.7 mM glucose and incubated for 30 min in 3 ml of Krebs-Ringer bicarbonate buffer containing 5.5 mM glucose with or without various test substances, at 37°C in an atmosphere of O₂-CO₂ (95:5, vol/vol). The microbeakers were shaken at 55 cycles per min throughout the preincubation and incubation periods.

Following incubation, 0.4 ml of the medium solution was collected into tubes containing 1000 KIU of aprotinin (gift from Bayer Company) and 2.4 mg of EDTA-2Na to prevent the degradation of glucagon and insulin. The samples were immediately frozen and kept at -20°C until assay. Immunoreactive glucagon (IRG) was measured by the dextran-coated charcoal method using 30-K antibody (Böttger *et al.*, 1973). Porcine glucagon (gift from Eli Lilly and Company) was used as the standard. Immunoreactive insulin (IRI) was measured by the solid phase method (Wide and Porath, 1966).

Neurotensin (Protein Research Foundation), diphenhydramine-HCl as an histamine H₁-receptor antagonist (Sigma Chemical Company), histamine-HCl and L-arginine-HCl (Nagoya Katayama Chemical Company) were obtained commercially. Metiamide as an histamine H₂-receptor antagonist was donated by the Smith, Kline & French Laboratories.

Analysis

Data were analyzed by Student's *t*-test, the criterion for significance being $p > 0.05$.

Results

In our *in vitro* system, L-arginine (20 mM) stimulated the release of IRG and

IRI from isolated pancreatic islets, values being significantly different from those of the control. Both histamine (100 μ M) and neurotensin (100 nM) stimulated IRG release without a significant effect on IRI release (Table 1).

The rate of IRG Release (pg/islet/30 min) from isolated islets was 68.9 ± 2.8 (mean $\pm$ SE) in the presence of 10 nM neurotensin and 118.7 ± 10.2 in that of 100 nM neurotensin (Fig. 1), significantly higher than the control value (52.8 ± 2.2). On the other hand, in the presence of 1 nM and 10 nM neurotensin the rate of IRI release (μ U/islet/30 min) was 10.6 ± 0.5 and 11.8 ± 0.4 , respectively, significantly lower than the control value (17.3 ± 0.9) and that of 100 nM neurotensin (17.2 ± 0.8).

As shown in Fig. 2, neurotensin-induced IRG release was completely blocked by 1 mM metiamide (51.0 ± 5.8), but not by 1 mM diphenhydramine (140.8 ± 8.3). Neither diphenhydramine nor metiamide significantly affected the IRI release in the presence of 100 nM neurotensin.

Discussion

The present *in vitro* study has demonstrated the stimulatory effects of neurotensin and histamine on pancreatic glucagon release in rats. This is consistent with the previous *in vivo* studies which showed neurotensin-induced hyperglucagonemia in rats (Brown *et al.*, 1976) and dogs (Ukai

Fig. 1. Effect of neurotensin (NT) in the concentrations of 1, 10 and 100 nM on glucagon release and insulin release from isolated islets. Values are shown as the mean $\pm$ SE. Numbers of experiments are shown in parentheses. Significant difference vs. controls (CT) is indicated by one ($p < 0.05$) and two asterisks ($p < 0.01$).

Fig. 2. Effects of diphenhydramine-HCl (DPH) and metiamide (MTA) on neurotensin (NT)-induced glucagon release from isolated islets. The concentrations of NT, DPH and MTA in the media were 100 nM, 1 mM and 1 mM, respectively. Values are shown as the mean $\pm$ SE. Numbers of experiments are shown in parentheses. Significant difference vs. controls (CT) is indicated by an asterisk ($p < 0.01$).

et al., 1976). However, as we reported previously (Shibata *et al.*, 1978; 1980), the effect of neurotensin on glucagon release depends upon the concentration of glucose in the incubation medium, being rather inhibitory in a low glucose concentration (1.67 mM) and most stimulatory in a high glucose concentration (16.7 mM). This is inconsistent with the results obtained by Dolais-Kitabgi *et al.* (1979), which showed the inhibitory effect of neurotensin with the high concentration of glucose or arginine and the stimulatory effect with the low concentration (3 mM) of glucose on glucagon release from isolated rat islets. The fact that when the concentrations was less than 100 nM, neurotensin suppressed insulin release from the isolated islets (Fig. 1), may be compatible with the results of Moltz *et al.*, (1977) using 3- to 4-day-old Wistar rats, but inconsistent with those of Dolais-Kitabgi *et al.*, (1979), using adult Sprague-Dawley rats. Although it is difficult to explain the dis-

crepancies in the results obtained by various investigators, we tentatively assume that the high concentration of medium glucagon caused by neurotensin might stimulate insulin release. If this assumption is correct, no inhibition of insulin release by 100 nM neurotensin might be explained by this mechanism. It is likely that the effect of neurotensin on the release of pancreatic hormones from the isolated islets may differ, depending upon not only the concentration of glucose, insulin, glucagon and somatostatin in the medium but also the age or the physiological condition of rats used in the experiments. This concept may partly account for a seemingly dual effect of neurotensin on the release of pancreatic hormones obtained in our *in vitro* system.

A good deal of similarity between the biological properties of neurotensin and those of histamine in both the central and peripheral nervous systems has been shown from various aspects such as prolactin

release (Livertun *et al.*, 1976; Rivier *et al.*, 1977), hypothermia (Bissette *et al.*, 1976; Shaw, 1971; Green *et al.*, 1976), hypotension and increased capillary permeability (Carraway *et al.*, 1973; Beaven, 1976). Both neurotensin and histamine are known to be present in high concentrations in the hypothalamus and the gastrointestinal tract (Carraway *et al.*, 1976; Kitabgi *et al.*, 1976; Green, 1964; Beaven, 1976). Neurotensin or its specific receptors may be widely distributed, possibly even in the pancreas, and may regulate the secretions of insulin, glucagon and somatostatin by functioning as a neurotransmitter or as a local modulatory factor. It was found in the present study that histamine stimulated glucagon release from the isolated islets of rats (Table 1), and this is consistent with the results reported by Pontiroli *et al.* (1979). In addition, neurotensin-induced glucagon release was completely blocked by metiamide, an H_2 -receptor antagonist, but rather stimulated by diphenhydramine, an H_1 -receptor antagonist, which had been reported before (Itatsu *et al.*, 1979), being inconsistent with the results of Brown *et al.* (1976) and partially compatible with the those of Nagai *et al.* (1978). According to the present data on 30 min incubation, diphenhydramine was likely to accelerate neurotensin-induced glucagon release. One explanation is that the affinity of the H_2 -receptors for neurotensin might be relatively increased by blocking the H_1 -receptors. Another is that the incubation time for 30 min might be too short for the H_1 -receptor antagonist to exert a full effect on the pancreatic A-cell response to neurotensin. The latter concept seems to be supported by the study of Bruce *et al.* (1979), in which the antral-strip response to histamine was stimulated during initial exposure but inhibited on longer exposure to diphenhydramine, while the response was always inhibited by cimetidine, an H_2 -receptor antagonist. Data obtained in the present study may be interpreted, at

least, as indicating that the H_2 -receptors may be involved in mediating the neurotensin-induced glucagon release.

To elucidate the mechanism by which the H_2 -receptor antagonist suppresses the neurotensin-induced glucagon release in the present system, possibilities such as the presence of competitive antagonism for the neurotensin receptor (Lazarus *et al.*, 1977; Rioux *et al.*, 1980) and indirect inhibition of the messengers or mediators through the H_2 -receptors (Dismukes *et al.*, 1975; Feldman and Richardson, 1978; Rioux *et al.*, 1980) must be taken into consideration.

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