Glucagon Potentiation of Insulin Secretion by the Perfused Rat Pancreas

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SUMMARY

The relationship between glucagon and insulin secretion was studied using the isolated perfused rat pancreas. Glucagon was found to have no stimulatory effect on the beta cells when glucose was absent from the perfusing medium. There invariably occurred, however, a substantial increase in the amount of insulin secreted (over and above that which was released in response to glucose alone) whenever glucagon and glucose were simultaneously administered to a pancreatic preparation. This potentiation of insulin release also occurred when tolbutamide was substituted for glucose as the stimulatory agent, thus indicating that glucagon may be active on some end step in the secretory process rather than being mediated via a metabolite of glucose. The action of glucagon was also found to occur regardless of whether or not puromycin was present in the perfusate (at a concentration which has previously been shown to inhibit insulin synthesis), suggesting that a major action of glucagon is to augment the release of preformed insulin. DIABETES *19*:420-28, June, 1970.

It has been well established that in vitro pancreas preparations release insulin in response to stimulation by glucose[1-6] and other agents such as tolbutamide.[2,3,7,8] Reports of the effectiveness of glucagon in promoting in vitro insulin secretion vary, however. Some investigators observed a stimulatory effect of glucagon only in the presence of glucose in the perfusing or incubating medium,[9-11] while others suggest that glucagon exerts its effect directly, even in the absence of glucose.[12,13] The purpose of this paper is to report further investigations of the relationship which exists between glucagon and insulin secretion by the perfused rat pancreas. In addition, evidence shall be presented supporting the view that a primary effect of glucagon is to enhance the release of prestored hormone from the beta cells. A possible mechanism by which glucagon exerts its action shall also be considered.

Winner of the 1969 Research Contest of the American Diabetes Association. This is Dr. Curry's prize-winning paper.

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MATERIAL AND METHODS

In the present experiments the pancreas with the adjacent stomach, spleen, and proximal portion of the duodenum was removed from male Long-Evans rats weighing between 300 and 400 gm. The tissue was then placed in a perfusion apparatus as previously described.[8,14] Perfusion of an artificial perfusate consisting of a modified Umbreit solution, containing 4 per cent human albumin from Cutter laboratories, was then immediately begun via the normal vasculature. The perfusate was allowed to recycle between the tissue and perfusate reservoir for a period of approximately ten minutes so that there occurred stabilization of temperature at 37° C. and equilibration in a gas mixture of 95 per cent O_2 and 5 per cent CO_2. During this period the flow rate was adjusted to approximately 10 ml./min. by regulating the mean perfusing pressure on the pump side of the arterial cannula at about 60 mm. Hg. At the end of the above stabilization period, designated zero time, recycling of the perfusing medium was stopped and the total portal vein outflow was continually collected in a series of graduated test tubes for either thirty or sixty-second intervals. This collection period ranged from thirty to sixty-five minutes in total. The first two collection samples (Minutes 0-1 and 1-2) served as baseline controls. At the end of the two-minute mark the stimulating substance (usually glucose) was infused for various periods of time into the tubing leading to the cannula in the celiac axis. In most of these experiments another substance (either glucagon or tolbutamide) was infused starting at the end of the fifteen-minute mark and this infusion period also varied, as shown in the accompanying graphs.

In those experiments concerned with the relationship between glucose and glucagon to insulin secretion, glucose infusion was begun at Minute 2 and continued

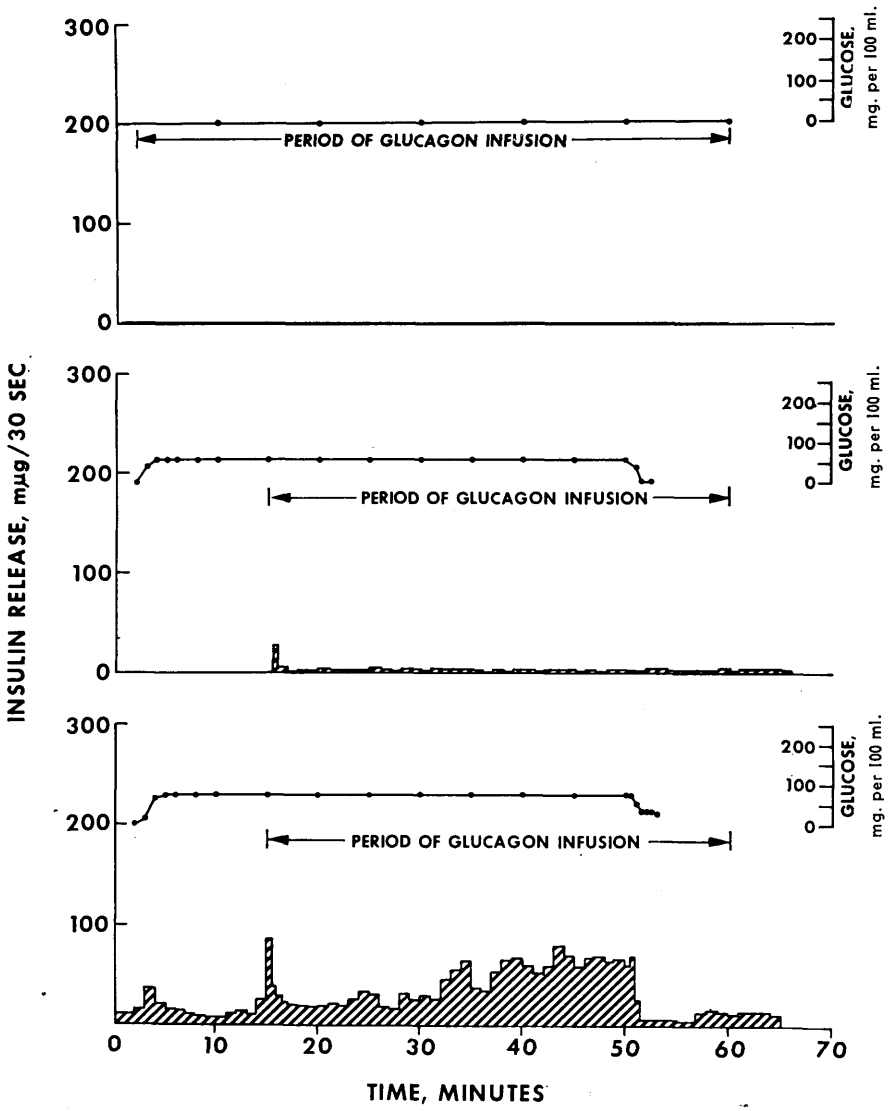

FIG. 1.
Three pancreas perfusions illustrating the time course of insulin release in response to continuous infusions of glucose and/or glucagon. The upper graph depicts insulin release in response to a fifty-eight-minute infusion of glucagon (10 μg./ml.) in the absense of glucose (one of four perfusions, each of which produced identical results). The middle graph illustrates insulin release as a function of a forty-eight-minute infusion of glucose (58 mg./100 ml.) beginning at Minute 2 and a forty-five-minute infusion of glucagon (10 μg./ml.) beginning at Minute 15. The lower graph shows insulin secretion in response to a forty-eight-minute glucose infusion (72 mg./100 ml.) beginning at Minute 2 and a forty-five-minute infusion of glucagon (10 μg./ml.) beginning at Minute 15. Since the glucose scale is contracted on these graphs for simplicity, it should be stressed that the venous glucose concentration varied only slightly during any one infusion.

until either Minutes 50 or 60. The concentration of the glucose infusate was varied so as to produce glucose levels in the perfusate ranging from 58 to 188 mg./100 ml. In these experiments glucagon* infusion was started at the end of Minute 15 and continued until either Minute 50 or Minute 60. Although concentration of glucagon in the venous effluent was not chemically determined, the rate of glucagon infusion was always adjusted so as to produce a stimulating concentration of 10 μg./ml. of perfusate. In four experiments glucagon was infused from Minutes 2 through 58 in the absence of glucose. In addition to the above, similar experiments were performed using tolbutamide (40 mg./100 ml.) in place of glucose as the stimulating agent.

Experiments were also carried out to observe the effects of mannoheptulose* on the stimulatory action of either glucose, glucagon or tolbutamide. In these instances mannoheptulose was added to the original perfusate in the reservoir so as to produce a concentration of 300 mg./100 ml. and the equilibration time was extended to twenty minutes. In the experiments utilizing tolbutamide,† this agent was infused from Minutes 15 through 28 at a rate so as to produce a perfusate concentration of 40 mg./100 ml. Puromycin‡ was used to

*Crystalline glucagon, Lot No. 258-234B-167-1, Eli Lilly and Company.

*D-mannoheptulose, Nutritional Biochemicals Corporation.
†Sodium orinase, The Upjohn Company.
‡Puromycin, Lot No. 5225B-61-C, Lederle Laboratories.

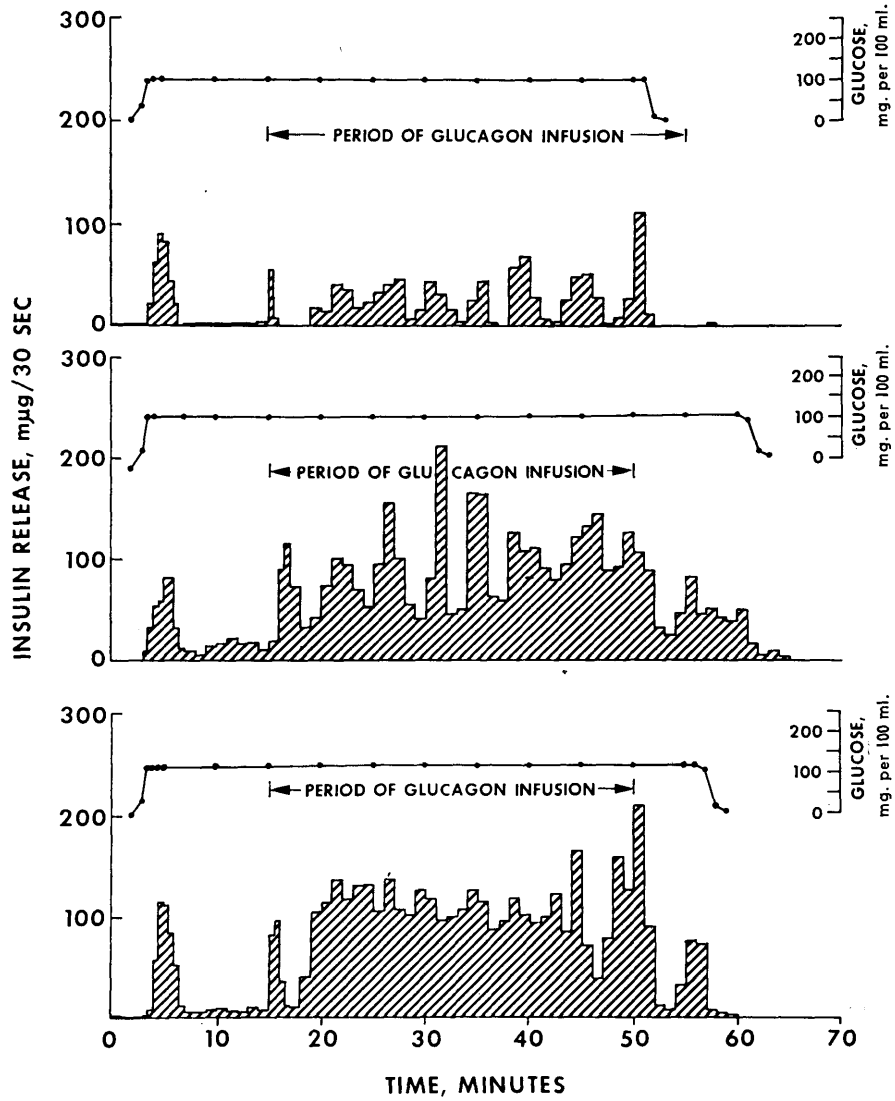

FIG. 2.
Three pancreas perfusions illustrating the time course of insulin release in response to continuous infusions of glucagon (10 μg./ml.) and glucose. The glucose level in the top graph was 100 mg./100 ml., that in the middle graph 107 mg./100 ml., and that in the bottom graph was 114 mg./100 ml.

study the effect of glucagon on insulin secretion during a period when insulin synthesis was blocked. In these experiments puromycin was added to the original perfusate at a concentration (80 μg./ml.) which inhibits > 95 per cent of the total pancreatic protein synthesis.[14] During these experiments the equilibration time was extended to thirty minutes.

Insulin concentration of the venous effluent was determined by the immunochemical method of Grodsky and Forsham[15] with pure rat insulin* as the reference standard. Total insulin release was calculated from the volume of venous effluent and its insulin concentration. Glucose concentration of the venous effluents was determined by the Somogyi-Nelson method.[16] The glucagon used in these experiments was slightly contaminated with insulin (equivalent to approximately 0.3 mμg. of rat insulin per μg. of glucagon), and all perfusions utilizing glucagon were corrected for this insulin value. This was done for each experiment by infusing the glucagon infusate into a test tube for thirty seconds at the same infusion rate used in the actual experiment. Perfusate solution was then added to produce a volume exactly comparable to that used for each thirty-second period of the perfusion involved (usually 5.0 cc.). Each "control" solution was analyzed for insulin contamination and the value obtained was subtracted from the total amount of insulin released during each thirty-second period of glucagon infusion during that par-

*Crystalline rat insulin, Lot No. R166, NOVO Laboratories.

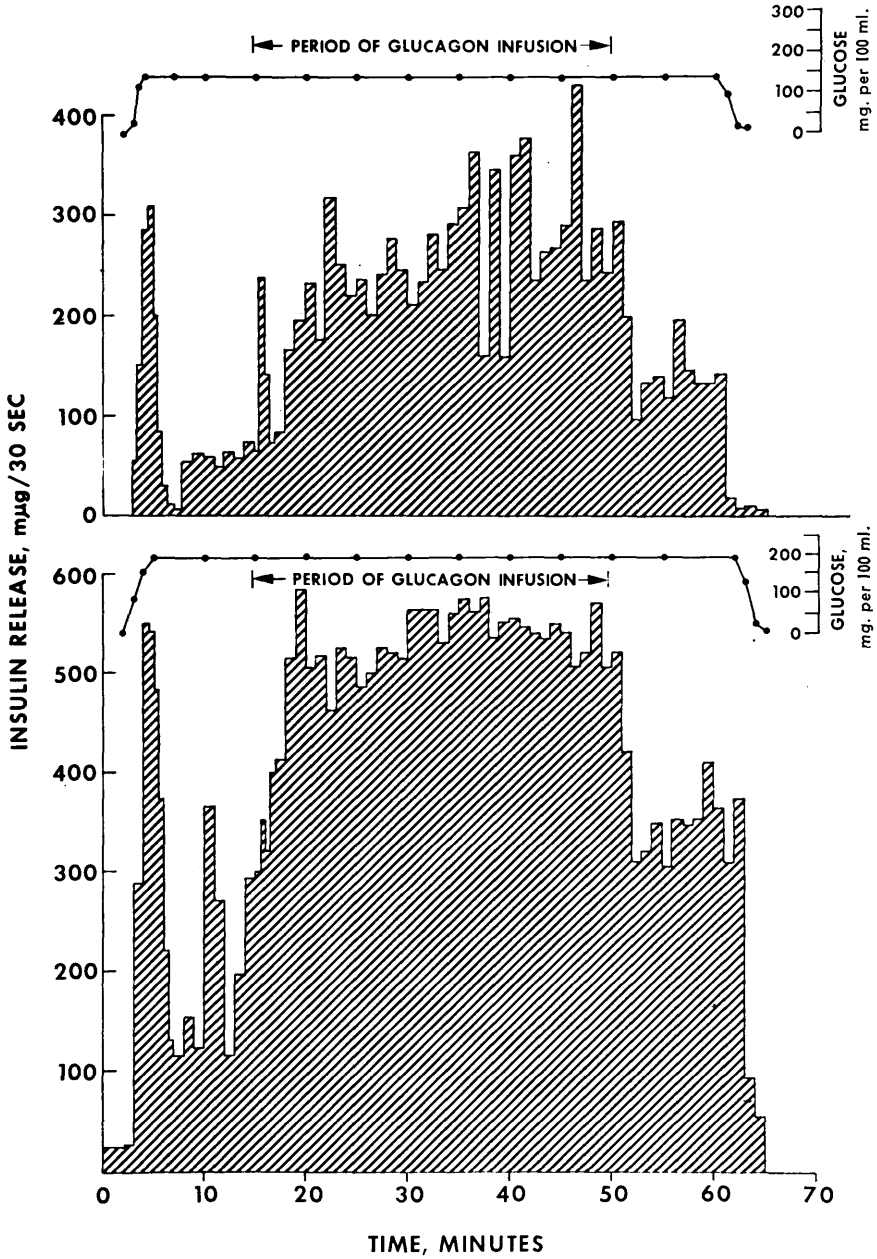

FIG. 3.
Two pancreas perfusions illustrating the time course of insulin release in response to continuous infusions of glucose and glucagon. The upper graph shows insulin release in response to a fifty-eight-minute infusion of glucose (135 mg./100 ml.) beginning at Minute 2 and a thirty-five-minute infusion of glucagon (10 μg./ml.) beginning at Minute 15, while the lower graph shows insulin release as a function of a continuous fifty-eight-minute glucose infusion (188 mg./100 ml.) beginning at Minute 2 and a thirty-five-minute infusion of glucagon (10 μg./ml.) starting at Minute 15.

ticular experiment. Any value greater than zero indicates actual insulin release and does not reflect insulin contamination of the glucagon.

RESULTS

In figures 1 through 3 are shown data relating insulin secretion to various perfusate concentrations of glucose (ranging from 0 to 188 mg./100 ml.) both in the presence and absence of glucagon. When glucose was absent from the perfusate, the top graph of figure 1, infusion of glucagon did not stimulate insulin secretion. Four perfusion experiments were performed using this design and the insulin observed in the venous effluent during the period of glucagon infusion, in each case, was balanced by the insulin contamination of the glucagon. When glucose was present in the perfusate at a substimulatory concentration (58 mg./100 ml., middle graph of figure 1), the infusion of glucagon produced only a small initial release of insulin which rapidly returned almost to basal levels. The

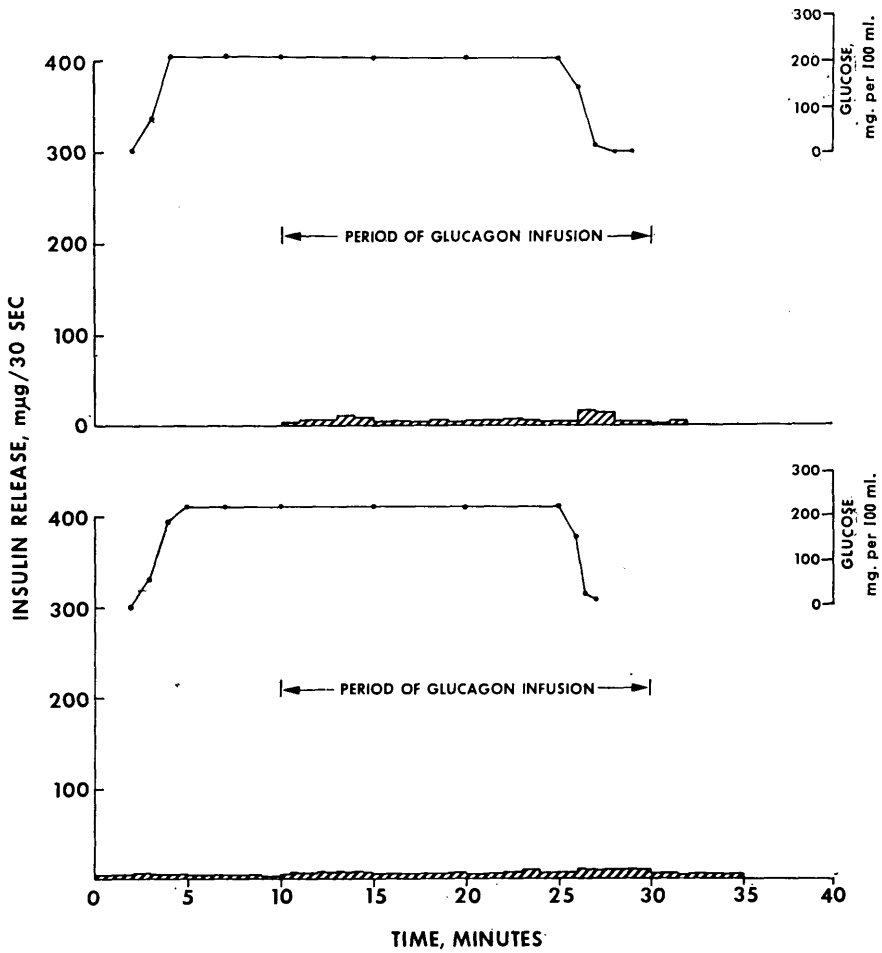

FIG. 4.
Two pancreas perfusions illustrating the time course of insulin secretion in response to infusions of glucose (200 mg./100 ml.) and glucagon (10 μg./ml.) in the presence of mannoheptulose in the perfusion medium at a concentration of 300 mg./100 ml.

effect of glucagon in producing an increased release of insulin became more and more pronounced as the glucose stimulus was increased above 58 mg./100 ml. (figure 1, bottom graph, and figures 2 and 3).

There are several interesting observations to be made from the graph at the bottom of figure 1. During the first thirteen minutes of glucose infusion (72 mg./100 ml.), when glucagon was absent, there occurred a slight release of insulin. This indicated that the lower threshold for glucose stimulation had been reached. Promptly after beginning the glucagon infusion at Minute 15, a rapid burst of insulin release was observed which corresponded to that seen in the middle graph of figure 1. When glucose infusion was stopped at Minute 50 while glucagon infusion was continued to Minute 60, there was a rapid decline in insulin secretion to near baseline levels which paralleled the cessation of the glucose stimulation, even though glucagon was still being presented to the pancreatic preparation. This lends further support to the view that glucagon alone does not cause insulin secretion.

From this experimental design it was impossible to determine if glucagon had any effect on the secondary release phase in addition to the small burst of release which was seen to occur promptly following the glucagon infusion. Therefore, similar experiments were performed in which glucagon infusion was stopped at Minute 50 (figures 2 and 3) while glucose infusion was allowed to continue to Minute 60. In these experiments, in addition to an increase in insulin release in response to the glucagon infusion, there was also a rapid decline in insulin release at about the Minute-51 mark which closely followed the discontinuation of the glucagon. In these instances the insulin release did not fall to baseline levels, however, a lower rate of insulin secretion was established in response to the continued glucose stimulation which was occurring in the absence of glucagon. These data suggest that glucagon does indeed augment glucose-stimulated insulin secretion by the perfused rat pancreas.

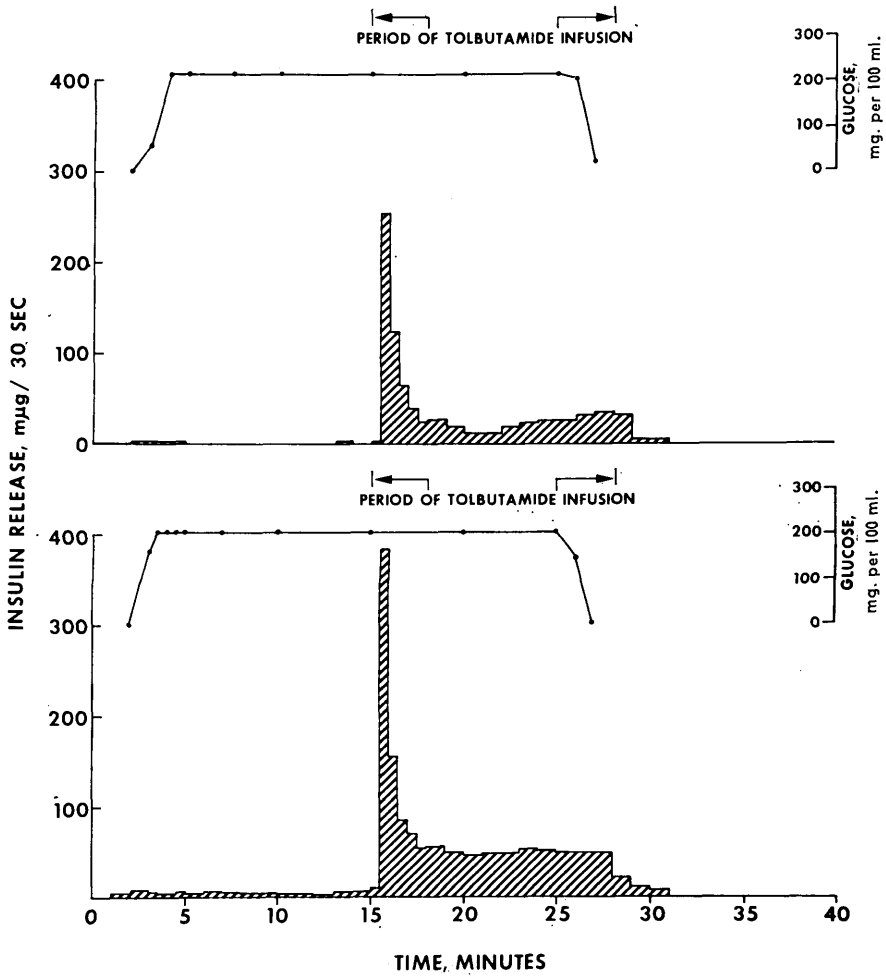

FIG. 5.
Two pancreas perfusions illustrating the time course of insulin secretion in response to infusions of glucose (200 mg./100 ml.) and tolbutamide (40 mg./100 ml.) in the presence of mannoheptulose (300 mg./100 ml.) in the perfusion medium.

The effect of mannoheptulose upon the stimulatory action of glucose and glucagon is shown in figure 4. Mannoheptulose was added to the perfusate at a concentration (300 mg./100 ml.) which completely blocked the effect of an adequate glucose stimulus (200 mg./100 ml.) that began at Minute 2. In addition, there was little or no increase in insulin release when glucagon infusion was begun at Minute 15.

The effect of mannoheptulose upon the stimulatory action of glucose and tolbutamide is shown in figure 5. Mannoheptulose again was added to the perfusate at a level (300 mg./100 ml.) which completely blocked the effect of a 200 mg./100 ml. glucose infusion that began at Minute 2. When tolbutamide infusion (40 mg./100 ml.) was begun at Minute 15, however, there was observed a rapid release of insulin which followed the same pattern that generally occurs in response to this agent.[8] Thus, mannoheptulose did not inhibit the insulin secretory process, but rather inhibited only the glucose stimulatory pathway.

The effect of glucagon upon tolbutamide-stimulated insulin release, in the absence of glucose, is shown in figure 6. Tolbutamide infusion was begun at Minute 2 and continued until Minute 30. Glucagon infusion occurred from Minutes 10 through 25. Although the insulin release during the second phase (in response to tolbutamide) was relatively low, there clearly occurred an increase in insulin secretion during the period of glucagon infusion. This increased rate of insulin secretion declined to its previous level following the cessation of the glucagon infusion.

In figure 7 are data showing the effect of puromycin on glucose and glucagon-stimulated insulin secretion. When compared to figures 1 and 2, it appears as if puromycin has no effect upon the early phase of insulin release. This is in agreement with an earlier

report by Curry, Bennett, and Grodsky[14] in which it was concluded that this early phase of insulin release represents the release of prestored insulin. The increased insulin release produced by glucagon during the secondary phase was not blocked by the presence of puromycin, since both the rapid increase in insulin secretion and the decline in secretion corresponding to the beginning and end of the glucagon infusion occurred in spite of the fact that puromycin was present. Augmentation of insulin release by the perfusion, illustrated in the top graph of this figure, is only marginal, probably due to the fact that the glucose level of the perfusate is so low (90 mg./100 ml.).

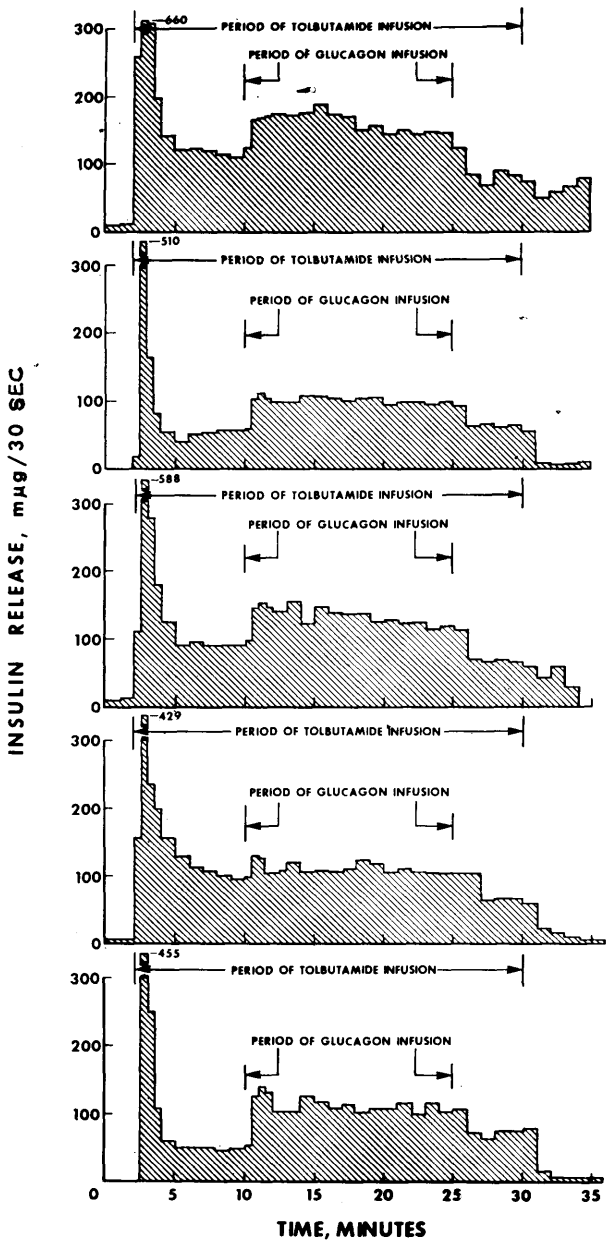

FIG. 6. Five pancreas perfusions showing the effect of glucagon on tolbutamide-stimulated insulin release in the absence of glucose. In each case tolbutamide infusion (40 mg./100 ml.) occurred from Minutes 2 through 30, while glucagon infusion (10 μg./ml.) was carried out from Minutes 10 through 25.

DISCUSSION

The data presented in this paper show that glucagon is a very effective agent in augmenting insulin release by the perfused rat pancreas. This in vitro stimulatory action of glucagon has been seen by many investigators.[9-13] The dependence of glucagon on the presence of an agent which stimulates insulin release is a matter of importance. As seen in figures 1 through 3, the enhancement of insulin release in response to glucagon infusion occurred only when glucose was also being presented to the pancreas. When glucose infusion was stopped while glucagon infusion continued, there occurred a decline in insulin secretion to prestimulatory levels that closely followed cessation of the glucose stimulus. In addition, promptly after the start of glucagon infusion, there was generally observed a rapid rise in the rate of insulin release which continued in a more or less sporadic manner until either the glucose or glucagon infusion was terminated. This sporadic release was confirmed by reassay of the perfusate and cannot be explained as being due to vagaries of the immunochemical insulin assay.

The fact that the glucagon augmentation of insulin secretion occurred regardless of whether or not puromycin was present to inhibit pancreatic protein synthesis (figures 1-3, 7) is of interest. Since it had previously been concluded that puromycin blocks insulin synthesis, thus reducing the magnitude of the second release phase,[14] and one now observes that puromycin does not inhibit glucagon potentiation during this phase, it would seem that the major action of glucagon is to increase the release of prestored insulin rather than to stimulate insulin synthesis and/or the release of newly synthesized hormone. These findings are in agreement with those of Taylor,[17] who found that glucagon did not stimulate the incorporation of labeled leucine into insulin.

Mannoheptulose, which inhibits phosphorylation of glucose,[18] completely blocked the action of glucose and the potentiation of glucose action by glucagon (figure 5), which is in agreement with previous reports.[2,9] The possibility that mannoheptulose was also blocking some process associated with the final release mechanism

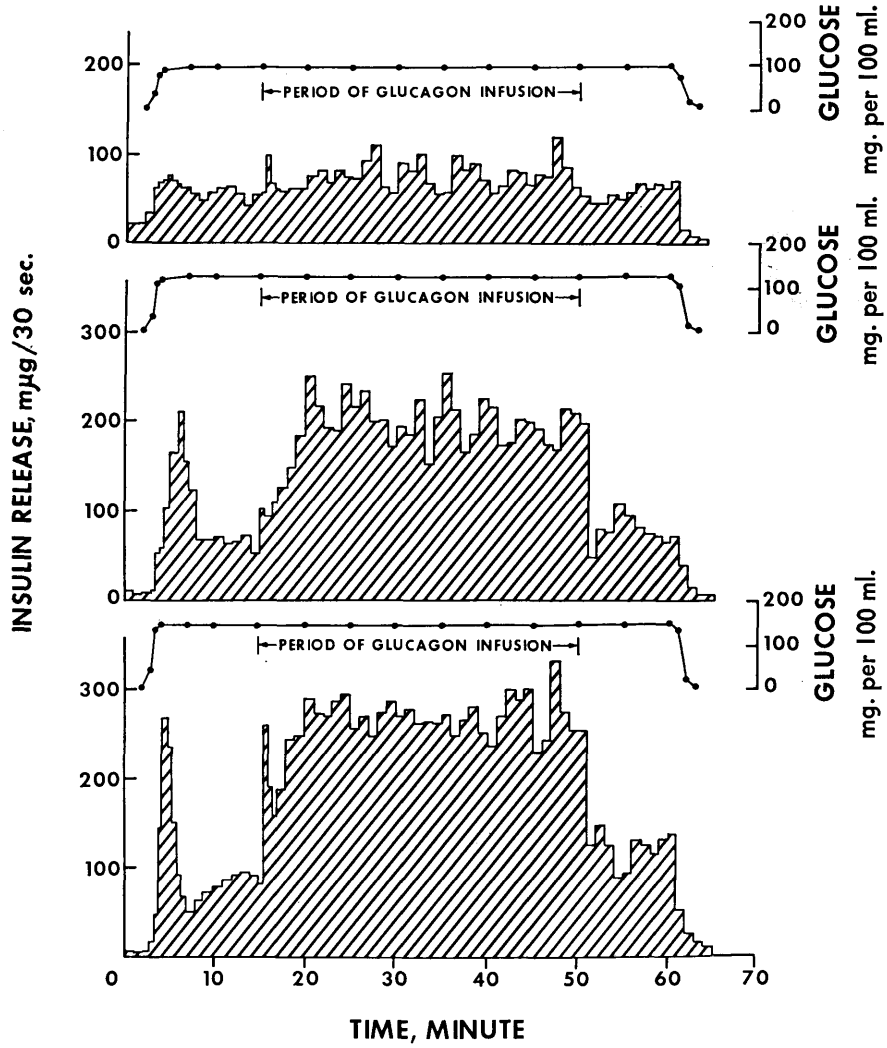

FIG. 7.
Three pancreas perfusions showing the time course of insulin secretion in response to continuous infusions of glucose and glucagon. The upper graph illustrates insulin release in response to a fifty-eight-minute infusion of glucose (94 mg./100 ml.) beginning at Minute 2 and a thirty-five-minute infusion of glucagon (10 μg./ml.) beginning at Minute 15. The middle experiment was carried out in the same manner, except that the glucose level plateaued at 125 mg./100 ml., as was the lower experiment with the exception that the glucose concentration used was 147 mg./100 ml. In all three experiments puromycin had been added to the perfusate thirty minutes prior to time zero and at a concentration (80 μg./ml.) which had previously been shown to inhibit > 95 per cent of the total pancreatic protein synthesis.

was excluded when it was shown that the pancreas was capable of secreting insulin in response to tolbutamide in the presence of mannoheptulose, a fact which had previously been observed by Coore and Randle.[2] Therefore the action of glucagon had to be either via some metabolite of glucose or upon the final secretory process itself. If this latter possibility were the case, then glucagon would also be expected to enhance the release of insulin regardless of the stimulatory agent. The observations that glucagon enhanced the release of insulin in response to tolbutamide (figure 6) strongly suggest that the potentiating effect of glucagon is mediated through some step in the secretory process other than being solely active on the glycolytic pathway. A recent report by Jarrett and Keen[19] also suggests that the mechanism of glucagon action on isolated islets is not associated with glycogenolysis. It should be emphasized, however, that the above reasoning holds only if one assumes that the mechanism for glucagon potentiation is the same regardless of whether the stimulatory agent is glucose or tolbutamide.

Although the mode of glucagon action remains in doubt, there is abundant evidence which suggests that it is associated with the production of cyclic AMP, which in turn potentiates the release of insulin.[9,10,20-23] Some investigators have observed potentiation of insulin secretion by agents, other than glucagon, which either stimulate the production of cyclic AMP, e.g., ACTH and TSH,[21,23,24] or decrease its degradation by inhibiting cyclic nucleotide phosphodiesterase, e.g., xanthines.[5,21,24-26] Insulin secretion is inhibited by alpha-adrenergic agents, such as epinephrine, which possibly act by directly inhibiting the production of cyclic AMP.[26] It therefore may be that cyclic AMP, which is recognized

to act on many enzymatic processes other than those of glycolysis and glycogenolysis,[27,28] is stimulated by glucagon and in turn has a potentiating effect on the final release process by activating an enzymatic system within the beta cell membrane. It is also possible, although probably less likely, that glucagon acts directly on the release mechanism to augment insulin secretion. These data agree with and further extend the observations of Malaisse[9] and Jarrett[19] by focusing attention on other enzymatic processes as possible modes of action of glucagon.

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