Effect of Norepinephrine on Insulin, Glucagon, and Somatostatin Secretion in Isolated Perifused Rat Islets

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SUMMARY

The rate of insulin, glucagon, and somatostatin secretion was measured from isolated rat islets maintained in a perifusion system. The effect of norepinephrine (NE) was simultaneously determined on the release rate of all three hormones. Norepinephrine was employed at an acute dose of 10 μM and in graded doses from 1 nM to 10 μM in the presence of high (22 mM) and low (1.4 mM) glucose concentrations. Under both high and low glucose conditions, insulin secretion was maximally inhibited at 10 μM NE concentration and was significantly depressed at 100 nM NE concentration. Under both high and low glucose conditions, glucagon release was maximally stimulated at 10 μM NE concentration and was significantly elevated at 10 nM NE concentration. Under high and low glucose conditions, somatostatin release was inhibited by 10 μM NE concentration and was significantly depressed at 100 nM NE concentration. During the initial maximal stimulation of glucagon, NE inhibition of somatostatin and insulin was prevented, possibly by the high level of glucagon released. A paracrine effect of glucagon on beta and delta cells is proposed. DIABETES 28:899–904, October 1979.

This study addresses the question of how somatostatin release is modulated and the putative paracrine role of the hormone. In preliminary studies, we noted that reserpine treatment of rats resulted in a 2½-fold increase of extractable pancreatic somatostatin and a 1½-fold increase of extractable insulin. From this observation we reasoned that norepinephrine (NE) may modulate the release of somatostatin in a fashion similar to the well-known catecholamine effect on insulin release.[1] Schauder et al.[2] reported that 11 μM epinephrine causes a 34% decrease in glucose-stimulated somatostatin release from batch-incubated, isolated rat islets. In the perfused dog pancreas, Samols et al.[3,4] tested the influence of 1 nM epinephrine on somatostatin release and found no effect. When epinephrine was infused in combination with propranol, somatostatin secretion was diminished. When epinephrine was infused in combination with phentolamine, somatostatin release was increased.

To further clarify the role of catecholamines on somatostatin release and islet paracrine physiology, we elected to study NE effects at both acute, high dose levels and graded dose levels (ranging from 1 nM to 10 μM) in an islet perifusion system. In addition, we studied the effects of NE in the presence of both low (1.4 mM) and high (22 mM) glucose concentrations. The questions under consideration were: (1) Does NE modulate somatostatin release? (2) Can a dose–response relationship be demonstrated for the effect of NE on somatostatin release? (3) Can paracrine effects be demonstrated by modulating islet hormone release with NE? (4) Are these effects dependent on glucose concentration?

MATERIALS AND METHODS

Materials. The incubation medium, RPMI 1640, was obtained free of bicarbonate, glutamine, and glucose (Gibco, Grand Island, NY). Prior to use the medium was brought to 20 mM Hepes (Sigma), 26 mM $NaHCO_3$, 2 mM glutamine (Gibco), and 100 mg/dl albumin (bovine serum albumin, Armour Pharmaceutical Company). The medium pH was adjusted to 7.4 with 1 N NaOH and then gassed with O_2/CO_2:95/5. d-Glucose (Dextrose, Sigma) was added as required. Norepinephrine (L-arterenol, Sigma) was made as a stock solution in 100 μM ascorbate and kept at 4°C until added to the incubation medium (which also contained 100 μM ascorbate) at the time of perifusion.

Perifusion technique. The perifusion technique was modified slightly from that described by Lacy et al.[5] To reduce the total lag time due to dead volume in the system, the efferent portion of the perifusion chamber (13 mm, Millipore) was fitted with a small stopper through which the efferent plastic tubing was passed. The system's dead volume was less than 1.5 ml and had a total lag time of less than 5 min

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when pumping at a rate of 0.25 ml/min with a polystaltic pump (Buchler Instruments).

Islets were isolated from 150–250-g Sprague-Dawley rats by the collagenase digestion technique of Lacy and Kostianovsky.[6] The pancreatic digest was applied to ficoll density gradients[7] for further enrichment of the islets. The ficoll was dialyzed[8] before use. Approximately 200 islets were hand picked, with the aid of a micropipette, from the ficoll gradient medium and transferred directly to each perifusion chamber, which was fitted with a 5-μm Millipore filter. The islets were preincubated in the chamber a minimum of 30 min in all experiments. The pumping rate was 0.25 ml/min, and 0.5-ml fractions were collected. The 0.25-ml/min pumping rate was selected to assure a somatostatin concentration within the assay range. In this series of experiments, the ranges from low (or depressed) to high (or stimulated) hormone concentrations were 140–860 mU/ml for insulin, 1300–6000 pg/ml for glucagon, and 50–200 pg/ml for somatostatin. With the exception of the first 20 min of preincubation, each fraction was assayed for all three hormones throughout the duration of the experiment.

Radioimmunoassays. Insulin was assayed according to Morgan and Lazarow,[9] glucagon according to McEvoy et al.,[10] and somatostatin according to Petersson et al.[11] To facilitate the assays, a common buffer was used for all three immunoassays and consisted of 0.07 M Na_2HPO_4, 0.14 M NaCl, 0.025 M EDTA, 0.01 M benzamidine HCl, and 0.002% merthiolate. The pH was brought to 7.6 with 5 N NaOH, and sampling was done with a dilutor dispenser (Hamilton). For insulin and glucagon assay, 50-μl aliquots were used, and 400 μl for somatostatin. The final reaction volume for all assays was 1 ml. Separation of free from bound hormone for all assays was done using polyethylene oxide 6000 (Polysciences). Antibodies against insulin, glucagon, and somatostatin were raised and characterized in our laboratory. ^{125}I-insulin and ^{125}I-glucagon were obtained from New England Nuclear. ^{125}I-somatostatin was labeled according to the method of Arimura.[12] The assay range for insulin is 2–60 μU, for glucagon 20–800 pg, and for somatostatin 4–512 pg. All assays were begun immediately on completion of the perifusion experiment. Single determinations of each fraction were done for each of the three hormones.

Data analysis. Two simultaneous perifusion channels were run in each experiment, with the second channel used as the control. The mean immunoreactive hormone released/minute/islet was calculated for the duration of the treatment and compared with the mean immunoreactive hormone release rate for the control channel during the same treatment interval. Subsequent use of the terms "insulin," "glucagon," and "somatostatin" refers to the immunoassayable hormone. Statistical analysis was performed using the paired Student's *t* test (two tailed), and values are expressed as mean ± SEM.

RESULTS

Effect of an acute dose of norepinephrine on insulin, somatostatin, and glucagon release in the presence of 22 mM glucose. The effect of an acute dose of 10 μM NE on the release rate of insulin, glucagon, and somatostatin in the presence of 22 mM (400 mg/dl) glucose was studied using the perifusion technique. The experimental protocol consisted of preincubating 200 isolated rat islets for a minimum of 30 min in the presence of 1.4 mM glucose before the collection of samples for assay. Perifusion of islets with 1.4 mM glucose was continued for an additional 20–30 min before changing the perfusate to 22 mM glucose for a period of 40 min. NE was then added to the high glucose medium, bringing it to a final concentration of 10 μM, and the islets were perifused for 30 min. The NE medium was then replaced by high glucose medium free of NE for an additional 30 min. The control channel received the same treatment; however, the islets were not exposed to NE. Data from a representative experiment are shown in Figure 1, and data from all experiments are summarized in Figure 2.

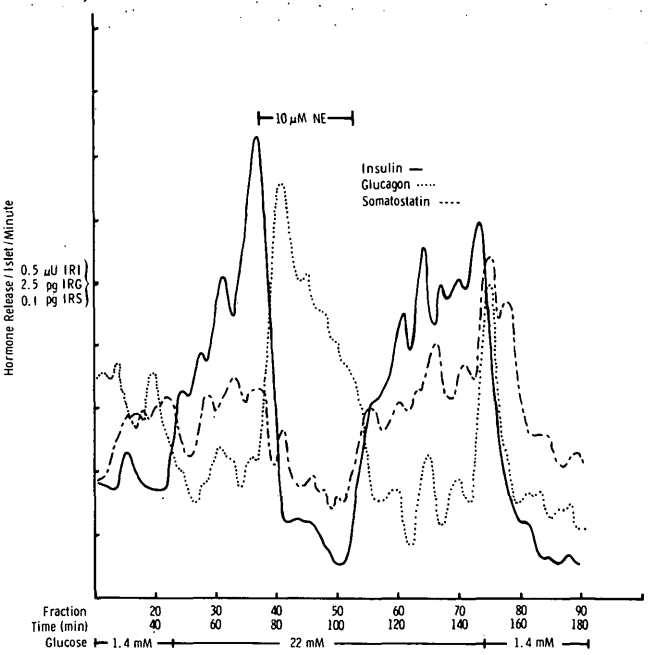
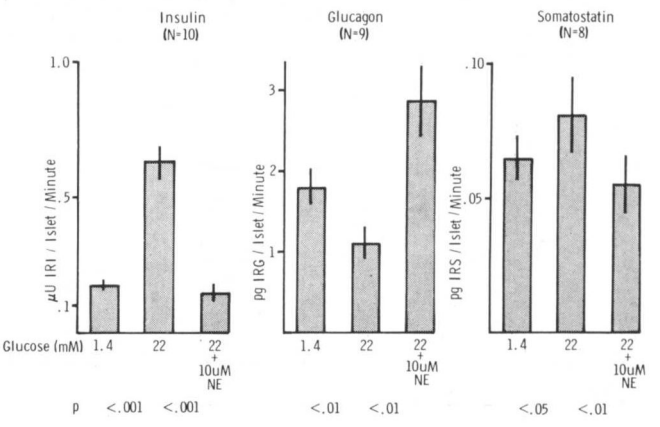


FIGURE 1. Time course of the effects of glucose and norepinephrine in the presence of 22 mM glucose on immunoreactive insulin, glucagon, and somatostatin release. Data are from the experimental channel in one representative experiment. One point on the ordinate is defined for each hormone.

As seen in Figures 1 and 2, in response to change from low to high glucose concentrations, the hormone release rate for insulin increased nearly fourfold, somatostatin increased 25%, and glucagon decreased 38%. In the presence of 10 μM NE and high glucose levels, the release rate

FIGURE 2. Summary of the effects of glucose and norepinephrine in the presence of 22 mM glucose on immunoreactive insulin, glucagon, and somatostatin release.



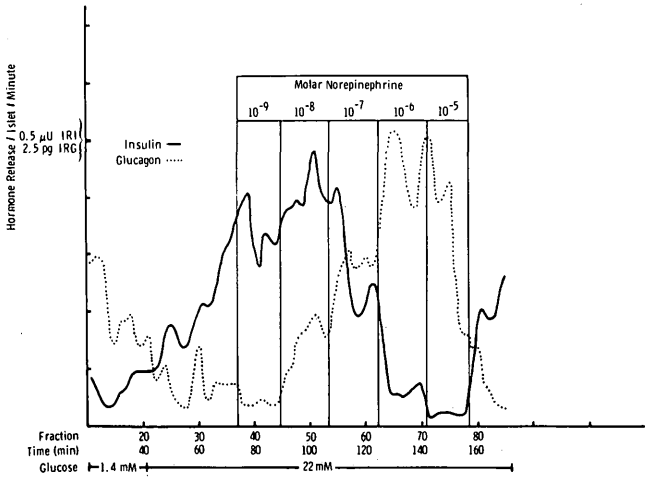
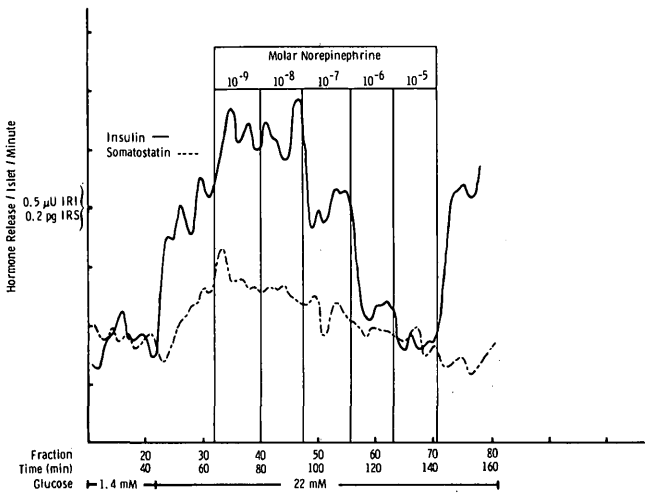


FIGURE 3. Effect of logarithmically increasing doses of norepinephrine on the release of immunoreactive insulin and glucagon in the presence of 22 mM glucose. Data are from the experimental channel in one representative experiment. One point on the ordinate is defined for each hormone.

for insulin was depressed 77%, somatostatin was depressed 36%, and glucagon was stimulated 153%. With the addition of NE, both insulin and somatostatin release immediately decreased and continued on a downward trend throughout the NE treatment. Although glucagon release in the presence of 10 μM NE suddenly increased, the initial high rate of stimulation, although significant, was diminished throughout the remaining portion of the treatment period. On cessation of NE treatment, the release rate for all three hormones immediately proceeded toward the levels of the untreated control channel.

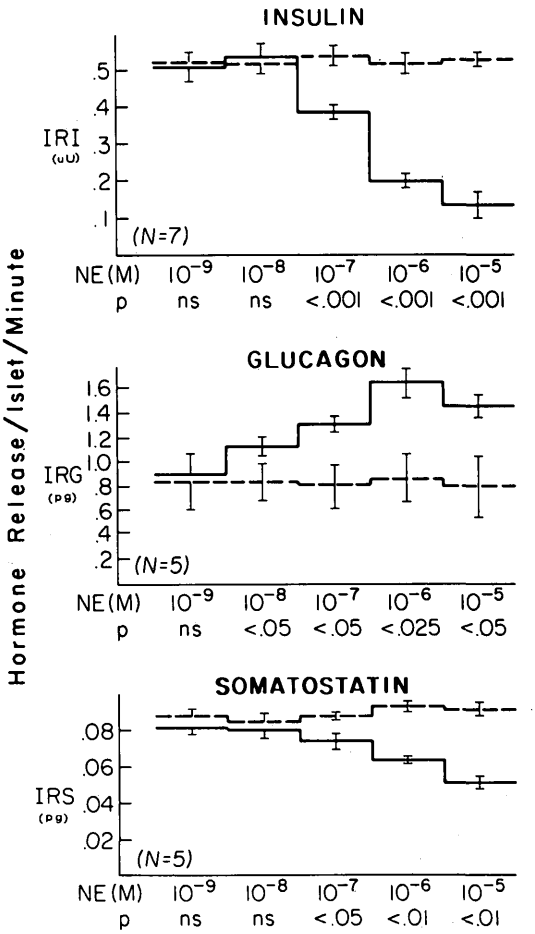
Whenever the glucose concentration was changed from low to high, glucagon and somatostatin release levels suddenly dropped and remained low for periods of up to 10 min before returning to their high glucose release rates. In contrast, whenever the glucose concentration was changed from high to low, there was a sudden pronounced release of both glucagon and somatostatin (Figure 1). The high release rate was sustained for about 8 min before hormone release from low glucose was resumed.

FIGURE 4. Effect of logarithmically increasing doses of norepinephrine on the release of immunoreactive insulin and somatostatin in the presence of 22 mM glucose. Data are from the experimental channel in one representative experiment. One point on the ordinate is defined for each hormone.



Effect of graded doses of norepinephrine on insulin, somatostatin, and glucagon release in the presence of 22 mM glucose. The protocol for these experiments was the same as in the acute dose experiments, except that NE was administered in five increasing concentrations ranging from 10^{-9} M (1 nM) to 10^{-5} M (10 μM). The period of treatment for each dose was 16 min. Data from a sample experiment for insulin and glucagon are presented in Figure 3, and insulin and somatostatin in Figure 4. Data from all experiments for each of the three hormones are summarized in Figure 5. The mean hormone release rate during the treatment interval for a given concentration of NE is provided along with the mean hormone release rate for the parallel control channel during the same treatment interval. An apparent log–dose response relationship was observed for all three hormones. The release rate for insulin and somatostatin decreased as a function of NE concentration through the range 10^{-7} M (100 nM) to 10^{-5} M (10 μM). For glucagon, the release rate increased as a function of NE concentration through the range 10^{-8} M (10 nM) to 10^{-5} M. In none of the experiments was there an effect of 10^{-8} M NE on either insulin or somatostatin.

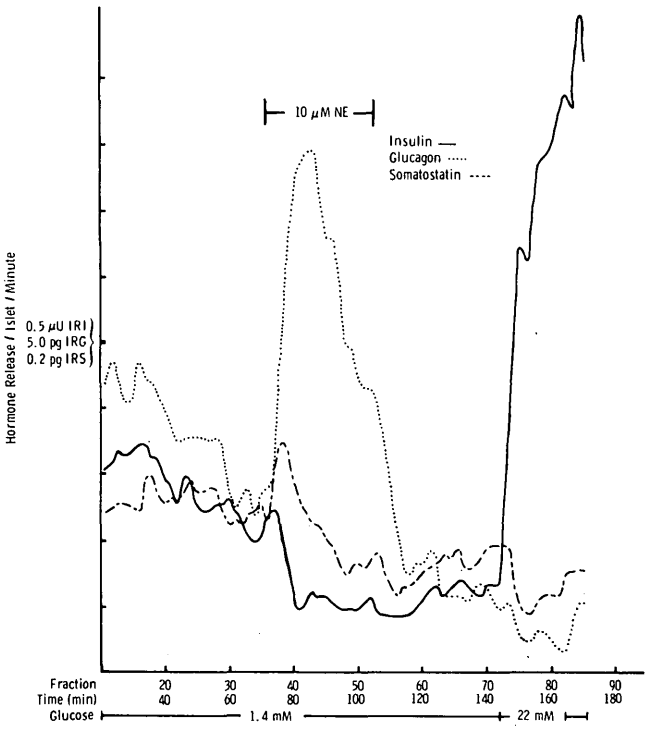
FIGURE 5. Summary of the log–dose response effect of norepinephrine on the release of immunoreactive insulin, glucagon, and somatostatin in the presence of 22 mM glucose. The release rate per dose of norepinephrine (—) is compared statistically to the amount of hormone released in the control channel (----) during the same time interval.



Effect of an acute dose of norepinephrine on insulin, somatostatin, and glucagon release in the presence of 1.4 mM glucose. The experimental format for determining the effect of an acute dose of NE (10 μM) on insulin, somatostatin, and glucagon release in the presence of low glucose (1.4 mM) was the same as that in the corresponding high glucose experiments. The islets were perifused throughout the entire experimental period with 1.4 mM glucose until the final 30 min when the concentration was increased to 22 mM to assess the glucose sensitivity of the cells. The treatment period for NE was 30 min. Again, the parallel control chamber received the same treatment as the experimental chamber except that no NE was added. Data from a sample experiment are shown in Figure 6, and data for all experiments are summarized in Figure 7.

Qualitatively, the results of an acute dose of 10 μM NE on islet hormone release in low glucose medium were similar to those seen for high glucose medium. Insulin and somatostatin release were depressed, whereas glucagon release was enhanced. The release rate was depressed 47% for insulin and 21% for somatostatin as compared with the control channel during the same treatment interval. Glucagon release was enhanced 153% as compared with the control channel during the same treatment interval. In comparison with the high glucose concentration experiments, the rate of decreased insulin release at onset of NE inhibition was less abrupt. In addition, after cessation of NE treatment, the insulin release rate remained depressed up to 30 min, during which time it slowly recovered to the release rate seen in the control channel. Response to glucose at the end of the experiment was brisk and unaltered from the control channel. A similar alteration in response to NE was observed for somatostatin. Somatostatin release was either unaltered or stimulated during the first 6–10 min of NE treatment, and then was notably depressed. Like insulin, somatostatin release remained depressed for up to 30 min following cessation of NE treatment before it finally achieved control channel release rates. The profile for glucagon release was unchanged from that seen in the high glucose concentration experiments. During the initial peak of glucagon release, the rate was as high as 9 pg/islet/min, giving a medium concentration of 7.4 ng/ml.

FIGURE 6. Time course of the effect of norepinephrine on immunoreactive insulin, glucagon, and somatostatin release in the presence of 1.4 mM glucose. The data are from the experimental channel in one representative experiment. One point on the ordinate is defined for each hormone.



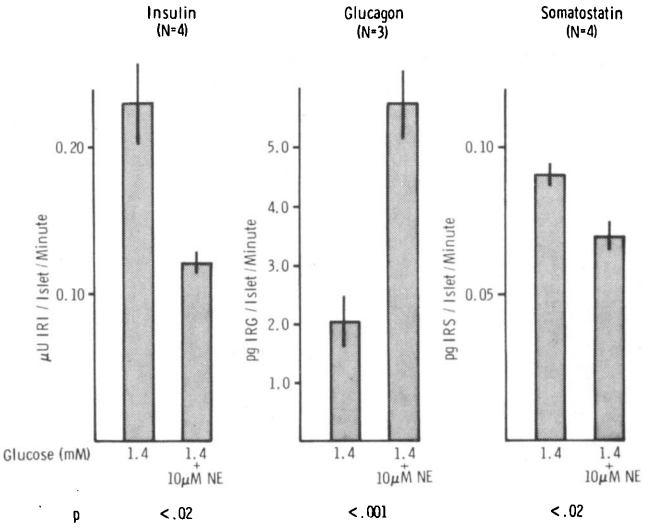


FIGURE 7. Summary of the effect of norepinephrine on the release of immunoreactive insulin, glucagon, and somatostatin in the presence of 1.4 mM glucose as compared with the untreated control channel during the same treatment interval.

DISCUSSION

The dual chamber perifusion technique proved to be an effective method for simultaneously studying the effect of NE on insulin, somatostatin, and glucagon release from isolated rat islets. A perifusion system was preferred over static incubations so that the released hormones would not accumulate. Increasing hormone concentrations in the medium could interact with the cells and potentially obliterate the effect of the drug being tested.

The effect of glucose on insulin, somatostatin, and glucagon release in perifused islets was consistent with previous reports.[5,13–19] That is, insulin release is markedly enhanced, somatostatin release mildly stimulated, and glucagon release inhibited. Islets, in some instances, were perifused for periods of up to 3½ h without significantly altering glucose sensitivity.

A consistent observation was made regarding somatostatin and glucagon release at the time the medium was changed from low to high glucose concentration. At that time there was a sudden depression of both somatostatin and glucagon release. This period of depressed hormone release lasted for about 10 min, then the hormone release levels returned to normal rates for high glucose levels. Similarly, at the time when low glucose medium replaced high glucose medium, there was a sudden high release rate for somatostatin and glucagon, lasting for about 8 min before the hormone release rate returned to normal for low glucose concentration. The change in osmolarity during the change

from low to high glucose concentration was less than 5%. The stimulation of somatostatin release has been reported previously by Schauder et al.,[14] and for glucagon by Assan et al.[10] Whether glucagon and somatostatin are responding to the rate of change in beta-cell activity or osmotic effects is unclear and currently under investigation in our laboratory.

An acute dose of 10 μM NE resulted in approximately 40% inhibition of glucose-stimulated somatostatin release. This change is similar in magnitude to that observed by Schauder[2] for 11 μM epinephrine-treated, batch-incubated islets. The inhibitory effect was dose-dependent, with the delta-cells demonstrating sensitivity to 10^{-7} M NE. Glucose-stimulated insulin release was similarly inhibited by 10 μM NE, but by a greater degree (80%). This change is consistent with previous reports in the literature.[2,17,18] The inhibition was prompt, sustained throughout the NE treatment, and readily reversed after removal of NE. Similar to delta-cells, the beta-cells were sensitive to 10^{-7} M NE.

In the presence of high glucose concentration, alpha-cells responded to 10 μM NE with a pronounced release of glucagon followed by a continuously elevated release throughout the treatment interval. The stimulatory effect was dose-dependent, with the alpha-cells showing sensitivity to 10^{-8} M NE, or 10-fold greater than observed for beta- and delta-cells. Thus, the alpha-cells showed a response to NE in the presence of a high glucose concentration and stimulated levels of insulin (approximately 600 mU/ml) and somatostatin (approximately 200 pg/ml). That is, NE was capable of stimulating glucagon release without first depressing somatostatin release. Exogenous somatostatin has been shown to inhibit norepinephrine-induced glucagon release,[19] and one might have anticipated that delta-cells would be as sensitive or more sensitive than alpha-cells. Alpha-cell insensitivity to somatostatin in isolated islets seems unlikely since antisomatostatin serum has been shown to increase glucagon release under similar conditions,[20] a report that we have confirmed in our laboratory.[21] Therefore, it would appear that 10^{-8} M NE is capable of overcoming any inhibitory effect that endogenous somatostatin may have on glucagon release.

The responses of all three hormones to an increased glucose concentration were reversed by norepinephrine. In addition, paracrine influences of somatostatin were not readily apparent in the responses observed. Rates of somatostatin release changed in the same direction as for insulin, thereby suggesting that somatostatin does not mediate insulin response to NE. Furthermore, the greater sensitivity of alpha-cells to NE compared with delta-cells suggests that somatostatin does not contribute to the response of glucagon to NE.

To test whether glucose modulated the NE effect on insulin, somatostatin, and glucagon release, the acute NE experiments were repeated in the presence of a low glucose concentration. The overall effect was the same as for the high glucose experiments. That is, insulin and somatostatin release was inhibited, and as described by Oliver et al.[19] in a similar experiment, glucagon was stimulated. However, the release profile for insulin and somatostatin in the low glucose experiments was altered from that described for the high glucose experiments. During the first few minutes of NE treatment, there was either no change or an elevation in hormone release rate. This period corresponded with the maximal release of glucagon. Glucagon medium concentrations during this peak release period were in excess of 7 ng/ml, then typically fell to less than half of that concentration for the duration of the treatment. Weir et al.,[22] using the isolated perfused dog pancreas, demonstrated maximum somatostatin and insulin release in response to a glucagon concentration of 10 ng/ml and significant stimulation with as little as 1 ng/ml. Therefore, it seems plausible that the absence of an immediate inhibitory response of insulin and somatostatin release to NE is due to the counterbalancing stimulatory effect of a high prevailing glucagon concentration. Subsequently, as the high glucagon level subsides, the NE effect becomes manifest.

From these experiments on isolated perifused rat islets we conclude that (1) glucose modulates the release of insulin, somatostatin, and glucagon, (2) NE modulates the release of insulin, somatostatin, and glucagon in the presence of both high and low glucose levels, (3) the NE effect is dose-dependent, (4) glucagon release is more sensitive to NE than insulin and somatostatin release, and (5) under certain conditions, glucagon may possibly influence somatostatin and insulin release through paracrine mechanisms.

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