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Effects of Neuromedin B on Insulin and Glucagon Release from the Isolated Perfused Rat Pancreas

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Abstract

The effect of neuromedin B (NMB) on insulin and glucagon release was studied in isolated perfused rat pancreases. Infusion of NMB (10 nM, 100 nM and 1 μM) did not affect the insulin release under the perfusate conditions of 5.5 mM glucose plus 10 mM arginine and 11 mM glucose plus 10 mM arginine, although 10 nM NMB tended to slightly suppress it under the perfusate condition of 5.5 mM glucose alone. The degree of stimulation of insulin release provoked by the addition of 5.5 mM glucose to the perfusate was not affected by the presence of 10 nM NMB. The glucagon release was slightly stimulated by the infusion of 100 nM and 1 μM NMB but not by 10 nM NMB under the perfusate condition of 5.5 mM glucose plus 10 mM arginine. The effect of C-terminal decapeptide of gastrin releasing peptide (GRP-10) was also examined and similar results were obtained ; 10 nM and 100 nM GRP-10 did not affect insulin release and 100 nM GRP-10 stimulated glucagon release under the perfusate condition of 5.5 mM glucose plus 10 mM arginine.

The present results concerning glucagon release are consistent with the previous results obtained with isolated perfused canine and porcine pancreas. However, the results regarding insulin release are not. Species differences in insulin release are also evident with other neuropeptides such as substance P and the mechanism of such differences remains fo be clarified.

Two mammalian bombesin family peptides, neuromedin B (NMB) and C-terminal decapeptide of gastrin-releasing peptide (GRP-10), are both decapeptides which differ in their amino acid sequence at positions 3, 6 and 9 (Reeve *et al.*, 1983, Minamino *et al.*, 1983 ; Okada *et al.*, 1984). Both peptides stimulate gestrin release (Guo *et al.*, 1987), secretion of pancreatic juice (Namba *et al.*, 1986) and smooth muscle contraction (Broccardo *et el.*, 1975 ; Mayer *et al.*, 1986) in experimental animals as does GRP. Concerning pancreatic hormone release, our recent *in vivo* and *in vitro* studies have demonstrated that NMB and

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GRP-10 clearly stimulate insulin release in dogs and that glucagon release was slightly stimulated by higher doses of GRP-10 and not by NMB (Kawai *et al.*, 1988). On the other hand, in isolated perfused rat pancreases the stimulatory effect of NMB and GRP-10 on insulin release was not clear (Otsuki *et al.*, 1987) in contrast to our results with isolated canine pancreas perfusion. Our previous study demonstrated that substance P stimulated insulin release from the isolated perfused canine pancreas and inhibited it from the rat pancreas (Chiba *et al.*, 1985). Therefore, we have carefully examined the effect of NMB and GRP-10 on insulin and glucagon release from the isolated perfused rat pancreas.

Materials and Methods

Peptides

NMB and GRP-10 were synthesized by a solid phase method described previously (Mukai *et al.*, 1987), and proved to be at least 95% pure by analytical reverse phase high-performance liquid chromatography on a silica gel plate (Mukai *et al.*, 1987).

Pancreas perfusion

The pancreases were isolated from male Wistar rats, weighing about 250 g, under pentobarbital anesthesia after an overnight fast. The isolated pancreas was perfused by the method of Grodsky *et al.* (1963) with slight modification. A Krebs-Ringer bicarbonate solution containing 4% dextran T-70 (Pharmacia Fine Chemicals, Uppsala, Sweden), 0.2% bovine serum albumin (BSA), 5 mM each of pyruvate, fumarate and glutamate, 5.5 mM glucose, and 10 mM arginine was bubbled with a 95% O_2-5% CO_2 mixture at 37°C. The flow rate of the perfusate was 2 ml/min. The peptide was dissolved in 0.9% saline containing 0.2% BSA and administered through a side-arm syringe at a rate of 0.1 ml/min. The venous perfusate was collected at 1-min intervals and stored at −40°C until the assay.

Assays

Insulin and glucagon were assayed by RIA, according to the methods of Herbert *et al.* (1965) and Faloona and Unger (1974) with E-7 antibody (kindly donated by Dr. H. von Schenck), respectively. In the insulin assay, porcine insulin was used as a standard. In our laboratory, the interassay variations were 6.1% and 10.9% for insulin and glucagon, respectively and the intraassay variations were 7.3% and 6.5%, respectively.

Statistics

The data were analyzed by analysis of variance, and the Newman-Keuls procedure for multiple comparisons was performed. A P value of 0.05 or less was considered significant.

Results

Effects NMB on insulin and glucagon release from the isolated perfused rat pancreas with different perfusate conditions (Fig. 1).

At first, the effect of NMB was examined under the perfusate condition of 5.5 mM glucose plus 10 mM arginine because this condition is close to a physiological condition and the same perfusate was used in the dog pancreas perfusion to examine the effect of NMB (Kawai *et al.*, 1988). However, 10 nM NMB did not affect either insulin or glucagon release. Under the perfusate condition of 5.5 mM glucose alone, 10 nM NMB tended to slightly suppress insulin release. Under the perfusate condition of 11 mM glucose plus 10 mM arginine, 100 nM NMB did not affect insulin release but slightly stimulated glucagon release.

Effects of different doses of NMB on insulin and glucagon release from the isolated perfused rat pancreas (Fig. 2).

The effects of higher doses of NMB were examined under the perfusate condition of 5.5 mM glucose plus 10 mM arginine.

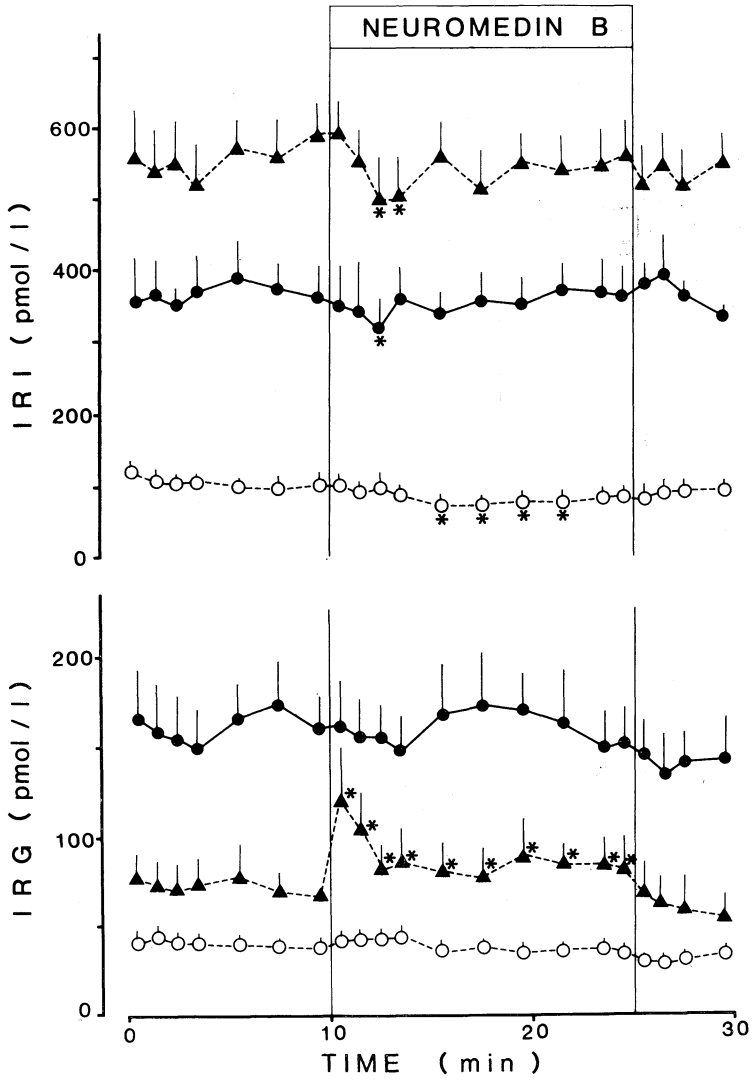

Fig. 1. Effects of NMB on insulin and glucagon release from the isolated perfused rat pancreas under different perfusate conditions. -●—●-; 10 nM NMB with the perfusate condition of 5.5 mM glucose plus 10 mM arginine (N=5), --○--○--; 10 nM NMB with the perfusate condition of 5.5 mM glucose (N=5), --▲--▲--; 100 nM NMB with the perfusate condition of 11 mM glucose plus 10 mM arginine (N=5). mean± SEM. *; $p<0.05$ vs. basal level (mean of preceding three points).

As shown in Fig. 2, 10 nM and 100 nM NMB did not affect insulin release although 1 μM NMB tended to slightly suppress it. Glucagon release was stimulated by both 100 nM and 1 μM NMB, although the stimulation was transitory.

Effect of NMB on the stimulation of insulin release by glucose from the isolated perfused rat pancreas (Fig. 3).

As an alternate type of experiment, the effect of 10 nM NMB on the stimulation of insulin release by 5.5 mM glucose was examined. When the degree of increase in glucose stimulated insulin release in the presence and absence of NMB was compared, it was found that 10 nM NMB did not have any effect.

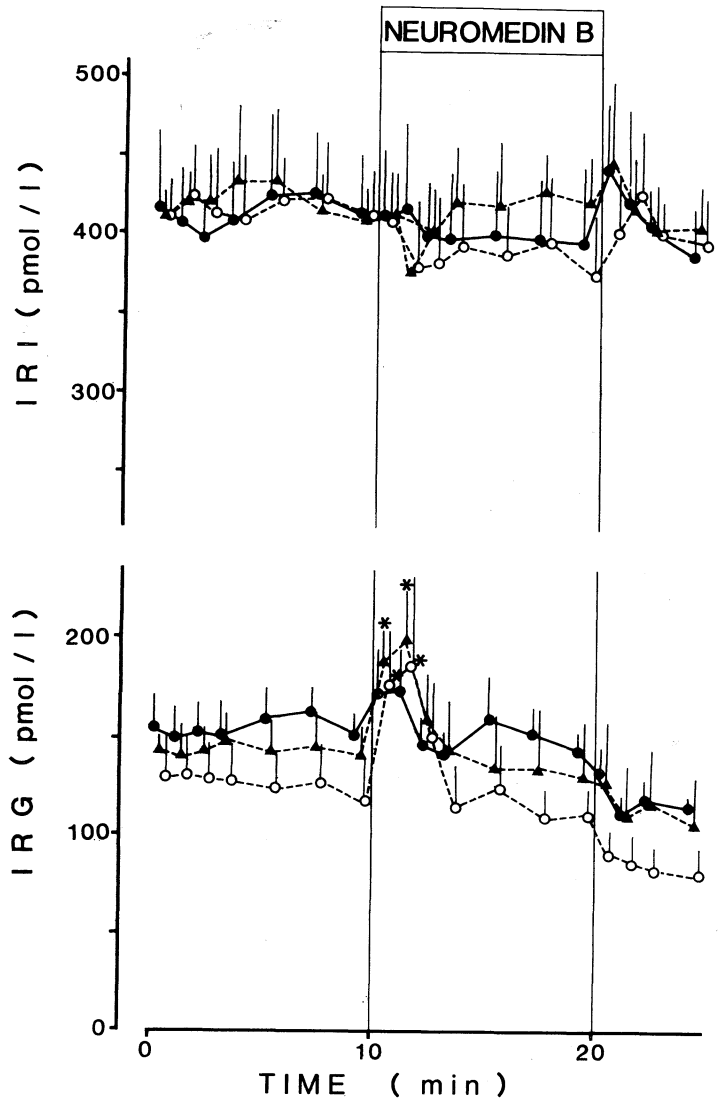

Fig. 2. Effects of different doses of NMB on insulin and glucagon release from the isolated perfused rat pancreas. -●—●- ; 10 nM NMB, (N=5), --▲--▲-- ; 100 nM NMB (N=5), --○--○-- ; 1 μM NMB (N=5), mean±SEM. The perfusate contained 5.5 mM glucose plus 10 mM arginine. * ; $p<0.05$ vs. basal level (mean of preceding three points).

Effects of GRP-10 on insulin and glucagon release from the isolated perfused rat pancreas. (Fig. 4).

The effect of another bombesin family decapeptide, GRP-10, was examined. 10 nM GRP-10 did not influence insulin release but tended to stimulate glucagon release under the perfusate condition of 5.5 mM glucose plus 10 mM arginine. 100 nM GRP-10 also did not affect insulin release and stimulated glucagon release under the perfusate condition of 11 mM glucose plus 10 mM arginine.

Discussion

In this paper, we demonstrate that NMB did not affect insulin release from the isolated perfused rat pancreas and that it

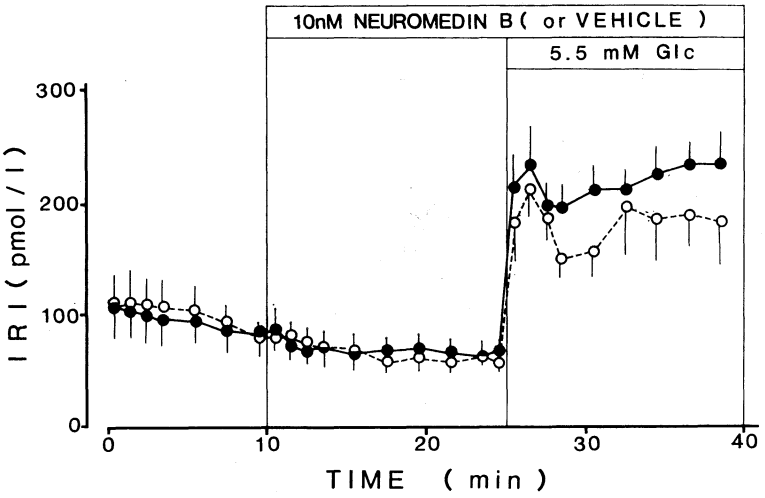

Fig. 3. Effect of NMB on the stimulation of insulin release by glucose from the isolated perfused rat pancreas. The basal perfusate contained 5.5 mM glucose. The increased insulin release in response to an additional 5.5 mM glucose in the presence and absence of 10 nM NMB was compared. -●—●-; 10 nM NMB (N=5), --○--○--; vehicle of NMB (0.9% saline containing 0.2% BSA, N=5), mean±SEM.

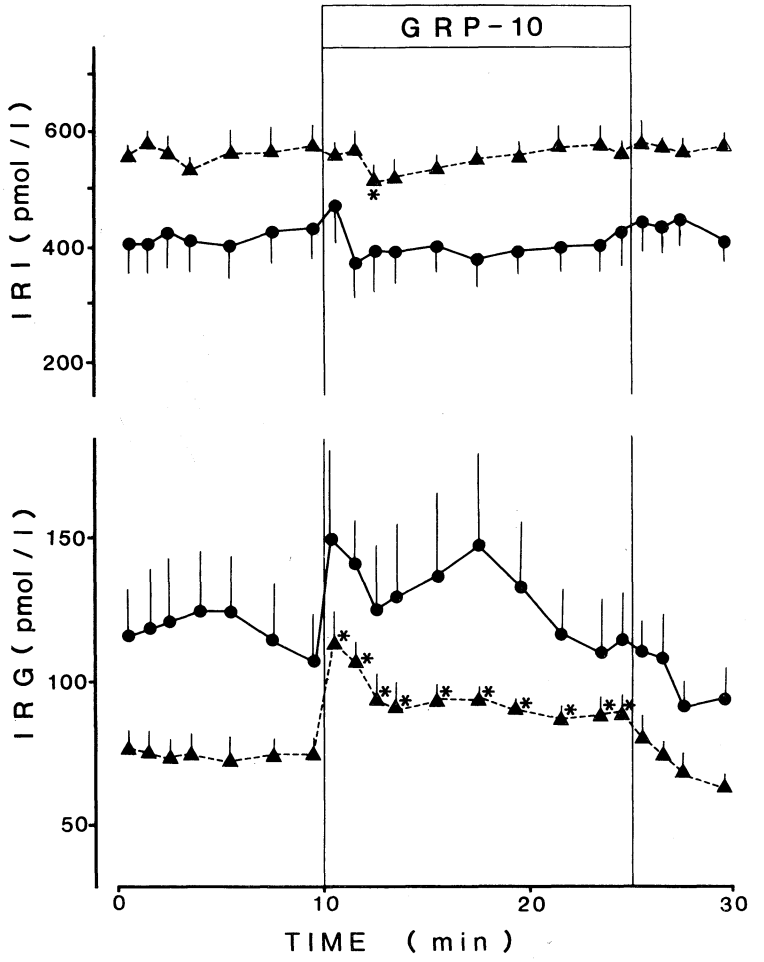

Fig. 4. Effects of GRP-10 on insulin and glucagon release from the isolated perfused rat pancreas. -●—●-; 10 nM GRP-10 with the perfusate condition of 5.5 mM glucose plus 10 mM arginine (N=4), --▲--▲--; 100 nM GRP-10 with the perfusate condition of 11 mM glucose plus 10 mM arginine (N=5), mean±SEM. *; $p<0.05$ vs. basal level (mean of preceding three points).

stimulated the glucagon release at pharmacological doses (100 nM～1 μM). GRP-10 also did not affect insulin release and stimulated the glucagon release.

Otsuki *et al.* (1987) have demonstrated that under the perfusate condition of 8.3 mM glucose, 1 nM GRP, 1 nM GRP-10 and 10 nM NMB provoke a transient (about 2 min) increase in insulin release and a successive slightly suppressive effect which terminates with a rebound-like increase. Except for an initial peak, our results were consistent with theirs, because we also found a slight suppression of insulin release and a rebound-like increase (Fig. 2). Taninato *et al.* (1978) demonstrated that bombesin (10^{-8}～10^{-6} M) inhibits insulin release from isolated pancreatic islets of rats incubated with high glucose medium. On the other hand, Martindale *et al.* (1982) reported that bombesin (10^{-9} M～10^{-7} M) did not affect basal insulin secretion from the isolated perfused rat pancreas but enhanced glucose-induced insulin secretion. In our study, 10 nM NMB did not enhance it (Fig. 3). The reason for this difference is unclear so far, although it is generally accepted that bombesin is more biologically potent than other bombesin family peptides.

In the *in vivo* study, a bolus injection of NMB (1 nmol/rat) or bombesin into the jugular vein of anesthetized rats caused a significant increase in plasma insulin by 30 min later but not within 10 min. Plasma glucagon and glucose did not change (Namba *et al.*, 1984). On the other hand, the intravenous infusion of GRP (4.3 pmol/min/rat) into the anesthetized rats increased basal plasma insulin, glucagon and glucose, and potentiated the plasma insulin response to glucose, while it inhibited the insulin response to arginine (Pettersson and Ahrén, 1988). There are some discrepancies between the two reports on rats.

In the dog pancreas perfusion experiment, we have found that the same vials of NMB and GRP-10 stimulate insulin release even with 100 pM (Kawai *et al.*, 1988). Knuhtsen *et al.* (1987) reported that 1 nM GRP stimulates insulin release from the isolated perfused porcine pancreas under the perfusate condition of 5 mM glucose.

Glucagon release was stimulated by 100 nM of NMB and GRP-10. In the dog pancreas perfusion, 10 nM GRP-10 stimulated glucagon release but 100 nM NMB did not (Kawai *et al.*, 1988). In the porcine pancreas perfusion, 10 nM GRP did not affect glucagon release (Knuhtsen *et al.*, 1987).

The same discrepant effect of neuropeptides on pancreatic hormone release has been found with substance P (Chiba *et al.*, 1985). Substance P stimulates insulin release from the isolated perfused dog pancreas and inhibits it from the isolated perfused rat pancreas. There is no species difference in the amino acid sequence of NMB and substance P in higher animals (Reeve *et al.*, 1983; Minamino *et al.*, 1983 and 1984; Krane *et al.*, 1988; Aronin *et al.*, 1983). Therefore, these differences in the effect of neuropeptides according to species are not ascribed to a species difference in the primary structure of peptides. In higher animals, a part of the sympathetic or parasympathetic effect on pancreatic endocrine and exocrine tissues is mediated by the release of peptidergic transmitter from the pancreas (Trimble, 1988). The degree of increase in GRP-release during the electrical stimuli to vagal nerve varies according to the species (Knuhtsen *et al.*, 1985). From these findings, it is speculated that the different endogenous bombesin family peptide concentration might bring about different metabolic memories in isolated perfused pancreases and cause a different response to exogenous bombesin family peptides. In addition to their direct action on B- and A-cells, these peptides might modulate the pancreatic hormone release through the contraction of pancreatic blood vessels because they stimulate the

contraction of smooth muscles. A species difference in sensitivity of pancreatic blood vessels to these peptides might cause a different response to pancreatic hormone release, although we observed only a small change in perfusion pressure during the infusion of these doses of NMB or GRP-10.

In summary, we have not found a significant stimulatory effect on insulin release by NMB and GRP-10 in the rat pancreas perfusion experiment under various perfusate conditions, which contrasts with results from experiments on dog and porcine pancreas perfusions, and the reason for this difference remains to be determined.

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