Glucagon Secretion from the Perfused Rat Pancreas

STUDIES WITH GLUCOSE AND CATECHOLAMINES

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ABSTRACT The isolated *in situ* perfused rat pancreas was used to study glucose and catecholamine control of glucagon secretion, and to investigate the possible role of endogenous cyclic AMP as a mediator of this secretory process. When perfusate glucose was acutely dropped from 100 to 25 mg/100 ml, glucagon was released in a biphasic pattern with an early spike and a later plateau-like response. 300 mg/100 ml glucose suppressed glucagon secretion to near the detection limit of the radioimmunoassay (15 pg/ml). When perfusate glucose was dropped from 300 to 25 mg/100 ml, a delayed, relatively small peak occurred suggesting persisting alpha cell suppression by prior high glucose exposure. 2-Deoxy *d*-glucose stimulated glucagon secretion and inhibited insulin secretion.

Glucagon was secreted in a biphasic pattern in response to both 2.7×10^{-7} M epinephrine and norepinephrine. The glucagon response to epinephrine was markedly suppressed by glucose at 300 mg/100 ml, and the biphasic response pattern was obliterated. Glucose evoked a two-phase insulin secretory pattern, and the second phase was markedly and rapidly inhibited by epinephrine. Pancreases were perfused with glucose at 300 mg/100 ml which was then lowered to 80 mg/100 ml. 5 min later, epinephrine was infused and definite blunting of the first-phase spike occurred. 10 mM theophylline produced modest rapid uniphasic stimulation of glucagon release, and, in addition, caused enhancement of epinephrine-stimulated glucagon release. An inhibitory influence upon epinephrine-stimulated glucagon was observed as well. Insulin secretion was stimulated by 10 mM theophylline, and this stimulation was inhibited by epinephrine.

This work was presented in part at the 33rd Annual Meeting of the American Diabetes Association, Chicago, Ill., 24 June 1973.

Dr. Weir is a recipient of a Daland Fellowship of the American Philosophical Society.

Received for publication 21 March 1974 and in revised form 9 August 1974.

INTRODUCTION

It has been well shown that glucose exerts important control over alpha cell secretion in a manner almost the reverse of glucose control over insulin release from the beta cell. At low glucose levels, glucagon secretion is enhanced and at higher levels suppressed. This has been demonstrated both in vivo (1–9) and in vitro (10–15). The details of the timing and magnitude of glucagon release in response to varying glucose concentrations remain incompletely defined.

Catecholamines may be as important as glucose in the control of glucagon secretion. Both epinephrine (16–18) and norepinephrine (17) have been shown to stimulate glucagon secretion. It is likely that epinephrine secretion by the adrenal medulla and norepinephrine secretion by sympathetic nerve terminals exert important physiological influences (5, 16–19). The interactions of glucose and catecholamines upon glucagon secretion have not yet been defined. In particular, it is not known whether catecholamine-induced glucagon secretion is influenced by the glucose concentration surrounding the alpha cell.

Very little is known about the mechanisms through which catecholamines stimulate glucagon secretion. It is reasonable to hypothesize that catecholamines exert their effect by stimulating adenylate cyclase leading to the generation of cyclic AMP. Unfortunately it is not yet possible to isolate pure enough alpha cell tissue for direct dynamic studies of enzyme activity and metabolite concentration. Indirect approaches are therefore necessary to learn more about the possible importance of cyclic AMP in glucagon secretion. Such an indirect approach would involve the use of theophylline, a methylxanthine which inhibits the destruction of cyclic AMP. Enhancement of catecholamine-stimulated glucagon release by theophylline would provide some support for the above hypothesis.

The present study was undertaken with the isolated perfused rat pancreas to analyze the dynamics of glu-

cagon secretion in response to changes of glucose concentration, and to investigate the effect of glucose upon catecholamine-stimulated glucagon release. In addition, theophylline was used to learn more about the mechanisms by which catecholamines influence alpha cell secretion.

METHODS

Materials. Glucagon antiserum 02K was kindly supplied by Dr. Roger Unger, and insulin antiserum (lot K4273) was obtained from Burroughs Wellcome Co. (Research Triangle Park, N. C.). [^{125}I]Glucagon was purchased from the Cambridge Nuclear Radiopharmaceutical Corporation (Billerica, Mass.), and [^{125}I]insulin was obtained from the Amersham/Searle Corp. (Arlington Heights, Ill.). Pork glucagon standard (lot GLF 599A) was supplied by Eli Lilly and Company (Indianapolis, Ind.), and rat insulin standard (lot R355) was supplied by the Novo Research Institute (Copenhagen, Denmark). Trasylol was obtained from FBA Pharmaceuticals (New York), and dextran T70 (lot 1730) from Pharmacia Fine Chemicals, Inc. (Piscataway, N. J.). Epinephrine was from Parke, Davis & Company (Detroit, Mich.), and norepinephrine was from Winthrop Laboratories (New York). 2-Deoxy *d*-glucose was obtained from Sigma Chemical Co. (St. Louis, Mo.). Theophylline (lot no. XGD) was from Mallinckrodt Chemical Works (St. Louis, Mo.).

Glucagon radioimmunoassay. The assay was a modification of the method of Unger, Aguilar-Parada, Muller, and Eisentraut (20). Glycine buffer (0.2 M, pH 8.8) contained 0.25% bovine albumin (fraction V) and 1% normal sheep serum (Grand Island Biological Co., Grand Island, N. Y.). The incubation mixture was prepared by consecutive addition of 50 μl glycine buffer, 50 μl Trasylol (500 U), 200 μl of either perfusate sample or pork glucagon standard in perfusate, 100 μl of antiserum 02K (1:1,200 dilution), and 100 μl of [^{125}I]glucagon, giving a total volume of 500 μl in a 10 × 75-mm disposable glass tube. After incubation for 2–3 days at 4°C, free glucagon was separated from antibody-bound glucagon by a dextran-charcoal separation technique. Assay sensitivity was usually about 15 pg/ml.

Insulin radioimmunoassay. The assay was a modification of the method of Albano, Ekins, Maritz, and Turner (21). A phosphate/albumin buffer with a pH of 7.4 was used. The incubation was prepared with the consecutive addition of 500 μl of phosphate buffer, 50 μl of perfusate sample or rat insulin standard in perfusate, 100 μl of Wellcome antiserum (1:40,000 dilution), and 100 μl of [^{125}I]insulin, giving a total volume of 750 μl in a 10 × 75-mm disposable glass tube. After incubation for 2–3 days at 4°C, free insulin was separated from antibody-bound insulin by charcoal separation. Assay sensitivity was 1–2 μU/ml.

Perfused rat pancreas preparation. The *in situ* isolated perfused rat pancreas preparation was a modification of the method of Penhos, Wu, Basabe, Lopez, and Wolff (22). Fasted male Charles River strain albino rats, weighing 350–600 g, were anesthetized with intraperitoneal Amytal Sodium (Eli Lilly and Company, 160 mg/kg). Renal, adrenal, gastric, and lower colonic blood vessels were ligated. The entire intestine was resected except for about 4 cm of duodenum and the descending colon and rectum. Therefore, only a small part of the intestine was perfused, thus minimizing possible interference by enteric substances with glucagon-like immunoreactivity. Perfusate was introduced via a polyethylene cannula entering the lower aorta just above the bifurcation. The upper abdominal aorta was ligated just below the diaphragm and perfusate samples were collected from a cannulated portal vein. The carcass was kept in a Plexiglas box maintained at 38°C.

The perfusate was a modified Krebs-Ringer bicarbonate buffer with 4% dextran T70 and 0.2% bovine serum albumin (fraction V), and was bubbled with 95% O_2 and 5% CO_2. A nonpulsatile flow, four-channel, roller bearing pump (Buchler polystatic, Buchler Instruments Div., Nuclear-Chicago Corp., Fort Lee, N. J.) was used, and a switch from one perfusate source to another could by accomplished almost instantaneously by switching a three-way stopcock only 15 cm proximal to the cannulation point of the aorta. In experiments done with methylene blue as a visual marker, it took 7–8 s after a switch for fluid from a new channel to reach the pancreas and an additional 7–8 s to leave the portal vein catheter.

Flow rate was monitored frequently and remained constant at 2–3 ml/min for the entire perfusion which usually lasted for about 70 min, depending upon the experiment. The first 20 min of each perfusion was always an equilibration period and will not be shown on any subsequent graphs. Samples from the portal vein catheter were collected over 30-s time intervals into glass tubes containing 4 mg of EDTA to protect against proteolytic degradation by pancreatic enzymes. These tubes were kept on ice and then frozen within 45 min. Perfusate was never recycled through the pancreas.

Epinephrine and norepinephrine were always added to the perfusate source 8 min before their infusion was initiated, to allow proper dispersion. All of the other pharmacological agents were added as the perfusate was made up, and these solutions were only used that day.

Perfusate glucose determinations. Perfusate glucose concentrations were measured with a Beckman glucose analyzer (model ERA-2001, Beckman Instruments, Inc., Fullerton, Calif.), which utilized glucose oxidase. For unknown reasons there was an error in measurement technique with analyzer results being 13% lower than true values, i.e., measurement of perfusate with known amount of added glucose. The error was consistent at glucose concentrations of 25, 80, and 300 mg/100 ml, and the glucose results reported are corrected values.

Data presentation. Glucagon secretion over a 30-s time period is depicted in subsequent graphs as a single point at the beginning of the time interval. For instance, a point at 2 min represents the glucagon concentration of perfusate collected during the 2–2.5 min time period. Each graphed point represents a mean of several perfusions, and brackets represent SEM. Total glucagon output over time was estimated by planimetry, and because of minor variations in flow, an assumed flow rate of 2.7 ml/min was used for the calculations. Both one and two tail Student's *t* tests were used to determine significant differences between groups.

RESULTS

Glucose control over glucagon secretion. In the experiment shown in Fig. 1, the preparations were preperfused for 20 min with perfusate containing 100 mg/100 ml glucose, and when glucose was dropped from 100 to 25 mg/100 ml, glucagon was released in a biphasic pattern. When perfusate glucose was changed back to 100 mg/100 ml, glucagon concentration returned

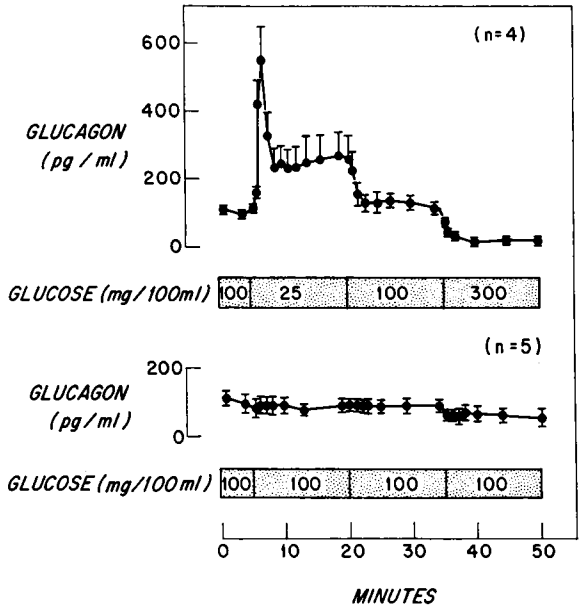

FIGURE 1 Upper panel, effects of varying glucose concentrations upon glucagon secretion. Lower panel, control perfusions showing minimal alteration of glucagon secretion following changes of perfusate sources.

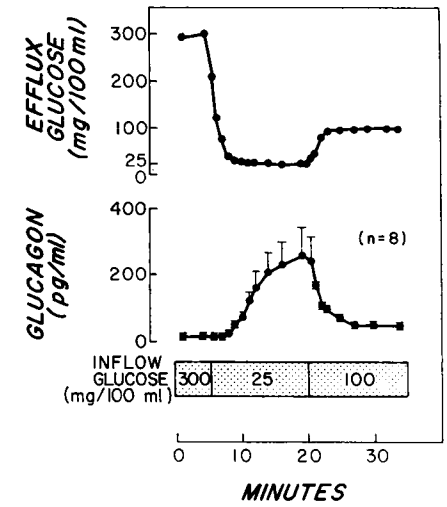

FIGURE 2 Upper panel, efflux glucose concentrations from one representative perfusion. Lower panel, glucagon secretory changes in response to glucose changes.

approximately to base line within 3 min. Then when glucose was raised to 300 mg/100 ml, glucagon secretion was suppressed within 5 min, down to the detection limit of the assay.

The lower panel of Fig. 1 represents a control study done to find if glucagon secretion was influenced by changing back and forth between three perfusate reservoirs, each containing a glucose concentration of 100 mg/100 ml. These changes were made at 5, 20, and 35 min with frequent sampling immediately following each. There was no serious perturbation of glucagon secretion after each change, but there was a gradual fall of glucagon output during the 50-min observation period from 112±38 to 57±16 pg/ml.

In the experiments shown in Fig. 2, pancreases were preperfused for 20 min with perfusate containing a glucose concentration of 300 mg/100 ml, which was then continued for the first 5 min of the observation period. After this 25-min exposure of the pancreas to glucose at 300 mg/100 ml, a change was made to perfusate with glucose at 25 mg/100 ml. 5 min after the change, efflux glucose had fallen to 31 mg/100 ml as measured in a representative perfusion. Under these conditions, glucagon output increased in a gradual uniphasic fashion, reaching only modest levels, without the rapid early secretory burst found after changing glucose concentration from 100 to 25 mg/100 ml (Fig. 1).

Glucagon and insulin response to 2-deoxy d-glucose. With the glucose concentration of the perfusate maintained at 100 mg/100 ml for the entire perfusion, addition of 80 mg/100 ml of 2-deoxy *d*-glucose produced prompt enhancement of glucagon secretion as seen in Fig. 3. In contrast to the two phases of release seen in Fig. 1 in response to low glucose, only a plateau-like pattern of release is seen. The base-line glucagon was 72±37 pg/ml rising to 207±57 pg/ml, following the addition of 2-deoxy *d*-glucose. The insulin concentrations depicted in the lower panel of Fig. 3 fell in response to 2-deoxy *d*-glucose, but did not rise again with termination of the infusion of the glucose analogue. The mean

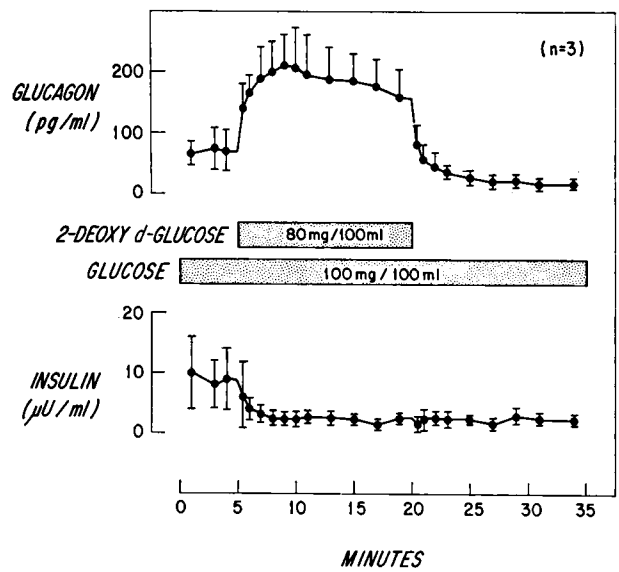

FIGURE 3 Glucagon and insulin secretion in response to 2-deoxy *d*-glucose.

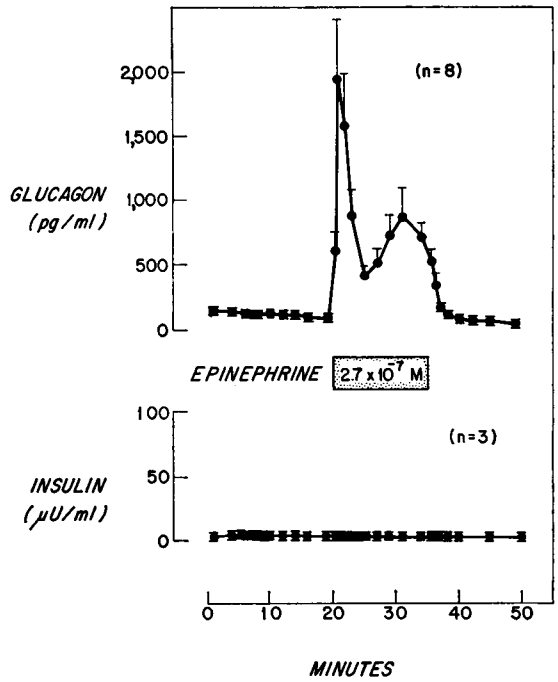

FIGURE 4 Glucagon and insulin secretion in response to epinephrine. Perfusate glucose maintained at 80 mg/100 ml.

insulin concentration for the three base-line time periods was 9±3 μU/ml, which was significantly higher ($P < 0.05$, one tail t test) than any of the insulin determinations during the last 12 min of the 15 min 2-deoxy *d*-glucose infusion.

Glucagon and insulin response to epinephrine. With glucose maintained at 80 mg/100 ml during the entire perfusion (including the preperfusion period), epinephrine was infused at a concentration of 2.7×10^{-7} M (50 ng/ml) (upper panel of Fig. 4), and stimulated glucagon release in a biphasic pattern. The glucagon concentration just before the addition of epinephrine was 90±19 pg/ml with the peak of first-phase glucagon release occurring 1 min later, reaching 1,959±464 pg/ml. In the eight individual perfusions, the first-phase peak occurred at 1 min after the epinephrine addition in six, and at 2 min in two. Also there was a second-phase glucagon response to epinephrine with the highest value reaching 885±229 pg/ml, 11 min after the change (Fig. 4). Total glucagon output during the 15 min of epinephrine as determined by planimetry was 32,451± 6,916 pg. No significant change of perfusate flow rate was detected as epinephrine was being infused.

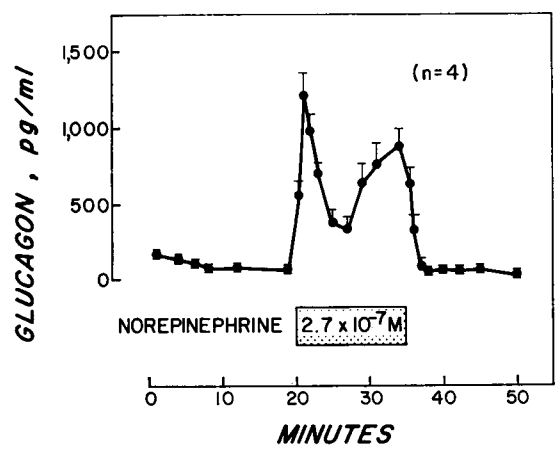

FIGURE 5 Glucagon response to norepinephrine. Perfusate glucose maintained at 80 mg/100 ml.

Insulin levels were determined in three representative perfusions as shown in the lower panel of Fig. 4. Insulin concentration during each was only 2–5 μU/ml, slightly above the detection limit of the assay, and did not significantly change during the epinephrine infusion.

Response of glucagon to norepinephrine. With perfusate glucose at 80 mg/100 ml, norepinephrine was infused at a concentration of 2.7×10^{-7} M (46 ng/ml). As can be seen in Fig. 5, norepinephrine produced a glucagon response pattern remarkedly similar to that elicited by the same molar concentration of epinephrine. Base-line glucagon immediately before initiating norepinephrine was 64±22 pg/ml, and the peak of the first phase was 1,213±153 pg/ml occurring 1 min later, which although lower, was not statistically different from the epinephrine-induced first-phase peak. The peak of the second phase was 823±110 pg/ml, reached 14 min after starting norepinephrine. As with epinephrine, there was an abrupt fall of glucagon secretion after stopping the norepinephrine with glucagon concentration dropping to 54±30 pg/ml only 3 min after the discontinuation. Total glucagon output during the 15 min of norepinephrine as determined by planimetry was 25,543±4,034 pg which was not significantly different from the output during the epinephrine infusion, 32,451± 6,916 pg. As with epinephrine, norepinephrine did not evoke any significant change of perfusate flow rate.

Effects of high glucose upon glucagon and insulin response to epinephrine. After an initial preperfusion and perfusion with glucose at 80 mg/100 ml (25 min total), perfusate glucose was raised to 300 mg/100 ml and continued for 30 min before being reduced back to 80 mg/100 ml. Epinephrine (2.7×10^{-7} M) was infused during the 20–35-min time interval of the experimental period. High glucose drastically influenced both the pattern and magnitude of glucagon release in response to epinephrine, (upper panel, Fig. 6). The biphasic pattern seen with glucose at 80 mg/100 ml (Fig. 4) was obliterated. Glucagon concentration was suppressed by this high glucose concentration close to the detection limit of the assay (16±6 pg/ml) just before epinephrine was begun; then glucagon levels rose gradually, reach-

ing their highest level of only 382±57 pg/ml 5 min after the switch, and then maintaining a plateau until the epinephrine was discontinued. Using planimetry, the total glucagon output during the 15-min period of epinephrine infusion at 300 mg/100 ml glucose was 11,127 ±1,338 pg. This was significantly lower than the output produced by epinephrine at 80 mg/100 ml glucose (32,-451±6,916 pg), $P < 0.02$ (two tail t test).

Insulin was released in a biphasic pattern in response to a glucose concentration of 300 mg/100 ml (lower panel, Fig. 6). Base-line insulin at 80 mg/100 ml was 1.6±0.5 μU/ml, and the first-phase insulin peak was 228±41 μU/ml 1 min after the initiation of glucose at 300 mg/100 ml. The highest second-phase level was 88± 14 μU/ml, reached just before epinephrine was begun, and epinephrine rapidly reduced the insulin concentration to 3–8 μU/ml. 30 s after the cessation of epinephrine, there was a brief burst of insulin output with the insulin level reaching 98±34 μU/ml.

Recovery of epinephrine-induced glucagon release after glucose suppression. Perfusate glucose concentration was maintained at 300 mg/100 ml during the 20-min preperfusion period and during the first 15 min of the experimental period. Perfusate glucose was then lowered to 80 mg/100 ml. Efflux glucose levels were determined for a representative perfusion as shown in Fig. 7. 2 min after this change, glucose had fallen to 89 mg/100 ml and by 5.5 min (30 s after beginning epinephrine) had reached 85 mg/100 ml. The pattern of glucagon release in response to epinephrine was in marked contrast to that seen without prior exposure to glucose at 300 mg/100 ml (Fig. 4), in that a high first-phase peak of glucagon was not seen. This difference was statistically significant at times 20.5 and 21 min ($P < 0.02$), two tail t test). It is noteworthy that there was no significant difference of glucagon output during the last half of the epinephrine infusion. Despite the high first-phase glucagon levels reached in Fig. 4, the first phase did not contribute greatly to the total glucagon output during the 15 min epinephrine infusion. In spite of the blunting of first-phase release in Fig. 7, the total glucagon output as determined by planimetry was 32,450±5,046 pg which was virtually identical to the total output of the experiments with no prior high glucose exposure (32,451±6,916 pg, from the experiments of Fig. 4).

Response of glucagon and insulin to theophylline. 10 mM theophylline was infused for 30 min, and an early peak of glucagon occurred which rose from a base line of 99±23 to 348±111 pg/ml, 2 min after the initiation of theophylline (Fig. 8). Glucagon secretion then fell by 15 min (after the start of theophylline) to levels significantly lower than base line, and then stayed lower at a significance of either $P < 0.05$ or < 0.10 (one tail t test) until the theophylline was discontinued. This apparent lowering of glucagon in response to theophylline may, however, be misleading because when perfusate was maintained at 100 mg/100 ml for a 50-min observation period (Fig. 1) glucagon secretion gradually fell (from 112±38 to 57±16 pg/ml). Thus, even without theophylline, glucagon secretion might have fallen to levels significantly lower than base line. It is, therefore, hazardous to suggest that theophylline suppressed glucagon

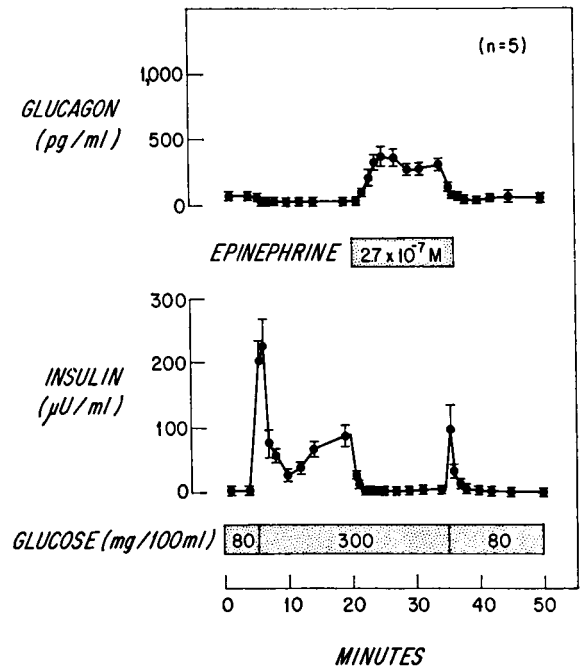

FIGURE 6 Effects of glucose at 300 mg/100 ml upon glucagon and insulin secretory responses to epinephrine.

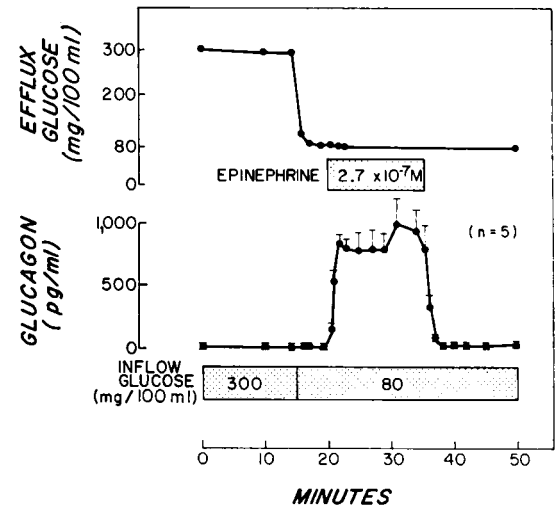

FIGURE 7 Effects of prior high glucose exposure upon glucagon secretory response to epinephrine.

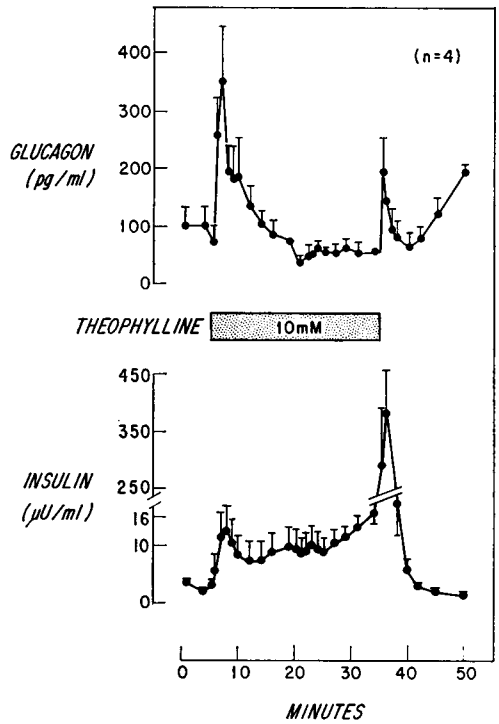

FIGURE 8 Glucagon and insulin secretion in response to theophylline. Perfusate glucose maintained at 80 mg/100 ml.

secretion in the last 15 min of the 30-min infusion. With cessation of theophylline, a brief burst of glucagon secretion occurred, reaching 195±58 pg/ml. This was significantly higher than base line (99±23 pg/ml) only at $P < 0.10$ (one tail t test), but was higher than the glucagon concentration just before theophylline discontinuation (55±21 pg/ml) at $P < 0.05$. There was then a further rise of glucagon secretion which reached 195±10 pg/ml, 15 min after the cessation of theophylline, which was significantly higher than base line, $P < 0.005$.

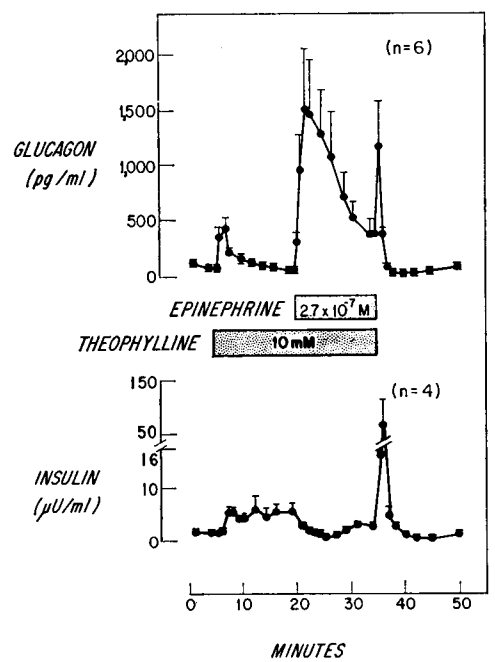

FIGURE 9 Glucagon and insulin secretion in response to theophylline and epinephrine. Perfusate glucose maintained at 80 mg/100 ml.

Insulin secretion was enhanced significantly by theophylline (lower panel, Fig. 8). Base-line insulin before theophylline was 2.0±0.4 μU/ml and during the 30 min infusion rose to levels four- to sixfold higher, all points being significantly higher at either $P < 0.05$ or < 0.10 (two tail t test). After cessation of the theophylline infusion, there was a marked rise of insulin secretion up to 385±74 μU/ml with its peak occurring 1 min after the cessation.

Glucagon and insulin responses to epinephrine and theophylline. With 10 mM theophylline alone (Fig. 9), a uniphasic increase of glucagon secretion occurred which essentially reproduced the results seen in Fig. 8. When epinephrine was then infused in addition to the 10 mM theophylline, a marked output of glucagon still occurred, but with a different pattern than with epinephrine alone (Fig. 4). In the presence of theophylline, the glucagon secretory response to epinephrine is characterized by a broad uniphasic peak and then, following simultaneous cessation of both epinephrine and theophylline, a brief burst of glucagon secretion.

The total glucagon output, as determined by planimetry, during the epinephrine infusion superimposed upon theophylline was 35,430±11,696 pg which was not statistically different than the total output during epinephrine alone, 32,451±6,919 pg. In Fig. 10, cumulative glucagon secretion, as determined by planimetry, is depicted for the first 1.5 min, and also the first 8.5 min

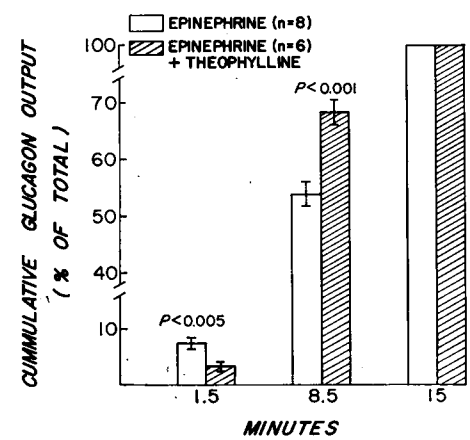

FIGURE 10 Comparison of cumulative glucagon output of epinephrine and epinephrine plus theophylline. Statistical significance determined by two tail t test.

of the epinephrine infusion, both with and without theophylline. These are shown as percent of total glucagon output over the 15-min epinephrine infusion period and quantify the differences in release patterns. A very significant inhibition of glucagon release by theophylline during the first 1.5 min is apparent. Then for the first 8.5 min, despite the early inhibition, epinephrine-induced glucagon secretion is enhanced by theophylline. Of note, there was no change of perfusate flow with either epinephrine or theophylline alone, or in combination.

Insulin responses are depicted in the lower panel of Fig. 9. Insulin secretion was significantly enhanced by theophylline alone as in Fig. 8, and then significantly suppressed after epinephrine superimposition to base-line levels, near the detection limit of the insulin radioimmunoassay. As with glucagon, after simultaneous discontinuation of both the epinephrine and theophylline, a burst of insulin secretion occurred rising from 2.5±0.5 μU/ml before the switch to 68±10 μU/ml a minute later.

DISCUSSION

When the glucose concentration of the perfusate was abruptly dropped from 100 to 25 mg/100 ml, the alpha cell responded with a two-phase pattern of glucagon output. The first phase occurred rapidly and was of short duration with a spike-like pattern, whereas the second phase was a plateau-like response pattern. A very similar response pattern was also observed when glucose was abruptly dropped from 80 to 25 mg/100 ml.[1] This biphasic glucagon response to low glucose is in some ways a mirror image of the beta cell which secretes insulin in a biphasic pattern in response to high glucose (23). It must be noted, however, that the second phase of glucagon secretion is a plateau whereas the second phase of insulin rises with time.

When the perfusate glucose was abruptly changed from 300 to 25 mg/100 ml, there was a gradual rise of glucagon release rather than a rapid first-phase peak. These experiments suggest that the alpha cell, which has been previously bathed in a glucose concentration of 300 mg/100 ml is considerably less responsive to the effects of a low extracellular glucose concentration, and that at least 10 min at a low glucose concentration is necessary for full responsiveness to occur.

It has not been known how effectively high glucose concentrations can suppress alpha cell secretion. As is amply shown in Figs. 1, 2, 6, and 7, 300 mg/100 ml glucose can suppress glucagon secretion to about the detection limit of the radioimmunoassay, i.e., 15 pg/ml. An important issue raised by these findings is whether local secretion of insulin is causing some of the effect. Data suggests that insulin may have a suppressive effect upon glucagon secretion (24–29), and the anatomical juxtaposition of alpha and beta cells may permit exposure of alpha cells to very high insulin concentrations, particularly after the islets are exposed to a high glucose concentration.

2-Deoxy *d*-glucose is an analogue of glucose which is nonmetabolizable and appears to block glycolysis by inhibiting the enzyme phosphohexoisomerase (30). Müller, Faloona, and Unger (25) injected this analogue into dogs and found a prompt rise of plasma glucagon. It was unclear from this in vivo work whether glucagon release was elicited by a direct effect of 2-deoxy *d*-glucose on the alpha cell, or whether the glucagon might have been released by a secondary effect, perhaps via activation of the sympathetic nervous system. Iverson's recent report of slight stimulation of glucagon release by 2-deoxy *d*-glucose in the isolated perfused canine pancreas (31) has been confirmed in the present study. These results support the hypothesis that alpha cell metabolism of glucose somehow restrains glucagon secretion (32). When glucose metabolism is inhibited either by blocking glycolysis or by glucose deprivation, glucagon secretion is enhanced. It was of interest that the glucagon release pattern in response to low extracellular glucose (Fig. 1) was biphasic whereas the response to 2-deoxy *d*-glucose was not (Fig. 3). A possible explanation for this difference is that 2-deoxy *d*-glucose might have an effect on a glucoreceptor which prevents a first-phase glucagon response. Significant inhibition of insulin release was found in response to 2-deoxy *d*-glucose, an observation also seen in incubated monolayer cell cultures of pancreas from newborn rat (33) as well as in organ culture of fetal rat pancreas (34). This inhibition of insulin release may have resulted in significant lowering of the local insulin concentration surrounding the alpha cell, which may have contributed to the enhanced glucagon release. Therefore, the 2-deoxy *d*-glucose effect on glucagon secretion could be caused by any or all of the following: interference with glucose metabolism, direct interaction with a glucoreceptor, or indirectly through insulin secretion.

Stimulation of glucagon secretion by epinephrine was first reported by Leclercq-Meyer, Brisson, and Malaisse in rat pancreatic slices (16). This finding has been confirmed both in vivo (18) and in vitro (17). Biphasic glucagon response was elicited by both epinephrine and norepinephrine at a concentration of 2.7×10^{-7} M, a concentration clearly higher than circulating levels of these hormones. Since autonomic nerve terminals are often anatomically adjacent to alpha cells (19), it is probable that the alpha cell is exposed to

[1] Weir, G. C., S. D. Knowlton, and D. B. Martin. Unpublished observations.

high local concentrations of norepinephrine. It is therefore possible that the 2.7×10^{-7} M concentration of norepinephrine used in the present study is physiologic.

Other workers have demonstrated suppression of insulin secretion in response to epinephrine, but this was not seen in this set of perfusions. The likely explanation for this is that insulin secretion was so low at a perfusate glucose concentration of 80 mg/100 ml that either insulin secretion could not be suppressed further or that assay sensitivity was not adequate to demonstrate a change.

Suppression of epinephrine-induced glucagon secretion by 300 mg/100 ml glucose again indicates a marked similarity between the alpha and beta cell. In both cell types, glucose not only is a primary determinant of hormonal secretion but also governs each cell type's ability to respond to other secretagogues. For instance, the beta cell can secrete far more insulin in response to arginine at high glucose concentrations than at low (35). This overall control by glucose of glucagon and insulin secretion is an elegant control system to maintain blood glucose homeostasis. When blood glucose rises, beta cell activity and capability increases, whereas alpha cell performance wanes, providing a hormonal milieu favoring reestablishment of normoglycemia. The reverse occurs if blood glucose falls.

A high glucose concentration in the perfusate produced the well known biphasic insulin release pattern (Fig. 6). When epinephrine was superimposed upon the second phase, insulin output was abruptly and efficiently inhibited. Epinephrine inhibition of insulin secretion has been previously shown both in vitro (36) and in vivo (37). When glucose and epinephrine were simultaneously discontinued, a brief spurt of insulin secretion was apparent. The explanation for this effect is unclear. Possibly beta cell responsiveness to glucose was enhanced briefly when epinephrine exposure was terminated. Burr, Balant, Stauffacher, and Renold (38), using perifused rat pancreatic slices were able to show that preperfusion with epinephrine enhanced the first phase of insulin secretion in response to glucose.

Pancreases were perfused with a high glucose concentration for 35 min before epinephrine stimulation (Fig. 7) to learn more about alpha cell recovery after glucose suppression. Even though extracellular fluid glucose concentration fell to close to 80 mg/100 ml by the time the epinephrine infusion had begun, the first-phase glucagon response was clearly blunted. It would be hazardous, however, to suggest that the second-phase response remains intact. The biphasic release patterns of glucagon and insulin are not yet understood and remain descriptive information. Perhaps the later glucagon release of these experiments contains glucagon which would otherwise have been in the first phase. It is difficult to know exactly how long alpha cell recovery after glucose suppression takes, but clearly glucagon was being secreted in large amounts 10 min after glucose in the efflux fell to near 80 mg/100 ml.

In vitro studies of the effects of phosphodiesterase inhibitors upon glucagon secretion have not been in agreement. Using incubated isolated rat islets, Vance, Buchanan, and Williams (39) were unable to show alteration of glucagon secretion using aminophylline. With the isolated perfused Chinese hamster pancreas, however, Frankel et al. (40) reported stimulation of glucagon release by theophylline, as did Rosselin, Jarrousse, Rancon, and Portha (41) using incubated slices of newborn rat pancreas, and Braaten, Schenk, Lee, McGuigan, and Mintz (42) using newborn rat pancreas monolayer cultures. Wollheim, Blondel, Rabinovitch, and Renold (43), however, also using newborn rat pancreas monolayer culture, found suppression of glucagon release by theophylline. The present study, using the isolated perfused rat pancreas, reveals that theophylline elicited uniphasic stimulation, but this was short-lived, lasting only the first 5 min of the 30-min infusion. During the last 15 min, glucagon secretion was lower than base line, and could represent an inhibitory effect of theophylline. The burst of glucagon secretion occurring after discontinuation of the theophylline was similar to the spike-like pattern of insulin release found after discontinuation of theophylline by Landgraf, Kotler-Brajtburg, and Matschinsky (44). This phenomenon, which has been termed the "off response", is not understood. We were concerned that the off response might have resulted from an acute osmotic change. Discontinuation of 10 mM theophylline, 10 mM cyclic AMP, and 10 mM adenosine[1] all elicited glucagon off responses. We have, however, been unable to reproduce glucagon off responses with sudden discontinuation of 20 mM arginine, or with an acute drop of mannitol concentration from 16.7 to 1.4 mM.[1] Insulin secretion was only modestly enhanced by theophylline presumably because of the relatively low glucose concentration of the perfusate, 80 mg/100 ml. A greater response would be expected with a higher glucose concentration. Theophylline in the present experiments produced both inhibition and enhancement of the epinephrine-stimulated glucagon secretion. Inhibition was seen in the first 1.5 min of the epinephrine infusion, but despite this, by 8.5 min, total secretion was clearly enhanced. The gradual fall-off of glucagon secretion in the final 6.5 min of the epinephrine infusion may represent alpha cell exhaustion, or possibly an inhibitory effect of theophylline had been reasserted.

The enhancement by theophylline fits the initial hy-

pothesis that epinephrine-stimulated glucagon secretion is mediated via cyclic AMP. With inhibition of phosphodiesterase, perhaps epinephrine can produce higher intracellular levels of cyclic AMP, which in turn leads to greater glucagon secretion. The early inhibitory effect of theophylline upon glucagon secretion is difficult to explain. One might suggest that the small amount of glucagon release, which occurred with theophylline alone, depleted a "pool" of glucagon which would have been available for rapid release by epinephrine. This possibility was, however, considered unlikely because of unpublished experiments showing no early inhibition of early epinephrine-stimulated glucagon release after exogenous cyclic AMP, a more potent glucagon secretagogue than theophylline.[1] Further attempts to explain this inhibitory phenomenon would be very speculative. Certainly in other tissues theophylline effects have been noted, which would be difficult to explain simply by phosphodiesterase inhibition (45).

Theophylline stimulation of insulin release in the above experiment was found and suppression by epinephrine of this slight enhancement was observed. The off response peak of these experiments was considerably lower than the off response found following discontinuation of theophylline alone (68 ± 10 vs. 385 ± 74 μU/ml, $P < 0.01$) (two tail t test). This difference may be secondary to an inhibitory effect of epinephrine which lingered after the epinephrine was discontinued.

ACKNOWLEDGMENTS

The authors thank Dr. Juan C. Penhos for assistance in the development of the perfused pancreas system; Dr. Robert C. Turner and Dr. Joseph Avruch for valuable encouragement and advice; Dr. G. W. G. Sharp and Dr. Lloyd Axelrod for helpful criticisms of the manuscript; and Ms. Dorothy Schoch for assistance with the preparation of the manuscript. Dr. A. A. Rossini of the Joslin Research Laboratory kindly helped with glucose measurements.

This work was supported in part by the National Institute of Arthritis, Metabolism, and Digestive Diseases, grants AM 13774-03 and AMO 5295-12, and research grants from the William F. Milton Fund and the John A. Hartford Foundation, Inc.

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