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Research Article

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Characterization of the Effects of Arginine and Glucose on Glucagon and Insulin Release from the Perfused Rat Pancreas

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ABSTRACT To characterize the mechanisms by which arginine and glucose affect pancreatic alpha and beta cell function, the effects of these agents over their full dose response, both alone and in various combinations, were studied using the perfused rat pancreas. Arginine (0–38 mM), in the absence of glucose, stimulated biphasic glucagon (IRG) secretion ($K_m \simeq 3$–4 mM) at concentrations less than 1 mM and caused nonphasic insulin (IRI) release ($K_m \simeq 12$–13 mM) but only at concentrations greater than 6 mM. Glucose (0–27.5 mM) alone stimulated biphasic IRI release ($K_m \simeq$ 9–10 mM) at concentrations in excess of 5.5 mM and caused nonphasic inhibition of IRG secretion ($K_i \simeq$ 5–6 mM) at concentrations as low as 4.1 mM. These results demonstrate fundamental differences in pancreatic alpha and beta cell secretory patterns in response to glucose and arginine and suggest that glucagon secretion is more sensitive to the effect of both glucose and arginine. Various concentrations of arginine in the presence of 5.5 mM glucose stimulated biphasic IRG and IRI release; IRG responses were diminished and IRI responses were enhanced compared with those seen with arginine in the absence of glucose. Glucose (0–27.5 mM) in the presence of 3.2 or 19.2 mM arginine caused similar inhibition of IRG secretion ($K_m \simeq 5$–6 mM) and stimulation of IRI release ($K_m \simeq 9$–10 mM) as that seen with glucose alone, although greater IRG and IRI release occurred. This augmentation of IRI secretion was greater than that expected from mere additive effects of glucose and arginine. Classical Lineweaver-Burk analysis of these results indicates that glucose is a noncompetitive inhibitor arginine-stimulated glucagon secretion and suggests that glucose and arginine affect pancreatic alpha and beta cell function via different mechanisms. In addition, comparison of simultaneous insulin and glucagon secretion patterns under various conditions suggests that endogenous insulin per se has little or no direct effect on IRG secretion and that endogenous glucagon does not appreciably affect pancreatic beta cell function.

INTRODUCTION

Although glucose and amino acids are important physiologic determinants of immunoreactive glucagon (IRG)[1] and immunoreactive insulin (IRI) secretion (1, 2), the mechanisms through which these agents act upon pancreatic alpha and beta cells, as well as their interrelationship, remain ill-defined. It has been suggested that glucose itself may directly stimulate insulin release via a glucoreceptor (3–7). Nevertheless, other data suggest that some metabolite of glucose may be the actual stimulant (8–12). A similar dichotomy of receptor vs. metabolism may also apply to the effects of glucose on glucagon secretion (13). Of the amino acids influencing glucagon and insulin secretion, arginine has been the most frequently studied (14–38); this amino acid, in the absence of glucose, directly stimulates glucagon secretion in vitro (17, 18). A similar effect on insulin secretion has not been consistently observed (15, 18, 19–23). However, in the presence of glucose, arginine is a potent stimulant of insulin release both in vivo (1, 16, 24, 25) and in vitro (15, 17, 19–21). This has led to the suggestion that arginine may cause insulin release merely by facilitating the action of glucose (19, 25, 26), rather than by an intrinsic mechanism as thought by some (21, 27, 28). In addition to these uncertainties, it is also unclear whether arginine and glucose affect pancreatic

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[1] *Abbreviations used in this paper:* IRG, immunoreactive glucagon; IRI, immunoreactive insulin.

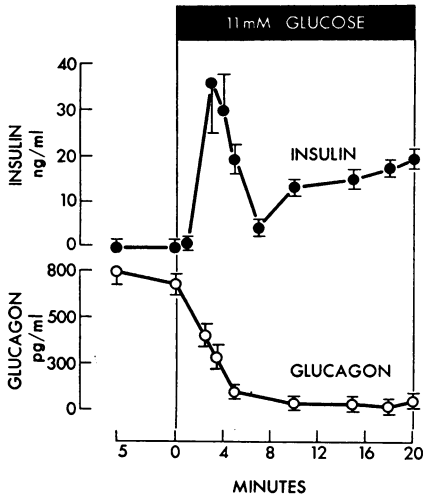

FIGURE 1 Effect of glucose on glucagon (○) and insulin (●) release from the in vitro perfused rat pancreas, mean ±SE ($n = 6$).

beta cell function via the same mechanism as they affect alpha cell function and vice versa.

Most of the previous studies investigating these questions either have been performed in vivo where it is not possible to study the effects of glucose and arginine in each other's absence and throughout their total dose response or have utilized in vitro systems not particularly suited for the simultaneous evaluation of glucagon and insulin secretion (29–32). Therefore, the present studies were undertaken using the perfused rat pancreas to characterize further the individual and combined effects of arginine and glucose on pancreatic alpha and beta cell function.

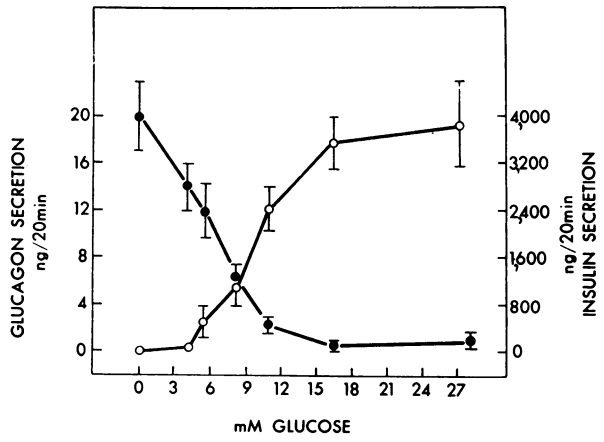

FIGURE 2 Comparison of effects of various concentrations of glucose on glucagon (●) and insulin (○) release from the in vitro perfused rat pancreas. Each point ($n = 6$) represents the total hormone released during a separate 20 min infusion of glucose, mean ±SE.

METHODS

Animals. Overnight-fasted, male Long-Evans rats, approximately 30-wk-old and 250 g in weight were used in all experiments.

Perfusion system. The technique for dissection of the rat pancreas and its perfusion have been previously described in detail (33, 34). In brief: Fasted rats were anesthetized with sodium pentobarbital; the pancreas, with adjacent stomach, spleen, and part of the duodenum were removed and subsequently perfused with a nonrecirculating medium consisting of 1% albumin, 3% dextran Krebs bicarbonate buffer, pH 7.4; perfusate was introduced into the celiac artery and total portal vein effluent was collected every 60 s; flow rates were maintained at 10 ml/min.

Experimental design. In experiments with either arginine or glucose alone, each agent was introduced via a side-arm syringe at 0 min after a 15–20-min equilibration period and perfusion was maintained for 20 min. In other experiments, arginine was introduced at 0 min into perfusate already containing glucose. Only in those experiments designed to study the combined effects of 19.2 mM arginine and glucose were both agents introduced simultaneously at 0 min. Each pancreas was exposed to only one concentration of glucose and arginine; sequential stimulations were not used in this study.

Glucagon and insulin determinations. Perfusate was collected in chilled tubes containing 15% EDTA and immediately frozen for subsequent determination of glucagon and insulin. Glucagon was measured by a modification of the Unger and Eisentraut immunoassay using the highly specific Unger 30K antiserum (35). Duplicate 0.2-ml aliquots of perfusate were added to 0.5 ml glycine buffer, pH 8.8, containing 0.25% human serum albumin, and to this mixture was added 0.4 ml antiglucagon antiserum (final dilution 1:40,000). After 72-h incubation, bound and free glucagon were separated with dextran-coated charcoal, and the free fraction was counted. The sensitivity of this assay is 20 pg/ml. The intra-assay coefficient of variation is 10% and the interassay coefficient is 15%. Insulin was measured by a solid-phase modification of the method of Grodsky and Forsham (36).

Calculations. Total glucagon and insulin released during experimental periods were determined from areas under the curves. K_m's (concentrations of agents resulting in half-maximal stimulation) and K_i's (concentration of agents causing half-maximal inhibition) were determined from analysis of dose-response curves using the method of least squares.

RESULTS

Glucagon and insulin responses to glucose (Figs. 1 and 2)

Infusion of glucose resulted in a gradual nonphasic diminution of IRG secretion and caused biphasic insulin release (Fig. 1). (Fig. 2 illustrates mean total IRG and IRI release from pancreases perfused individually for 20 min with glucose (4.1–27.5 mM). In the absence of glucose, IRG secretion averaged (mean ±SE) 1.15± 0.11 ng/min during the 20-min experimental period, whereas insulin release was undetectable. The lowest concentration of glucose studied (4.1 mM), a concentra-

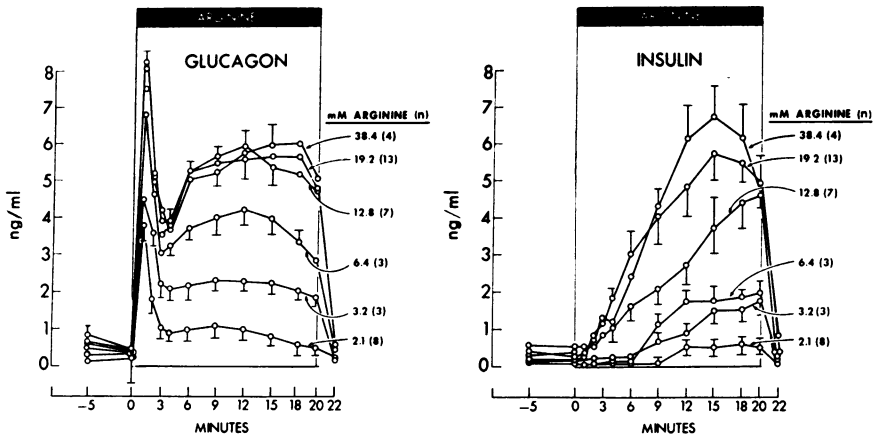

FIGURE 3 Effects of arginine on glucagon and insulin release from the in vitro perfused rat pancreas, mean ±SE.

tion similar to the fasting plasma glucose level of the rat (75 mg/100 ml), diminished glucagon secretion approximately 20%. Maximal inhibition of IRG release (> 95%) was seen at 16.7 mM glucose (300 mg/100 ml). Half-maximal inhibition of IRG release occurred between 5 and 6 mM glucose. Insulin release was first detectable at 5.5 mM glucose (100 mg/100 ml); release at lower glucose concentrations, though most likely present (33), was below the sensitivity of our assay. Maximal IRI release occurred around 16.7 mM glucose (300 mg/100 ml) and half-maximal release occurred between 9 and 10 mM glucose (160–180 mg/100 ml). Thus, glucose at physiologic concentrations inhibited IRG release and stimulated IRI secretion. Moreover, at these glucose levels the pancreatic alpha cell appeared to be more sensitive than the beta cell to the effects of glucose, both in terms of apparent threshold and K_m.

Glucagon and insulin responses to arginine (Figs. 3 and 4)

In the absence of glucose, arginine stimulated biphasic IRG secretion, whereas IRI responses were monophasic, even at concentrations of arginine causing maximal secretion (Fig. 3). Characteristically, both IRG and IRI responses tended to diminish toward the end of the arginine infusions (21). Total IRG and IRI responses during 20-min infusions of arginine at concentrations varying from 2.1 to 38.4 mM are shown in Fig. 4. (Note: the scale used in Fig. 4 differs from that used in Fig. 2.) At concentrations that stimulated little or no IRI release, arginine caused appreciable IRG secretion. Maximum responses for both phases of IRG release occurred at approximately 13 mM arginine. The K_m for both phases of arginine-stimulated glucagon release was 3–4 mM. Little insulin release was observed during infusions of arginine at concentrations less than 6 mM. Maximal IRI responses occurred at 19–20 mM arginine. The K_m for arginine-stimulated IRI release was approximately 13 mM. Despite differences in sensitivity to arginine, maximal IRG and IRI responses were quantitatively similar (900 ng/20 min). Thus, pancreatic alpha and beta cell responses to arginine differed in both secretory pattern and sensitivity: Glucagon responses to arginine were biphasic, whereas insulin release was monophasic. Glucagon secretion was more sensitive to the effects of arginine than was insulin release, similar to results observed with glucose.

Interaction of glucose and arginine on glucagon and insulin secretion

To characterize further the effects of glucose and arginine on IRG and IRI secretion, responses to the in-

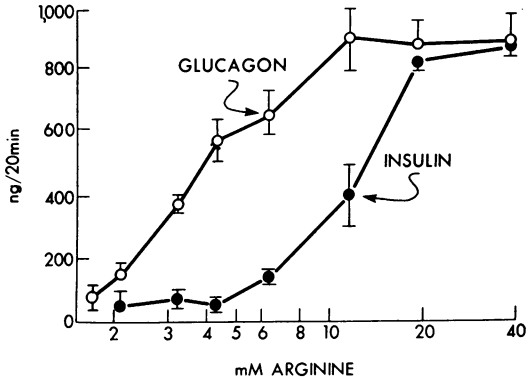

FIGURE 4 Comparison of effects of various concentrations of arginine on glucagon (○) and insulin (●) release from the in vitro perfused rat pancreas. Each point represents the total hormone released during 20 min infusions of arginine, mean ±SE. Data derived from results shown in Fig. 3.

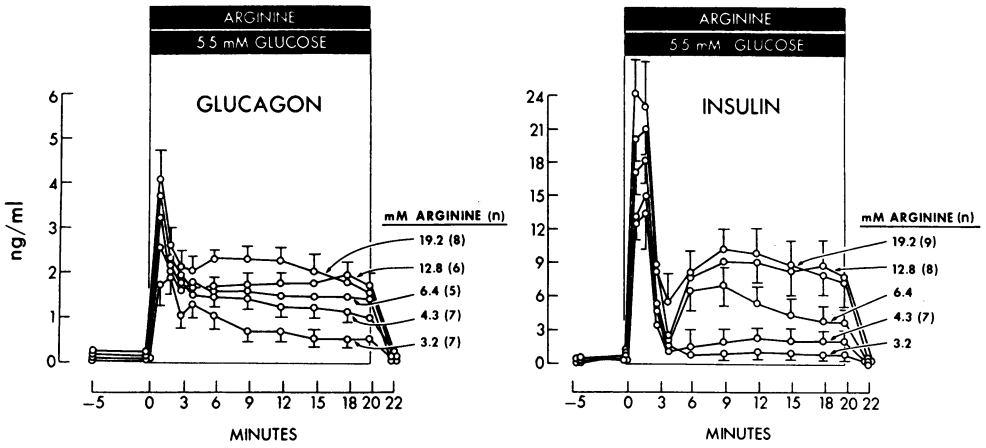

FIGURE 5 Effects of various concentrations of arginine on glucagon and insulin release from the in vitro perfused rat pancreas in the presence of 5.5 mM glucose, mean ±SE.

fusion of varying concentrations of one agent in the presence of a constant amount of the other were studied.

Glucagon and insulin responses to arginine in the presence of 5.5 mM glucose (*Figs. 5 and 6*). In the presence of 5.5 mM glucose, arginine stimulated biphasic release of both IRG and IRI (Fig. 5). Similar concentrations of arginine in the absence of glucose had not resulted in biphasic IRI release (Fig. 3), the latter being a characteristic of IRI responses to glucose. Less IRG and greater IRI release occurred at each concentation of arginine alone (Fig. 6). Glucose did not affect the K_m for IRG release (3–4 mM); however, the K_m for IRI release decreased from 12–14 mM arginine, seen with arginine alone, to 5–6 mM arginine. Thus, the addition of glucose to arginine altered both the sensitivity and the release pattern of IRI responses to arginine, whereas glucose did not alter these parameters for IRG responses to arginine despite its having an inhibitory effect.

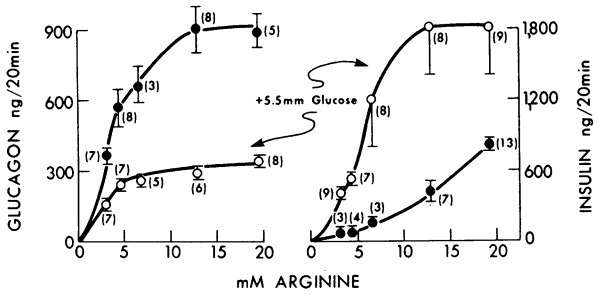

FIGURE 6 Glucagon and insulin responses to arginine in the presence of 5.5 mM glucose (○) and in the absence of glucose (●). Each point represents the total hormone released during 20 min infusions of arginine, mean ±SE. Data derived from results shown in Figs. 3–5.

Glucagon and insulin responses to glucose in the presence of 3.2 or 19.2 mM arginine (*Figs. 7–9*). Both IRG and IRI responses to combined glucose-arginine infusion were biphasic at glucose concentrations greater than 4.1 mM (Fig. 7). Increasing glucose concentrations had opposite effects on IRG and IRI release, decreasing IRG responses while augmenting IRI responses.

In the presence of 3.2 mM arginine, maximal inhibition of IRG release (> 99%) occurred between 10–27.5 mM glucose (Fig. 8). The *Ki* was 5–6 mM glucose, similar to that observed with glucose alone. Thus, although more IRG was released in the presence of arginine than in its absence, the inhibitory effect of glucose appeared to be the same whether or not arginine was present.

Little IRI release occurred during infusion of 3.2 mM arginine at glucose concentrations below 4.1 mM (75 mg/100 ml); at higher concentrations of glucose, IRI responses to arginine progressively increased through 27.5 mM glucose, the highest concentration studied. At these concentrations of glucose, perfusate IRI levels were already elevated before infusion of arginine. It seemed appropriate, therefore, to acquire dose-response data for IRI release using another experimental design in which glucose and arginine were added simultaneously. For these studies, glucose at various concentrations up to 27.5 mM was introduced simultaneously with 19.2 mM arginine. IRI responses to combined stimulation of glucose and arginine were greater than those seen at comparable concentrations of glucose in the absence of arginine (Fig. 9). Nevertheless, both the K_m and the concentration of glucose causing maximal release (9–10 mM glucose and 15–17 mM glucose, respectively) were similar under both conditions.

Thus, addition of arginine augmented IRI responses above the maximum obtainable with glucose alone but

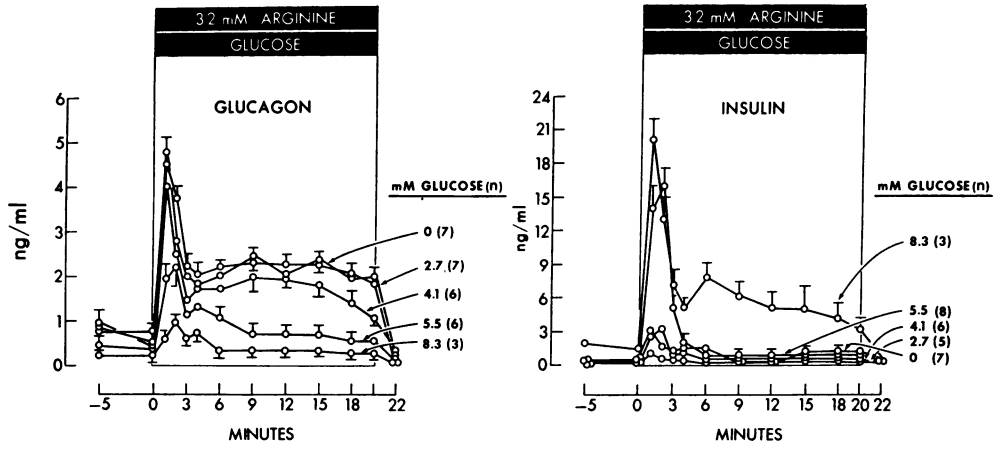

FIGURE 7 Effect of various concentrations of glucose on glucagon and insulin responses to 3.2 mM arginine, mean ±SE.

did not alter beta cell sensitivity to glucose. Similarly, addition of arginine augmented IRG release during glucose infusion without altering alpha cell sensitivity to glucose. Analysis of dose-response data obtained at near maximal velocity for IRI and IRG secretion using classical Lineweaver-Burk double reciprocal curves yielded results that are consistent with a noncompetitive interaction of glucose and arginine on *both* pancreatic alpha and beta cell function.

DISCUSSION

The present investigation was undertaken to characterize further the mechanisms through which glucose and arginine affect pancreatic alpha and beta cell function. By using a perfused rat pancreas preparation, it was possible to study the effects of each agent independently as well as in combination with the other over its entire dose response. The insulin-glucose dose-response relationship observed in the present study using the perfused rat pancreas is similar to that which we have previously described for both phases of insulin release (37) and to results obtained in other systems. Thus, Coore and Randle (8) using rabbit pancreas pieces, Malaisse, Malaisse-Lagae, and Wright (38) using rat pancreas segments, and Montague and Taylor (39) using isolated rat iselets, have also found the K_m for glucose-stimulated insulin release to be approximately 9–10 mM (160–180 mg/100 ml).

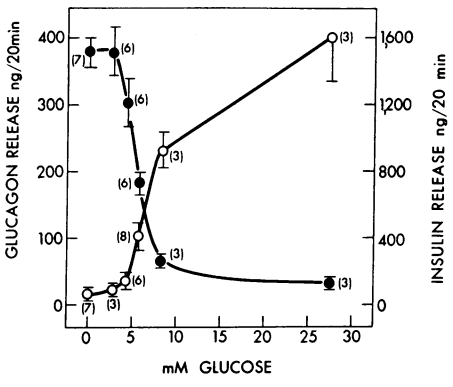

FIGURE 8 Effect of glucose on glucagon (●) and insulin (○) responses to arginine. Each point represents the total hormone released during separate 20 min infusions of 3.2 mM arginine in the presence of various concentrations of glucose, mean ±SE. Data derived in part from results shown in Fig. 7.

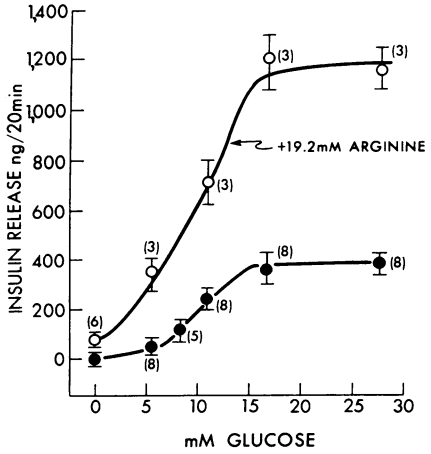

FIGURE 9 Insulin responses to glucose in the presence of 19.2 mM arginine (○) and to glucose alone (●). Each point represents the total hormone released during separate 20 min infusions of various concentrations of glucose, mean ±SE.

The effects of glucose on glucagon secretion are in close agreement with in vivo observations in man (2) and dogs (40), as well as with in vitro studies employing the perfused pancreas technique (41–44) which indicate that the pancreatic alpha cell is quite responsive to glucose. The K_i for glucose-induced inhibition of glucagon release observed in the present study (5–6 mM or 90–110 mg/100 ml) was lower than the K_m for glucose-induced insulin (9–10 mM), suggesting that at or around physiologic levels glucose is a more effective regulator of the alpha than of the beta cell. These observations contrast with the relative alpha cell insensitivity to glucose seen in studies employing isolated islets (29, 31) and pancreatic segments (30, 32), in which glucose levels of 16.7 mM (300 mg/100 ml) have often resulted in little or no suppression of glucagon secretion (32). Such results may be due to damage of pancreatic tissue during isolation or to the presence of proteolytic factors from acinar tissue remnants (30). Thus, it would appear that these in vitro systems may be less than ideal for the study of glucagon secretion.

The present study confirms that arginine in the absence of glucose is capable of stimulating insulin (17, 19, 21, 22). The failure of other investigators (15, 20, 23, 26) to find this effect is most likely attributable to the use of substimulatory concentrations of arginine and static incubation systems which may not be as sensitive for detecting low levels of insulin release as the isolated perfused pancreas. The intrinsic ability of arginine to stimulate insulin release, however, is small compared with that of glucose. This is evident from both the relative magnitude of insulin responses to each agent and the observed lower K_m for glucose-stimulated insulin release (9–10 mM) compared with that of arginine 12–14 mM). In contrast to its effects on the beta cell, arginine was an extremely potent stimulus of glucagon secretion. At concentrations less than 1 mM, arginine stimulated glucagon release with half-maximal responses around 3–4 mM arginine. This glucagon-arginine dose-response relationship is similar to the dose response observed for a mixture of amino acids approximating the composition of normal rat serum (44). It is possible, therefore, that glucagon responses to arginine may be representative of pancreatic alpha cell responses to amino acids in general.

Extrapolation of our in vitro data to in vivo situations requires caution. However, the present findings indicate that amino acids or glucose at physiologic concentrations found in the postabsorptive state (< 2 and < 5.5 mM, respectively) individually stimulate little or no insulin release but have a significant effect on glucagon secretion. This suggests that, in the rat, and perhaps in man (45), basal insulin secretion in the postabsorptive state is determined by other factors, including the synergistic effect of glucose and amino acids (21, 27). Our studies indicate that these levels of glucose and amino acids may play a greater role in regulating basal glucagon secretion.

One of the prime questions under consideration in the present study was whether glucose and arginine exert their effects through a common mechanism. Several lines of evidence derived from the present results indicate that they do not. Firstly, insulin responses to glucose were biphasic, whereas arginine, even at concentrations stimulating maximal secretion, caused monophasic insulin release. Secondly, the maximal release for glucose- and arginine-stimulated insulin release differed. Also, the addition of glucose to arginine and vice versa resulted in insulin release that exceeded the maximum obtained with either agent alone. This augmentation could not be accounted for simply by an additive effect as previously suggested (19, 26), and in most instances occurred without appreciable change in K_m for either glucose or arginine. Classical Lineweaver-Burk analysis, though possibly an oversimplified application to a complicated coupled stimulus-secretion system, indicated a noncompetitive interrelationship between the two agents. Although glucose and arginine may each affect insulin secretion by more than one mechanism (3, 5, 26, 37), this lack of competition suggests that glucose and arginine are not acting at the same receptor sites on the beta cell.

Glucagon responses in the present study suggest that glucose and arginine also act via different mechanisms on the alpha cell. Most obvious was the fact that glucose inhibited glucagon secretion while arginine stimulated it. Neither the K_i for glucose-induced inhibition nor the K_m for arginine-induced stimulation of glucagon release was appreciably affected by various combinations of these agents. In fact, according to classical Lineweaver-Burk analysis, glucose was found to be a noncompetitive inhibitor of arginine-induced glucagon secretion. Thus, data derived from study of both insulin and glucagon secretion suggest that glucose and arginine exert their effects at different sites. In terms of the receptor-stimulation theory (3, 5), these results support the presence of separate glucose and arginine receptors on both the alpha and beta cell. Indeed, recent reports demonstrating that arginine is not metabolized to a significant degree by mammalian islets (46, 47) and that a nonmetabolizable analogue of arginine can stimulate glucagon and insulin release (28) make the existence of an arginine receptor an attractive hypothesis.

A second question considered in the present study was whether glucose and arginine each affected pancreatic beta and alpha cells in a similar manner; that is, does arginine stimulate insulin and glucagon release and does glucose stimulate insulin release and inhibit

glucagon secretion by a similar mechanism? Given the possibility that differences intrinsic to the nature of highly specialized alpha and beta cells may govern their responses, several observations are of interest. Firstly, glucose caused biphasic insulin release and inhibited glucagon release nonphasically: Glucagon release decreased gradually and the secretory pattern was not the reciprocal of that observed for insulin release. Secondly, both alpha and beta cells demonstrated different sensitivities to glucose and to arginine. For arginine, the K_m for glucagon release (2–4 mM) differed from that of insulin release (12–14 mM). Similarly, the K_i for glucose-induced inhibition of glucagon release (5–6 mM) differed from the K_m for glucose-induced insulin release (9–10 mM). Thus, both the pattern of secretory response and the sensitivity of each cell to glucose and arginine suggest that each of these agents acts via a different mechanism on each cell.

It has been suggested that, under physiologic conditions, insulin may directly inhibit glucagon release and that glucagon may directly stimulate insulin release (48). Certain observations of the present study cast doubt that these are important actions of the endogenous hormones. Firstly, at concentrations of glucose causing biphasic insulin release, there was no parallel biphasic inhibition of glucagon secretion. Secondly, increasing concentrations of arginine in the presence of a constant amount of glucose (5.5 mM) caused progressive augmentation of both insulin *and* glucagon without any change in alpha cell sensitivity to arginine. These results suggest that insulin itself, independent of its action on glucose, has no direct effect on glucagon secretion. Observations in vivo also support the same conclusion: Neither infusion of glucose, which raises endogenous plasma insulin levels (49), nor administration of pharmacologic amounts of exogenous insulin prevents the glucagon response to a subsequent fall in plasma glucose (2, 49). Indeed, whether insulin is required for, or contributes appreciably to, the suppressive effect of glucose on glucagon secretion (50) is open to question. In studying glucagon secretion from perfused pancreases of rats made diabetic with streptozotocin, Laube et al, found no effect of infused insulin on glucagon release (42), and Pagliara et al. found that glucose was able to inhibit glucagon release in the absence of demonstrable insulin secretion (44). In general, studies showing a suppressive effect of insulin on glucagon secretion (50) have been performed in vivo where effects of insulin other than those directly on the alpha cell may be operative.

Pharmacologic doses of glucagon stimulate IRI release both in vivo (48) and in vitro (33). However, in the present study, stimulation of endogenous glucagon release by low concentrations of arginine to levels 50 times those seen in peripheral venous blood in man did not result in insulin release even in the presence of glucose (50 mg/100 ml). Thus, unless secreted glucagon bypasses the pancreatic beta cell, it seems unlikely that glucagon itself, at concentrations seen under physiologic conditions, directly stimulates insulin secretion.

In conclusion, the present study characterizes quantitatively the pancreatic alpha and beta cell responses to glucose and arginine. Glucagon secretion is more sensitive to the effects of both arginine and glucose than is insulin secretion. Glucose and arginine appear to interrelate but act via different mechanisms on both insulin and glucagon secretion. Although arginine itself directly stimulates insulin release, it does so in a manner different from that of glucose. Glucose is a noncompetitive inhibitor of arginine-stimulated glucagon release. Endogenous insulin and glucagon at the levels produced in these studies appear to have little or no effect on each other's secretion.

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