Glucose Modulation of Amino Acid-Induced Glucagon and Insulin Release in the Isolated Perfused Rat Pancreas

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A B S T R A C T Interactions between glucose and argi-
nine and a mixture of 20 amino acids found in normal rat
serum were studied in the isolated perfused rat pancreas
of normal rats, with release of immunoreactive glucagon
and insulin as parameters. Secretion of both pancreatic
hormones was low during the steady state, whether glu-
cose (5 mM) was included in the perfusion medium or
not. This glucose concentration significantly stimulated
insulin release twofold and resulted in an 80% inhibition
of basal glucagon release. Arginine and the amino acid
mixture were potent stimulants of both hormones. Se-
cretion of both hormones followed identical biphasic re-
sponse patterns after addition of arginine or the amino
acid mixture. However, stimulation of insulin release
occurred only when glucose was included, whereas both
phases of glucagon release were elicited in the absence
of glucose and markedly reduced in its presence. The
dose-dependency curves of hormone release due to argi-
nine on one hand and the amino acid mixture on the
other differed substantially: with arginine, release of
insulin and glucagon was linear between a concentration
of 0.3 and 20 mM. In contrast, the amino acid mixture
resulted in half-maximal release for both hormones
between a concentration of 3 and 4.5 mM, and maximal
release between 6 and 8 mM. The dose-dependencies of
glucose modulation of α- and β-cell activity were also
different: when the amino acid mixture was maintained
at 15 mM and glucose varied (0–6.25 mM), no insulin
release occurred until glucose was above 2.5 mM, whereas
incremental inhibition of glucagon occurred through
the complete dose range. It was also observed that glu-
cose inhibition of amino acid-stimulated glucagon re-
lease was dissociated from glucose-dependent increase of
insulin release.

These studies indicate that: (a) the α-cell, like the
β-cell, secretes at a low basal rate; (b) hypoglycemia
per se is a weak stimulus for glucagon secretion com-
pared to the high efficacy of a physiologic amino acid
mixture; (c) glucose plays opposite roles in the mecha-
nisms leading to amino acid-induced hormone release
from the α- and β-cells, functioning as an inhibitor in
the first case and a permissive agent in the second, and
(d) the data are compatible with the postulated existence
of glucose and amino acid receptors in both the α- and
β-cells.

INTRODUCTION

Glucagon and insulin are considered to be important
hormones for the maintenance of minute-to-minute fuel
homeostasis of the organism (1). The α- and β-cells,
the source of these hormones, are chemoreceptor cells
with a broad spectrum capable of rapid response to al-
most any alteration in the body's supply of assorted
nutrients. Since the actions of insulin and glucagon on
peripheral tissues in regard to carbohydrate, protein,
and fat metabolism are clearly antagonistic, it is not
surprising that the mechanisms responsible for the secre-
tion of these two hormones are likewise regulated in
opposite ways; in general terms, conditions which stimu-
late α-cells usually inhibit β-cells and vice versa. Both
glucose and fatty acids, for example, cause insulin re-
lease, whereas glucagon release appears to be inhibited
by these substances (2–4). Amino acids, however, seem
to present an exception to this general rule, since they
lead to hormone release from both α- and β-cells (1,
5–7). In spite of this overall understanding of α- and
β-cell physiology, there is little detailed knowledge
about possible complex interactions of the major small
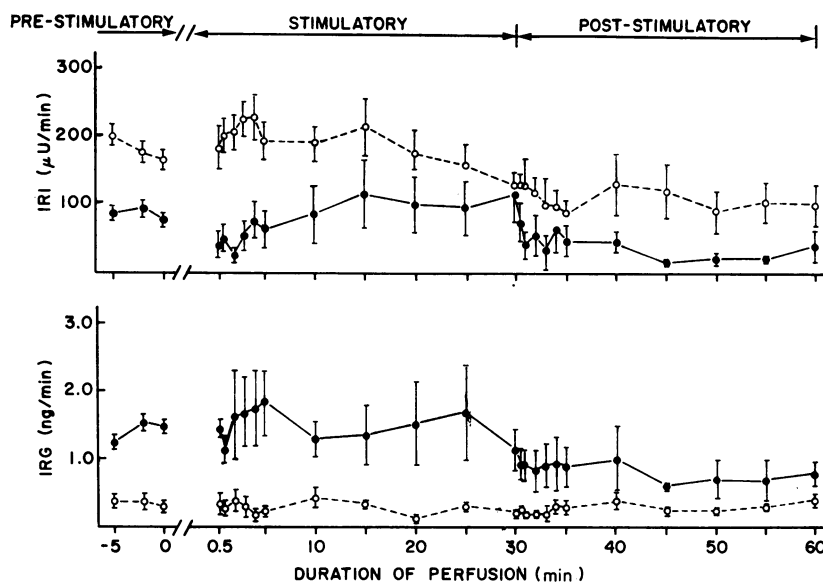calorigenic molecules (i.e. amino acids, fatty acids,

FIGURE 1 Basal insulin and glucagon release from the pancreas perfused with and without glucose. The pancreas was perfused for 75 min without changing the composition of the perfusate but the reservoir was switched at 0 and 30 min as in perfusions with the amino acid stimulants. After 10 min preperfusion, three samples were taken (t_{-5}, t_{-2}, and t_0). The data recorded in the preperfusion period represents the mean $\pm$SEM of all experiments performed in this study and also of those obtained in a parallel study [4] (insulin release without glucose, $n = 84$; insulin release $+5$ mM glucose, $n = 76$; glucagon release without glucose, $n = 95$ and glucagon release $+5$ mM glucose, $n = 76$). The values given during the time periods $t_{0.5}$–t_{30} and $t_{30.5}$–t_{60} are the means $\pm$SEM of the rates of release of both hormones and correspond to the stimulatory and poststimulatory time periods in perfusions performed with amino acids (insulin release without glucose, $n = 8$; insulin release $+5$ mM glucose, $n = 6$; glucagon release without glucose, $n = 3$; and glucagon release with 5 mM glucose, $n = 3$). See also Table I.

——, Insulin and glucagon release without glucose; - - -, insulin and glucagon release with 5 mM glucose; IRI, immunoreactive insulin; IRG, immunoreactive glucagon.

ketone bodies, and sugars) at the cellular or biochemical level.

It seems particularly important to comprehend the multiple functions that the glucose molecule might serve in regulating the chemosensitivity of the α- and β-cells, since it has become increasingly clear that glucose modulates the responsiveness of these cells to other stimuli, in addition to its well-known direct insulin-releasing action from β-cells. The multiple actions of glucose on islet cells have been explained in various ways. The frequently quoted unifying concept proposed by Edwards and Taylor (2) attributes glucose stimulation of β-cells on one hand and glucose inhibition of α-cells on the other to increased provision of fuel or of critical metabolites and cofactors, i.e. insulin release occurs when the β-cells are supplied with energy and an optimal metabolite and cofactor constellation, whereas glucagon release ensues when the energy level (i.e. ATP and P-creatinine) is lower in α-cells. This general notion has been extended by Unger and Lefebvre and collaborators (1, 5, 8), who believe that in the α-cells the provision of metabolic

energy from glucose, and consequently the inhibitory action of glucose, are insulin-dependent.

Another widely accepted view holds that the two types of islet cells are fundamentally different physiologically: α-cells are considered as inherently active cells, i.e. as pacemaker cells normally curbed by glucose levels in the physiological range, whereas β-cells require external stimuli to become activated (1). To submit these various concepts to further experimental testing, the present physiological and pharmacological studies were initiated with the isolated perfused rat pancreas. This paper is the first report of this series of studies. The major objective was to assess the possible role of glucose in regulating the function of α- and β-cells when stimulated by arginine or a mixture of 20 natural amino acids and to elucidate the kinetics of glucagon and insulin release in the isolated perfused rat pancreas to these stimuli as an initial step before correlation of hormonal release with possible biochemical changes occurring within the α- and β-cell.

TABLE I

Mean Average Basal Insulin and Glucagon Release in the Presence and Absence of Glucose*

Condition	No. of perfusions	n	$t_{-5}-t_0$ insulin	No. of perfusions	n	$t_{0.5}-t_{30}$ insulin	No. of perfusions	n	$t_{30.5}-t_{60}$ insulin
			$\mu U/min$			$\mu U/min$			$\mu U/min$
−Glucose	84	252	81 ± 3	8	88	74 ± 10	8	88	42 ± 5§
+Glucose (5 mM)	76	228	179 ± 11	6	66	193 ± 9	6	66	110 ± 5§
‡P			<0.001			<0.001			<0.001
			Glucagon, ng/min			Glucagon, ng/min			Glucagon, ng/min
−Glucose	95	285	1.42 ± 0.10	3	33	1.51 ± 0.07	3	33	0.84 ± 0.04§
+Glucose (5 mM)	76	228	0.37 ± 0.01	3	33	0.31 ± 0.03	3	33	0.29 ± 0.02‖
‡P			<0.001			<0.001			<0.001

* The average rates of insulin and glucagon release were calculated from the data shown in Fig. 1. The values given during the time period $t_{-5}-t_0$ are the mean $\pm$SEM of the rates of release at time points t_{-5}, t_{-2}, and t_0 for both hormones. The values given during the time periods $t_{0.5}-t_{30}$ and $t_{30.5}-t_{60}$ are the mean $\pm$SEM of the average rates of release of both hormones when perfusions were performed with and without 5 mM glucose (see legend to Fig. 1).

‡ Degree of significance between perfusions with and without glucose.

§ Significantly different, $P < 0.001$ from $t_{-5}-t_0$ and $t_{0.5}-t_{30}$.

‖ Significantly different, $P < 0.01$ from $t_{-5}-t_0$.

METHODS

Animals and perfusion system. Male Sprague-Dawley rats weighing 300–400 g, fed ad libitum with Purina rat chow (Ralston Purina Co., St. Louis, Mo.) and water were used in all experiments. The animals were purchased from Holtzman Co., (Madison, Wisc.) or Zivic-Miller (Allison, Pa.).

The pancreas was isolated and perfused by the procedure described by Grodsky et al. (9) with minor modifications (10). After an overnight fast, the animals were atropinized (0.1 mg/kg i.p.) and anesthetized with pentobarbital (45 mg/kg i.p.). Atropine was given since it prevented respiratory problems that occasionally arose during the surgical procedure. The pancreas was isolated en bloc with the spleen, stomach, and proximal portion of the duodenum. Polyethylene cannulae were inserted into the celiac artery and portal vein, and the organ complex was placed in an incubator and perfused at a constant 35°C without recycling the medium. The flow rate was frequently measured and was maintained constant between 5 and 6 ml/min. The perfusate contained routinely the following components: NaCl, 120 mM; KCl, 4.7 mM; MgSO₄, 0.8 mM; CaCl₂, 2.5 mM; KH₂PO₄, 1.2 mM; NaHCO₃, 25 mM; and dextran 7–8%. Other additions were made as described in the text below or in the legends of the appropriate figures and tables. Before perfusion, the medium was warmed to 35°C and gassed with a mixture of O₂ and CO₂ (95:5). The resulting pH was 7.4.

Perfusion with amino acids in the presence and absence of basal glucose. For each experimental condition, at least six separate perfusions were performed, half of which had 5 mM glucose present throughout. This concentration of glucose was selected since it has minor effects on basal insulin release (11, 12). In all experiments in which glucose and/or amino acids were studied, the pancreas was perfused for 15 min before exposure to the amino acid stimulant. The period of stimulation lasted 30 min and was followed by another control period of 30 min by perfusion in the absence of the amino acid(s). The transitions be-

tween different conditions by switching from one circulation medium to the other were rapid and did not result in detectable changes of flow rate.

Dose-response curves for insulin and glucagon release were obtained with L-arginine-HCl in concentrations of 0.3, 1, 5, 10, and 20 mM and with a mixture of 20 amino acids in proportions found in normal rat serum (13), at levels of 1, 3, 6, 10, and 15 mM. The amino acid mixture was conveniently added as a 154.7 mM stock solution stored at −20°C. The composition of this stock solution was as follows (all concentrations recorded in millimoles): alanine, 18.76 (12.10%); arginine HCl, 8.03 (5.20%); aspartic acid, 1.74 (1.10%); citrulline, 3.99 (2.58%); glutamic acid, 5.18 (3.36%); glycine, 12.75 (8.27%); histidine, 3.35 (2.17%); isoleucine, 4.04 (2.62%); leucine 6.97 (4.52%); lysine HCl, 15.87 (10.3%); methionine, 2.06 (1.34%); ornithine HCl, 2.98 (1.93%); phenylalanine, 3.49 (2.26%); proline, 14.91 (9.20%); serine, 24.40 (15.90%); threonine, 11.54 (6.85%); tryptophan, 3.17 (2.06%); valine, 8.62 (5.60%). Cystine and tyrosine were separately added to the perfusion medium in solid forms because of solubility problems. The relative concentrations of these two amino acids were always 1.9 and 2.2%, respectively.

In the above experiments, 0.5-ml samples were obtained at suitable intervals from the cannula in the portal vein, briefly cooled on ice, and frozen for storage at −20°C until assayed. 25 samples were taken in each experiment; of these 3 were during the preperfusion period of 15 min (at t_{-5}, t_{-2}, and t_0) to obtain the basal insulin and glucagon release levels; after the switch to the medium containing the stimulant under study, samples were collected 0.5 min after the change, then every minute for 5 min, and finally every 5 min for the remaining 25 min of stimulation. The same schedule of sampling was applied when the circulation was switched back to the control solution to study the poststimulatory activity. The rates of insulin and glucagon release were calculated by multiplying the concentration of the respective sample by the flow rate, which was measured at frequent intervals. Total release during 30 min of
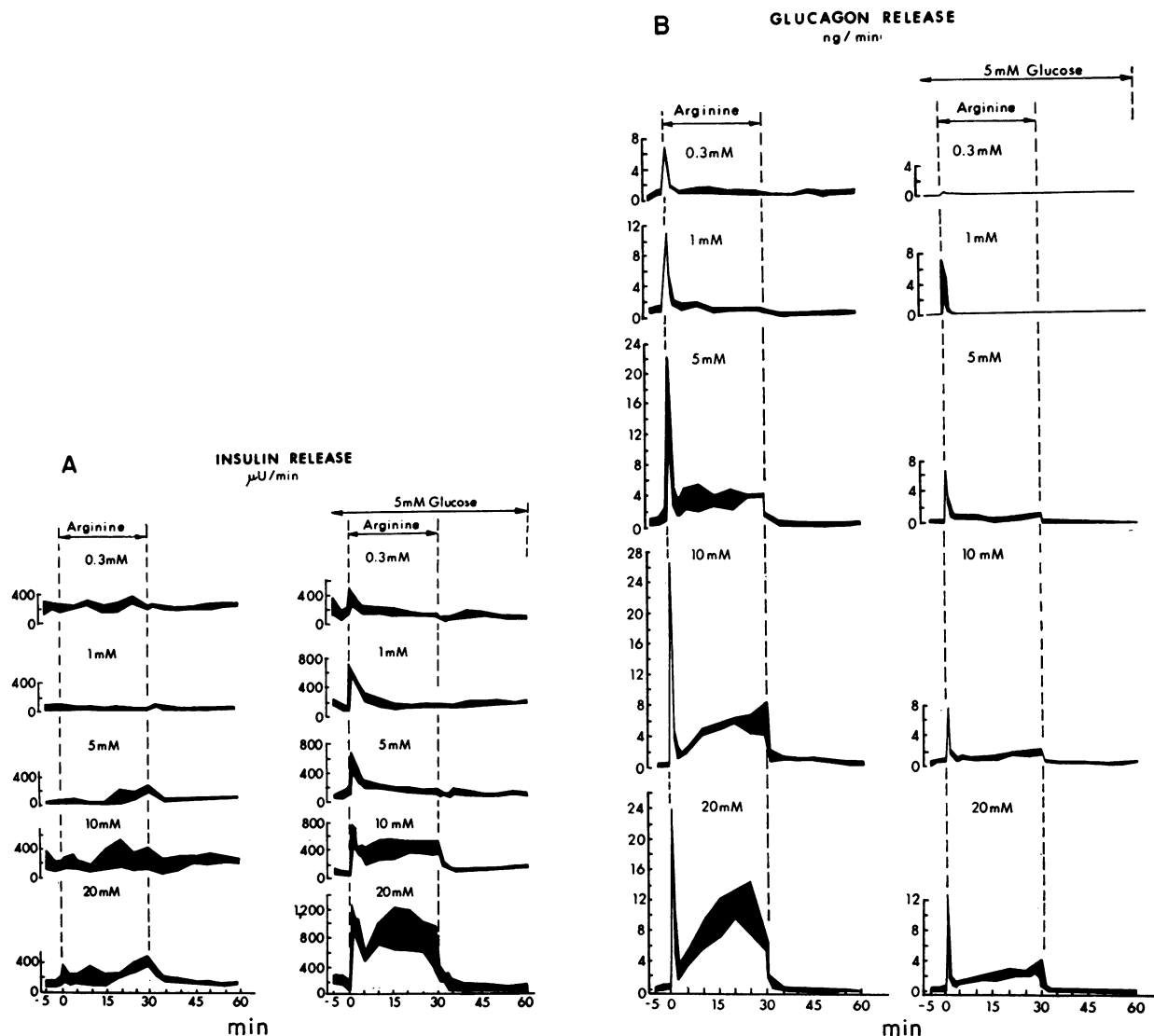
FIGURE 2 Glucose modulation of the α- and β-cell responses due to arginine and an amino acid mixture. The pancreas was perfused for 75 min. After preperfusion for 15 min (t_{-15}-t_0) the circulation was switched to a solution containing the stimulus in concentrations as indicated (t_0-t_{30}). The poststimulatory responsiveness was tested by switching back to the preperfusion fluid (t_{30}-t_{60}). In each panel are shown the data obtained with and without basal

exposure to a stimulus was obtained by planimetry of the means of the profiles of usually three perfusions. The net release during the period of stimulation was calculated by deducting the average secretion observed during the preperfusion period. This seemed justified since the fluctuations of the very low basal secretion rate for both hormones were small relative to the magnitudes of responses seen in the presence of effective stimuli.

Immunoassay procedures. Insulin was measured in duplicate by the double-antibody procedure of Hales and Randle (14), with porcine insulin as standard. To the buffer in the standard curves, Krebs-Henseleit-dextran was added in the same concentration as in the sample tubes.

Glucagon was measured also in duplicate by a double-antibody procedure with a guinea pig antiglucagon antibody developed in our laboratory. This antibody has slight cross-reactivity with gut glucagon, but it gives comparable values for serum to those obtained with Unger's antibody 30 K.[1] Porcine glucagon was used as a standard. [^{125}I]porcine glucagon (500–600 μCi/μg) was prepared by a slight modification of the method of Hunter and Greenwood (15). In the assay, 100 μl of sample or standard was preincubated with 400 μl of buffer containing 0.2 M glycine, pH 8.8, 1% bovine serum albumin (Sigma Chemical Co., St. Louis,

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[1] Manuscript submitted for publication.

C INSULIN RELEASE
μU/min

D GLUCAGON RELEASE
ng/min
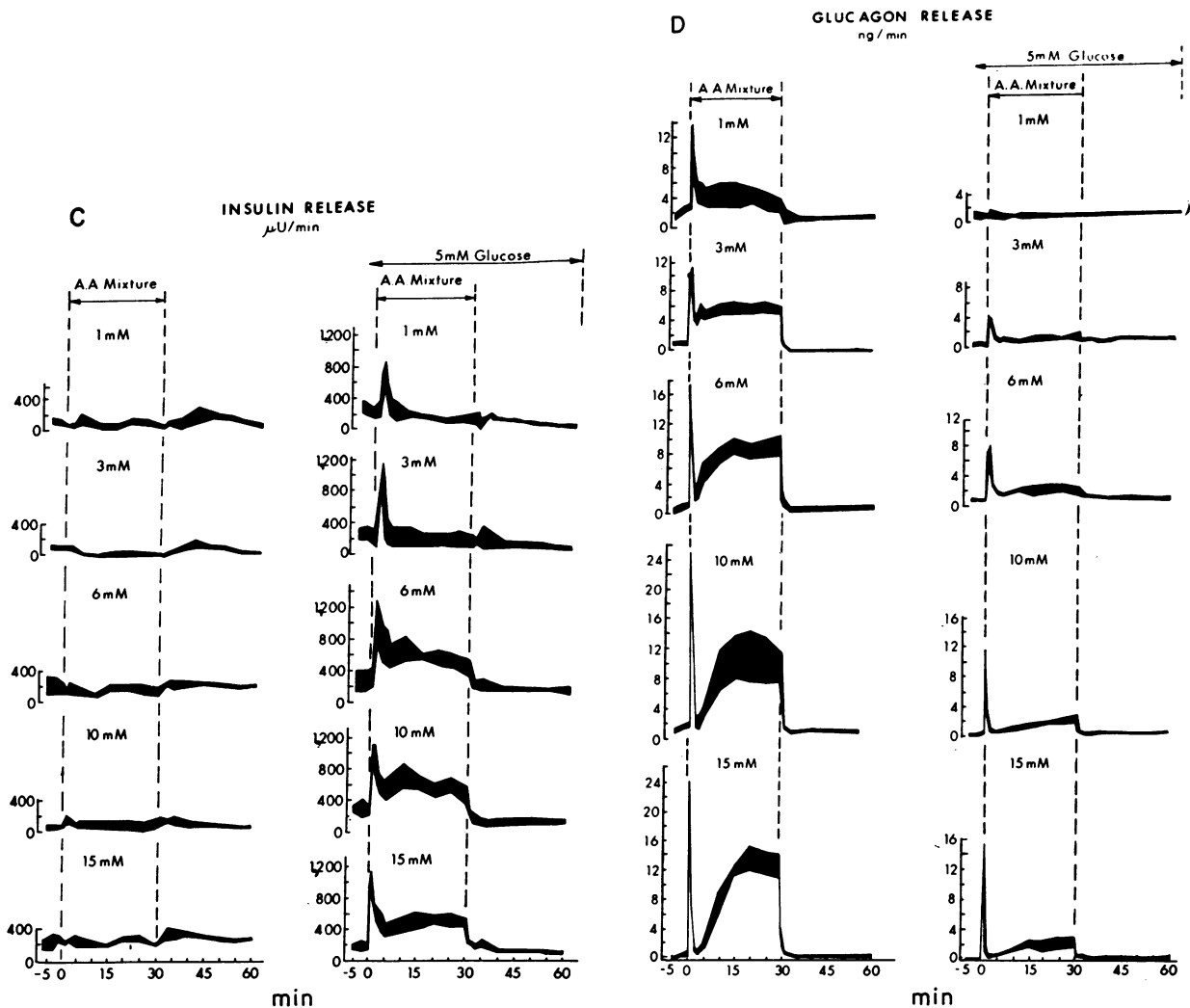
glucose, which was present throughout the entire 75 min. A and B: insulin and glucagon release profiles due to arginine; C and D: insulin and glucagon release profiles due to the amino acid mixture (see Method section concerning the composition of the mixture). For each condition, three experiments are recorded. The widths of the bands represent two SEM's.

Mo., fraction V) and 1,000 kallikrein inactivator units Trasylol/ml (FBA Pharmaceuticals, New York). The final antibody dilution during this step was 1:32,000. In the standard curves Krebs-Henseleit-Dextran was added in the same concentrations as in the sample tube. The tubes were incubated for 4 days at 4°C; the preincubation extended over 24 h and the incubation with ^{125}I lasted for 72 h. Separation of bound from free glucagon was accomplished by precipitation of the bound with goat anti-guinea pig gamma globulin and normal guinea pig serum. The lower limit of detection of the assay system is 5.0 pg. The standard deviation of the intrassay variability is 8% and that of the interassay variability 12%.

All substances added to the perfusion medium were tested at the highest concentration used for possible interference in the immunoassays.

RESULTS

Basal glucagon and insulin release. Secretion of both pancreatic hormones was low during the steady state, whether basal glucose (5 mM) was included in the perfusion medium or not (Fig. 1, Table I). This basal release varied substantially both among individual experiments and during the course of a single perfusion. However, the fluctuations of the base line would appear to be a minor problem, considering the high rates of release seen during amino acid stimulation.

Perfusion with 5 mM glucose doubled insulin release during each period corresponding to the prestimulatory $(t_{-5}-t_0)$, stimulatory $(t_{0.5}-t_{30})$, and poststimulatory

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phases ($t_{30.5}$–t_{60}) of perfusion when amino acids were included as stimulants (Fig. 1, Table I). The average rates of insulin release (in microunits per minute, mean ±SEM) when compared to perfusion with 5 mM glucose were: t_{-5} to t_0, 81±3 ($n = 252$) vs. 179±11 ($n = 228$); $t_{0.5}$ to t_{30}, 74±10 ($n = 88$) vs. 193±9 ($n = 66$); and $t_{30.5}$ to t_{60}, 42±5 ($n = 88$) vs. 110±15 ($n = 66$); for all values, $P < 0.001$.

Within both types of perfusion (with or without glucose), the average insulin release rates corresponding to the prestimulatory and stimulatory phases were virtually identical. However, in the poststimulatory phase, the average insulin release rate for both conditions was significantly lower than the corresponding prestimulatory and stimulatory periods (Fig. 1, Table I).

Perfusion with 5 mM glucose inhibited basal glucagon secretion. The average rates of glucagon release (in nanograms per minute, mean ±SEM) when compared to perfusions with 5 mM glucose were: t_{-5} to t_0, 1.42±0.10 ($n = 285$); vs. 0.37±0.01 ($n = 228$); $t_{0.5}$ to t_{30}, 1.5±0.07 ($n = 33$) vs. 0.31±0.03 ($n = 33$) and $t_{30.5}$ to t_{60}, 0.84±0.04 ($n = 33$) vs. 0.29±0.02 ($n = 33$), for all values $P < 0.001$. As with insulin, within both types of perfusion (with or without glucose), the average glucagon release rates corresponding to the prestimulatory and stimulatory phases did not differ. However, there was a significant decrease in the average glucagon release during the poststimulatory phase in the perfusions without glucose ($P < 0.001$, Table I). Low rates of basal insulin and glucagon release were also observed in an independent study with the isolated perfused canine pancreas (6).

We felt that this data justified the subtraction of the prestimulatory (t_{-5} to t_0) insulin and glucagon release rates, obtained from each individual perfusion when calculating rates of release above basal levels in the presence of an amino acid stimulus.

The effect of arginine and other individual amino acids on α- and β-cells. Arginine, in concentrations from 0.3 to 20 mM, had little to no effect by itself in increasing insulin secretion. It is particularly striking that in the absence of glucose the initial hormone peak is clearly absent (Fig. 2A). However, when 5 mM glucose was included in the perfusate, graded stimulation of insulin release occurred readily with increasing concentrations of the amino acid and the biphasic pattern previously described for glucose and many other stimulants was present (6, 11, 12). The peak of the first phase was obtained between 30 and 60 s after the switch with all concentrations and the nadir occurred between 3 and 5 min. A sustained second phase was observed with 10 and 20 mM arginine. After 30 min of continued perfusion with both arginine and 5 mM glucose, the switch to 5 mM glucose alone resulted in a rapid de-

crease in the secretion rate. The rate decreased with first-order kinetics, showing a half-time of approximately 10 s. Release during the poststimulatory phase was as low as during preperfusion.

The responses of the α-cells obtained with increasing concentrations of arginine differ dramatically from the responses of the β-cells (Fig. 2B). Glucagon release was markedly stimulated by arginine alone and was attenuated by addition of 5 mM glucose. There were two phases, as observed for insulin. The peak response for the first phase occurred within 30 s and returned to almost basal secretory levels within 2–3 min. A sustained second phase of release was seen with 10 and 20 mM arginine. Although 5 mM glucose inhibited glucagon release over the entire concentration range, biphasic responses were still maintained with 5–20 mM arginine. The decay of release after removal of the stimulus both with and without glucose was as rapid as seen for the insulin response, and reached basal levels in approximately 1 min.

In selected studies with 3 mM asparagine or alanine, both in the presence and absence of 5 mM glucose the α- and β-cell responses were quantitatively and qualitatively comparable to the results seen with corresponding concentrations of arginine (data not shown).

The present results with arginine agree generally with data from other in vitro studies and particularly with the findings of Iversen, who used the isolated perfused dog pancreas (6), and with the findings of Levin, Grodsky, Hagura, Smith, and Forsham (16), who also employed the isolated perfused rat pancreas. However, in the latter study, arginine had a more pronounced effect on insulin release even in the absence of glucose, particularly at the very high levels used (16).

Studies with a mixture of 20 amino acids. Since unphysiologically high concentrations of arginine and of other individual amino acids were used in the experiments just described (10–200 times the physiological levels) the studies were repeated with a mixture of 20 natural amino acids in concentrations and proportions found in normal rat serum (13). Serum concentrations of total amino acids after an overnight fast are approximately 3 mM (13) and are likely to increase to 4.5–6 mM postprandially.

The insulin release profiles with the amino acid mixture were qualitatively similar to those observed with arginine (Fig. 2C). No stimulation of release occurred in the absence of glucose, even at the highest amino acid concentrations (15 mM). With 5 mM glucose, the amino acid mixture stimulated insulin release with a single initial peak at 1 and 3 mM and a biphasic response at 6, 10, and 15 mM.

Secretion stopped rapidly on removal of the stimulus, as noted with arginine. Again, no poststimulatory hy-
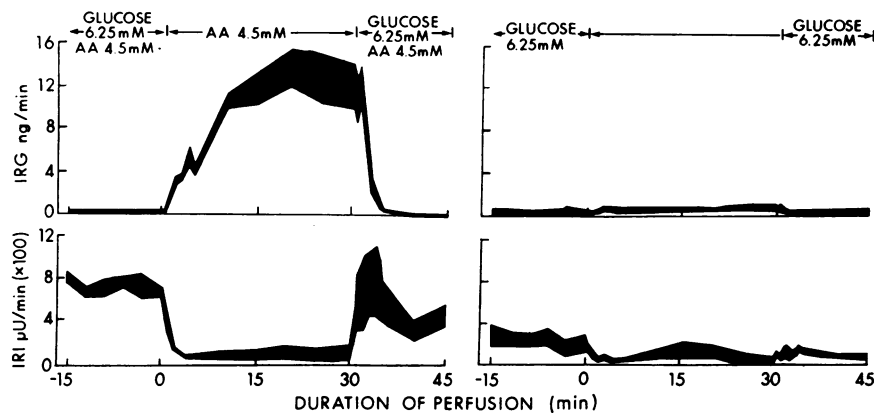
FIGURE 3 Effect of acute hypoglycemia on insulin and glucagon release with and without amino acid stimulation. The pancreas was perfused for 75 min. The panel on the left illustrates insulin and glucagon release when the amino acid mixture is maintained throughout the entire perfusion period and glucose is added and removed as indicated. The width of the bands represent two SE ($n = 4$). The panel on the right illustrates insulin and glucagon release in the absence of an amino acid stimulus when glucose is added and removed as indicated ($n = 3$). The first point (t_{-15}) is obtained 15 min after starting the perfusion.

perresponsiveness to glucose became apparent. In this regard, arginine and the amino acid mixture differ markedly from high glucose, leucine, isoleucine, ketone bodies, cetrain fatty acids, and α-keto-monocarboxylic acids, all of which lead to poststimulatory hyperresponsiveness to basal glucose (17). The data indicate that the sensitization of the β-cells to basal glucose is not simply a consequence of sustained excitation and secretion. It is conceivable that hyperresponsiveness to glucose is induced only by those substances that enhance glucose metabolism concomitant with triggering release. Consistent with this explanation are recent findings in our laboratory that show that in isolated islets, 10 mM L-isoleucine markedly stimulates lactate production from basal glucose, whereas the same level of arginine and of the mixture of 20 amino acids has little effect on lactate formation.[2]

As with arginine alone, glucagon release was markedly and progressively enhanced by increasing concentrations of the amino acid mixture; the biphasic pattern occurred with concentrations as low as 1 mM (Fig. 2D). All secretion profiles were drastically suppressed by the presence of 5 mM glucose.

The effect of acute hypoglycemia on α- and β-cells previously exposed to physiological levels of amino acids (4.5 mM) and glucose (6.25 mM) was tested (Fig. 3). There was a definite delay after the switch to the glucose-free solution before the glucagon response became apparent. The release profile was monophasic, lacking the pronounced initial spike seen on exposure to amino acids. In contrast to the glucagon response, the first

[2] Unpublished data.

peak was present in the insulin profile when basal glucose was reintroduced.

In the control experiments (amino acids omitted) abrupt aglycemia produced a comparatively minor glucagon response (Fig. 3). This experiment suggests that the tonic influence of plasma amino acids and the acute fall in blood glucose may be the primary factors responsible for the release of glucagon during hypoglycemia in vitro. The above factors together with inputs from the autonomic nervous system may in combination be responsible for glucagon release during hypoglycemia in vivo (18).

Characteristics of dose-response curves due to arginine and the amino acid mixture. The concentration dependency of glucagon and insulin release provoked by arginine and amino acids, both in the absence and presence of basal glucose, exhibits features of probable physiological significance, as illustrated in Fig. 4. The total hormone release during exposure to the stimulant is recorded, neglecting the multiphasic kinetics of release. The system, which responds to arginine plus glucose by releasing insulin, was not saturated by arginine, even at 20 mM. Unlike arginine, the amino acid mixture together with 5 mM glucose resulted in half-maximal secretion at 4.5–5 mM and maximal secretion with 6–8 mM levels of amino acid, concentrations within the physiologic range. The steepness of the curve obtained with the mixture suggests the possibility of cooperative effects among the different amino acids. That this might be the case is also suggested by the results of corresponding in vivo studies (7, 19). The relative contribution of the individual amino acids to the overall response remains to be unraveled.

IRI (mU/30 m⁻¹)

IRG (ng/30 min)

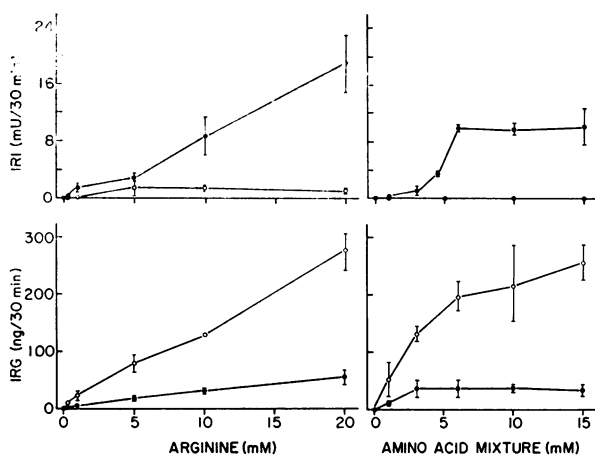ARGININE (mM)   AMINO ACID MIXTURE (mM)

Figure 4 Dose and glucose dependency of insulin and glucagon release by arginine and an amino acid mixture. This is a synopsis of the data presented in Fig. 2. The mean ±SEM of total hormone release during 30 min of exposure to the stimulus is recorded. It was obtained by planimetry of the profiles presented in Fig. 2. Correction was made for basal release in the absence of the stimulus by extrapolating from the average secretion observed in the preperfusion period. For more details see the Methods section.

Basal glucose inhibited glucagon release due to arginine and to the amino acid mixture by approximately 80% at all levels of the stimulants. As with insulin, the dose-response curves demonstrate the failure of arginine

to saturate the system involved in glucagon secretion, whereas the amino acid mixture with or without glucose resulted in near-maximal stimulation within the physiologic range of amino acid concentration.

Concentration dependency of glucose actions allowing α-cell stimulation and leading to β-cell inhibition. In all studies described above, a constant level of 5 mM glucose was employed. The permissive and inhibitory action[3] of glucose on β-cells and α-cells, respectively, was further investigated by maintaining the amino acid mixture at a level of 15 mM and perfusing with increasing concentrations of glucose (0–6.25 mM) (Fig. 5, Tables II and III).

The permissive action of glucose in allowing insulin release by amino acids revealed a clear threshold of the effect at 2.5 mM glucose; no insulin release occurred below this concentration (Fig. 5, Table II). In contrast, the inhibition of α-cell activity became manifest at the lowest glucose levels used (1.25 mM) and further increased up to 6.25 mM, at which point glucagon secretion was 6–7% of the secretion rate of the perfusions without glucose (Fig. 5, Tables II and III).

Both the permissive and the inhibitory actions of glucose on β- and α-cells, respectively, manifest themselves

[3] The terms "permissive and inhibitory actions of glucose" seem reasonable in a physiologic sense but are not meant to imply any knowledge about the molecular mechanisms underlying the phenomena described by them.

Table II

Dose-Dependency of Glucose Action on the Two Phases of Insulin and Glucagon Release Caused by 15 mM Amino Acid Mixture

Glucose	Insulin*			Glucagon*		
	1st phase 0–3 min	2nd phase 3–30 min	Total 0–30 min	1st phase 0–3 min	2nd phase 3–30 min	Total 0–30 min
mM	*mU/3 min*	*mU/27 min*	*mU/30 min*	*ng/3 min*	*ng/27 min*	*ng/30 min*
0	0	0	0	17.20 ±1.40	247.00 ±31.00	264.20 ±31.00
1.25	0	0.06 ±0.06	0.06 ±0.06	6.90‡ ±0.88	203.00 ±53.00	209.90 ±53.00
2.50	0.72‡ ±0.14	0.34 ±0.31	1.06‡ ±0.45	4.23‡ ±0.38	150.00‡ ±42.00	154.23‡ ±42.00
3.75	2.20‡ ±0.37	6.52‡ ±0.95	8.75‡ ±1.21	4.60‡ ±0.34	35.60‡ ±17.30	40.20‡ ±17.60
5.00	1.83‡ ±0.36	8.20‡ ±1.22	10.03‡ ±1.55	5.40 ±0.60	29.60‡ ±10.00	35.00‡ ±10.50
6.25	2.69‡ ±0.53	20.22‡ ±3.48	22.91‡ ±4.00	4.54‡ ±0.77	13.00‡ ±4.35	17.54‡ ±4.10

* The values represent the mean ±SEM of the integrated secretion rates of insulin and glucagon above base line as obtained by planimetry of the areas under the curves extending over the indicated time periods ($n = 3$ for each condition).

‡ Significantly different from control rates obtained in the absence of glucose ($P \leq 0.02$).

more readily in the first peaks than in the sustained phases of the respective hormone-releasing profiles (Table II). The first phase of insulin release due to 15 mM amino acids is maximal at 3.75 mM glucose, while the second phase continues to increase up to the highest glucose level used (6.25 mM) (Fig. 5, Table II). The α-cells are even more sensitive to the action of glucose; maximal inhibition of the first phase of the glucagon response is already reached at the lowest glucose levels tested (1.25 mM). Glucose suppression of the second phase of glucagon secretion was, however, less effective, and inhibition was incremental over the entire range of glucose concentrations (Fig. 5, Tables II and III). The most dramatic change occurred when the glucose level was increased from 2.5 to 3.75 mM.

These data might indicate that in the absence of stimulated insulin release, glucagon and insulin release during the first phase are unrelated, but do not preclude an interdependence in the secretion of the two hormones during the second phase, when the rates of insulin release are high (Fig. 6). These results imply that the glucose effect on α-cells may at least in part be independent of the insulin-releasing capacity. It is, however, not possible with the present information to determine whether the low base-line levels of insulin might not suffice to facilitate glucose entry into the α-cell, leading to inhibition.

It is noteworthy that in the prestimulatory phase, without amino acids in the perfusate, significant suppression of glucagon secretion occurs only at 5–6.25 mM concentrations ($P < 0.01$ for both) (Table III). In the poststimulatory phase, except for the first minute after removal of the amino acid stimulus, secretion rates were lower than in the prestimulatory phase and no further inhibition of glucagon release was apparent with increasing glucose concentrations (Table III).

DISCUSSION

Two aspects of the present results deserve detailed comment; (a) the physiological role of glucose as primary modulator of α- and β-cell function, and (b) the molecular mechanisms possibly underlying the multiple actions of glucose on islet cells.

Physiologic modulation of α- and β-cell function by glucose. The picture of physiological α- and β-cell function evolving from the present study and from the data in the literature may be sketched as follows: in the absence of external stimuli, which would include circulating substrates and possibly stimulatory inputs from the autonomic nervous system (18) and other unknown humoral factors, both α-cells as well as β-cells are in a state of rest, exhibiting low basal rates of hormone release only a few percent of their secretion potential. The data are incompatible with the view that
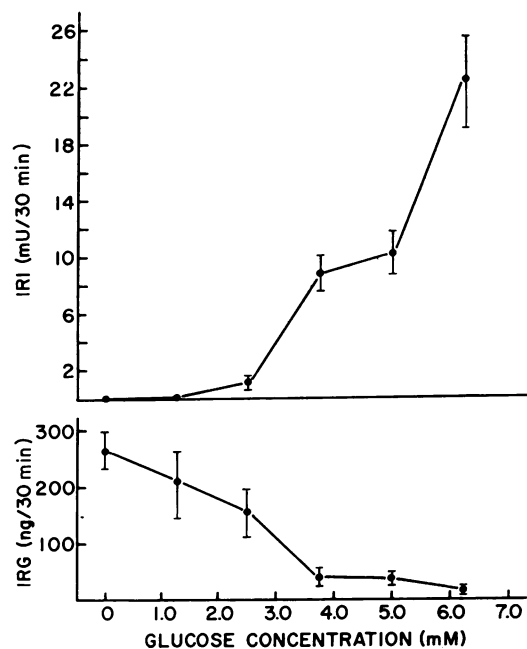

FIGURE 5 Dose-dependency of the modulator action of glucose on insulin and glucagon release due to 15 mM amino acid mixture. Total hormone release during 30 min of exposure to the amino acid mixture is recorded as a function of the glucose concentration in the perfusion medium. Each value represents the mean ±SEM of three perfusion experiments and was obtained by planimetry of the area under the releasing profiles at each glucose concentration. Correction for basal release was made as described in the legend to Fig. 4, except in the case of insulin with the 6.25 mM glucose level, in which condition the base line was obtained experimentally by perfusing the pancreas for 75 min with 6.25 mM glucose in the absence of the amino acid mixture (see Table II).

α-cells have substantial spontaneous activity when deprived of glucose (1). If the latter hypothesis were correct, one could expect aglycemic perfusion of the pancreas to result in a marked elevation of glucagon release. The consistent decrease in basal glucagon release with 5 and 6.25 mM glucose (Fig. 1, Table III), comparable to that observed in the perfused canine pancreas (6), would appear to be relatively unimportant by itself and may merely be indicative of the operative principle of glucose suppression of α-cell function. It would appear that α-cells, like the β-cells, are equally maintained in a state of rest provided external stimuli are absent.

The slow resting rate of hormone release, which amounts to only a few percent of the rate observed during physiological stimulation, is not necessarily artifactual, i.e. an indication of cell damage as a result of unfavorable extracorporeal conditions. Slow basal hormone release might well be analogous to the spontaneous physiological release of acetylcholine packets from

TABLE III

Dose-Dependency of Glucose Inhibition of

Sampling time, min glucose in perfusate	Prestimulatory phase				1st phase					2nd phase (Stimulatory phase, amino acid mixture (15 mM))				
	−5	−2	0	Mean ±SEM	0.5	1	2	3	Mean ±SEM	4	5	10	15	20
mM														
0	0.73 ±0.17	0.72 ±0.19	0.85 ±0.17	0.77 ±0.04	21.73 ±2.20	8.30 ±0.56	1.34 ±0.19	0.95 ±0.16	8.11 ±2.60	1.26 ±0.42	1.94 ±0.55	7.48 ±1.56	11.87 ±0.64	13.53 ±1.63
1.25	0.80 ±0.07	0.76 +0.09	0.81 ±0.06	0.79 ±0.02	12.75 ±0.56	2.39 ±0.38	0.58 ±0.16	0.72 ±0.25	4.11‡ ±1.31	0.66 ±0.32	1.58 ±0.74	7.11 ±3.16	9.74 ±2.56	10.60 ±2.00
2.50	0.66 ±0.04	0.68 ±0.06	0.75 ±0.14	0.70 ±0.03	8.95 ±0.42	1.56 ±0.20	0.45 ±0.04	0.55 ±0.06	2.88‡ ±1.07	0.50 ±0.78	0.86 ±0.32	3.21 ±1.42	7.60 ±2.48	8.65 ±2.18
3.75	0.72 ±0.07	0.76 ±0.08	0.81 ±0.02	0.76 ±0.03	8.88 ±0.51	2.24 ±0.16	0.63 ±0.01	0.42 ±0.04	3.04‡ ±1.04	0.48 ±0.01	0.56 ±0.05	1.35 ±0.31	2.11 ±0.78	2.61 ±1.04
5.00	0.55 ±0.04	0.44 ±0.11	0.39 ±0.07	0.46‡ ±0.05	11.27 ±1.85	1.38 ±0.42	0.61 ±0.53	0.41 ±0.17	3.67‡ ±1.50	0.45 ±0.02	0.57 ±0.11	1.04 ±0.23	2.10 ±0.46	1.64 ±0.82
6.25	0.35 ±0.06	0.25 ±0.07	0.30 ±0.06	0.30‡ ±0.03	6.67 ±1.40	0.96 ±0.08	0.48 ±0.05	0.42 ±0.05	2.13‡ ±0.66	0.42 ±0.03	0.50 ±0.08	0.64 ±0.11	0.80 ±0.12	0.90 ±0.18

* The results presented in this table are from the experiments described in Fig. 5. Glucagon values are recorded in nanograms per minute.
‡ $P \leq 0.01$ by Student t test when compared to aglycemic perfusion.
§ Mean ±SEM of poststimulatory phase was calculated by omitting the transitional 30.5 and 31-min values.

the nerve terminal in the absence of a nerve impulse, as manifested by miniature end plate potentials (20).

In the absence of glucose, α-cells are sensitized, able to respond readily to physiological levels of amino acids, whereas the β-cells are unresponsive to this major group of fuel molecules. This nonresponsiveness of the β-cells devoid of glucose extends to almost all physiologic stimuli, studied so far, including fatty acids, ketone bodies, secretin, pancreozymin, glucagon, and certain metabolites of amino acids. Glucose profoundly alters the chemosensitivity of both kinds of cell, but oppositely. Secretion of glucagon, induced by amino acids or by enteric hormones (6), is partially inhibited by physiologically low glucose levels (1–2 mM), and is almost entirely blocked by normal sugar levels (5–6 mM).

Glucose sensitizes the β-cells to secretory stimuli, in contrast to its action on α-cells. It was shown here with amino acids and in a parallel study with DL-B-hydroxybutyrate[4] that there is a threshold close to 2.5 mM for the permissive action of glucose that allows other molecules to release insulin, and that the glucose effect increases dramatically at levels exceeding this threshold. This should be physiologically advantageous. It would make little sense if the β-cells responded with insulin output to other molecules if the blood sugar were dangerously low, i.e., 2.5 mM or less. Glucose can therefore be considered as a safety switch, turning off the β-cells during hypoglycemia and sensitizing them when the blood sugar is normal or higher. As the blood sugar

falls, α-cells become deinhibited and the stimulatory factors gain increasing influence, thus curbing hypoglycemia. Similar ideas concerning the modulating role of glucose in β-cell responsiveness were derived from in vivo studies in man, using arginine, tolbutamide, and glucagon as secretagogues (21, 22).

What is the physiological significance of the biphasic glucagon response to amino acid stimulation? Since the temporal profile of amino acid-induced glucagon release resembles that of insulin release due to high glucose or amino acids plus basal glucose, one cannot help asking whether the biphasic glucagon release pattern might have similar significance as attributed to the biphasic insulin secretion pattern, and whether the widely accepted two-compartment model of insulin release applies to glucagon secretion from the α-cells as well. It is difficult to describe physiologic conditions that might cause substrate-induced biphasic glucagon release due to rapid rises in amino acids in blood, in contrast to the frequently encountered dramatic changes of the blood sugar levels. The vampire bat is probably one of the few mammals exhibiting large and rapid fluctuations in serum amino acids, which occur after a blood meal.[5] However, the possibility that neuronal and enterohormonal factors might modulate α-cell function in ways that demand the capacity for an abrupt, biphasic response must be considered in most other species.

With the information currently available, it is impossible to explain the mechanism of the biphasic response

[4] Manuscript in preparation.

[5] George Cahill, Jr. Personal communication.

			Post-stimulatory phase											
25	30	Mean ±SEM	30.5	31	32	33	34	35	40	45	50	55	60	Mean§ ±SEM
13.00	12.33	8.77	3.54	1.83	0.70	0.48	0.29	0.16	0.32	0.20	0.13	0.15	0.20	0.29
±1.51	±0.78	±1.17	±0.33	±0.24	±0.10	±0.11	±0.15	±0.16	±0.17	±0.20	±0.13	±0.15	±0.12	±0.06
10.43	11.55	7.38	1.73	0.75	0.23	0.18	0.14	0.12	0.12	0.18	0.20	0.15	0.21	0.17
±1.86	±1.60	±1.04	±0.53	±0.11	±0.12	±0.07	±0.05	±0.05	±0.04	±0.03	±0.08	±0.02	±0.01	±0.01
9.10	9.30	5.60‡	1.55	0.50	0.34	0.29	0.25	0.20	0.27	0.24	0.26	0.25	0.26	0.26
±1.54	±1.40	±0.96	±0.42	±0.06	±0.08	±0.05	±0.04	±0.04	±0.04	0.26	±0.07	±0.06	±0.06	±0.01
3.18	2.98	1.89‡	0.82	0.49	0.32	0.27	0.32	0.27	0.26	0.25	0.10	0.12	0.15	0.23
±0.99	±0.83	±0.32	±0.18	±0.12	±0.02	±0.01	±0.05	±0.03	±0.04	±0.02	±0.04	±0.03	±0.03	±C.06
2.21	1.97	1.42‡	0.67	0.58	0.22	0.26	0.27	0.27	0.37	0.20	0.23	0.19	0.21	0.25
±0.79	±0.93	±0.24	±0.04	±0.04	±0.02	±0.02	±0.03	±0.04	±0.13	±0.06	±0.07	±0.03	±0.04	±0.05
0.93	0.98	0.74‡	0.39	0.41	0.33	0.26	0.26	0.23	0.25	0.28	0.22	0.25	0.24	0.26
±0.17	±0.15	±0.06	±0.05	±0.06	±0.06	±0.05	±0.05	±0.07	±0.06	±0.06	±0.08	±0.07	±0.04	±0.01

of the α-cells. Like biphasic insulin release by the β-cells, an equally unsettled issue, multiphasic glucagon release might be due to multiple pools of the hormone, feedback control by the released glucagon itself or by unknown intracellular regulators (i.e. cyclic nucleotides, ions, or metabolites), or possibly even various combinations of the above mechanisms.

It remains also to be seen whether there are pathologic states, analogous to the adult-onset form of diabetes where the first phase of insulin release is blunted, in which the first phase of glucagon release is similarly affected.

The molecular mechanisms of the multiple actions of glucose in islet cells. How does the glucose molecule accomplish these multiple effects on α- and β-cells? First, on must answer the question of whether the effects of glucose on α- and β-cells are direct or indirect. Samols, Tyler, and Marks (23) propose that glucose suppression of glucagon secretion is a consequence of elevated insulin levels at the α-cells, and they also imply that amino acid-induced insulin release depends conversely upon elevated glucagon concentrations at the β-cells. At first glance, the data in this paper and in the literature (1, 2, 5–7, 23) seem to be compatible with this view. However, a closer look at the results of the present study reveals that the rates of glucagon and insulin release are not always inversely related, as one would expect if this concept were correct. In particular, low glucose levels inhibit the first phase of amino acid-induced glucagon release without augmenting basal in-

sulin release. More convincingly, it was found in a parallel study with pancreases of both alloxan- and streptozotocin-diabetic rats that glucose suppression of α-cell function is independent of insulin (24).

From the present data, it seems unlikely that amino acid-induced insulin release is in any way dependent upon concomitant glucagon secretion, since insulin secretion is maximal when glucagon release is low or almost ceases. Furthermore, there are physiological stimulants with no effect on glucagon release but with powerful insulin-releasing action (e.g. β-hydroxybutyrate[4]).

Assuming then, in contrast to most current beliefs, that glucose acts directly on α- as well as on β-cells, one has to consider a priori the following possible mechanisms for its action. (The mechanism could be the same in each type of cell except for an opposite sign.) They are: (a) transport of glucose into the cell; (b) phosphorylation of glucose; (c) further metabolism of glucose by the cell; (d) interaction of glucose with a receptor on the external surface of the cell; and (e) various combinations of the above.

Extensive studies have been performed to differentiate which of the above molecular mechanisms might be the basis of the permissive action of glucose on the β-cells (11, 17, 25–29), whereas analogous studies of the glucose modulation of α-cell function are scanty (2, 30). This disproportionate availability of knowledge on the two types of cells is because in rat and mouse, the two species preferentially studied, the contribution of α-cells to the islet tissue mass is only 15–30%, compared to
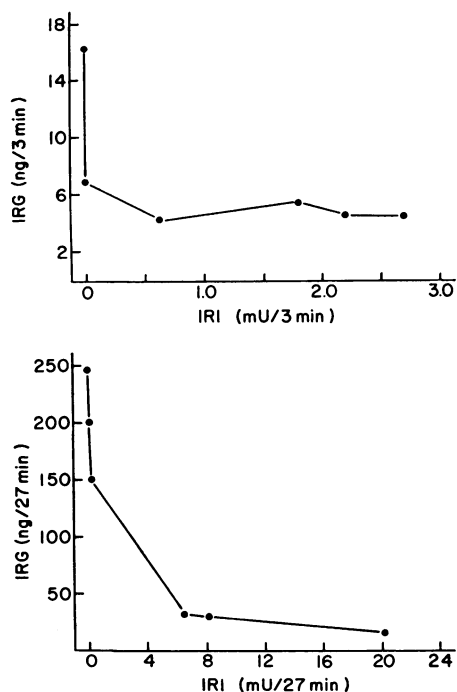
FIGURE 6 Relationship between insulin and glucagon in the first phase and the second phase of release above basal secretion rates caused by 15 mM amino acid mixture and increasing concentrations of glucose (see Table II).

is pertinent in this context that neither 5 mM mannose nor 20 mM fructose, which are suitable fuels for the islets, can substitute for glucose in its capacity to sensitize the β-cells to stimulation by isoleucine (17). Other evidence that the permissive action of glucose is almost certainly not due to provision of energy is derived from studies with the isolated perfused pancreas, in which it was found that ATP and P-creatine levels of islet tissue were the same in the absence and presence of glucose (11). The observations that metabolite profiles within the islets change only very sluggishly or not at all with glucose and are not correlated with insulin release give further indirect support for an alternate hypothesis explaining glucose-dependent insulin secretion (11).

Despite the lack of corresponding knowledge concerning the biochemistry of α-cell metabolism, we feel it is fruitful to outline a general working hypothesis for further studies. This hypothesis invokes glucoreceptors and amino acid receptors common to both the α- and β-cell (Fig. 7). In the case of the β-cell, occupation of amino acid receptor sites does not result in insulin release unless at least some of the glucoreceptors are also occupied. Occupation of a high proportion of the glucoreceptors can cause release in the absence of amino acids, but not vice versa. In contrast, in the case of the α-cell, occupation of amino acid receptors alone can cause glucagon release, whereas occupancy of glucoreceptors results in a conformational change in the amino acid receptors that blocks glucagon release. In this
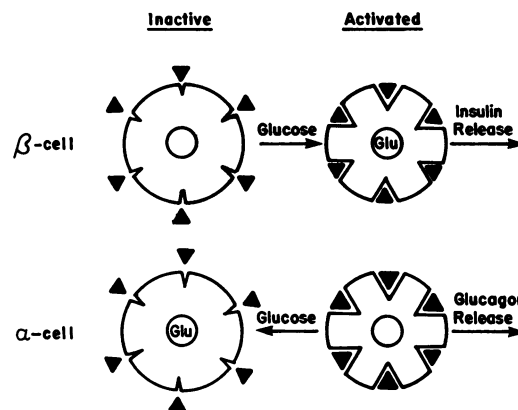
75–60% in the case of the β-cells, (the remaining 10% is contributed by stroma [31, 32]). Therefore, the available data have usually been interpreted as being representative for the β-cells. Although this is debatable, we will follow this approach in the following discussion.

Carrier-mediated transport across the cell membrane, as shown to exist in islets, is probably not the critical glucose-sensitive step of β-cells, because fructose, which is unable to stimulate insulin release in the rat, penetrates as rapidly as glucose, presumably via the same carrier system (11). Further, mannoheptulose blocks insulin release by glucose without interfering with glucose penetration (25).

If phosphorylation of glucose were the essential event, one would expect that both fructose and 2-deoxyglucose would function as triggers for insulin release, whereas sorbitol and xylitol, sugar alcohols that are not phosphorylated, should not; however, just the opposite is the case (11).

The argument in favor of the third, more popular alternative, that of a mechanism dependent on further glucose metabolism, is also not convincing. The most compelling evidence against the general metabolism hypothesis is the recent demonstration with isolated perifused islets that releasing and fuel function of glucose can be completely dissociated with iodoacetate (26). It

FIGURE 7 Activation and inhibition of amino acid receptors by glucose in β-cells and α-cells, respectively. Amino acid sites are depicted arbitrarily by a set of notches, amino acids by triagonal shapes, and the glucose site by a central circle. The glucose molecule is represented by the abbreviation GLU. In the β-cells, addition of glucose makes the amino acid sites accessible to the agonists, whereas in the α-cells, addition of glucose drastically decreases affinity of the agonists to their receptor sites. The "activated" receptor leads to hormone release.

model the intensity of release of each hormone is dependent upon the relative concentrations of both glucose and amino acids. This hypothesis is simple, compatible with our data and the data in the literature, and is open for further rigorous testing.

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