Electrical and Secretory Manifestations of Glucose and Amino Acid Interactions in Rat Pancreatic Islets

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SUMMARY

Interactions between glucose and amino acids in rat pancreatic islets were studied by recording the intracellular membrane potential and spike discharges from single islet cells and by measuring insulin release from the isolated perfused pancreas. It was found that L-isoleucine requires the presence of basal glucose (5 mM) in order to increase spike discharge from islet cells and depolarize the cell membrane. Similarly basal glucose is needed for insulin release by L-isoleucine. A physiological mixture of twenty amino acids also required the presence of basal glucose in order to increase spike activity and insulin release. In contrast to L-isoleucine the amino acid mixture did not depolarize the β-cells. Iodoacetate, at concentrations previously shown to block glycolysis completely, did not interfere with any of these permissive actions of glucose, nor did iodoacetate alter the well known electrical manifestations of high levels of glucose itself (i.e. depolarization and increased spike discharge).

These data show that glucose plays a pre-eminent role as regulator of islet cell function, governing the efficacy of amino acids as β-cells stimulants. The results are most easily interpreted if one assumes that glycolysis is not required for glucose to exert its action. DIABETES *24*:489-96, May, 1975.

The interrelationship of biophysical and metabolic events in pancreatic β-cells leading to insulin release is poorly understood.[1,2] Even though the secretory response to glucose is associated with simultaneous increases in electrical activity, [1,3] glucose utilization [4] and O_2 consumption, [5] it has become clear by using iodoacetate to completely block glycolysis that the fuel and releasing functions of glucose can be dissociated.[6,7] To unravel further the relation of electrical events in β-cells to metabolism and hormone secretion it seemed logical to examine the effects of iodoacetate on electrical activity.

Glucose not only directly stimulates insulin release but also is required at a nonstimulatory level to allow release by a wide variety of other agents, including L-isoleucine.[8] Enhancement of glycolysis by L-isoleucine is completely blocked by iodoacetate.[7] In the present study we have investigated whether changes in the electrical and secretory responses of the β-cells to isoleucine and to a mixture of twenty amino acids at blood plasma levels show a parallel dependency on the presence of basal glucose. In addition, we have used iodoacetate to decide whether or not glycolysis is required for glucose to permit amino acids to release insulin.

The results further support the view that bioelectrical and secretory events are physiologically coupled and that both phenomena are independent of the fuel function of glucose.

MATERIALS AND METHODS

Animals and electrical recordings

Isolated pancreatic islets were prepared by the collagenase procedure[9] from male Sprague-Dawley rats (250-350 gm.) fed ad libitum. For the electrical studies, a selected group of large islets (200-400μ in diameter) was placed in a small depression in a black Plexiglas platform in a thermostated chamber and was continuously superfused at a flow rate of 10 to 12 ml. per minute with a phosphate-buffered salt solution as the basic medium[3] supplemented with pyruvate and lactate. These two metabolites were added as possible alternate fuels when glucose metabolism was blocked by iodoacetate. The medium was continuously gassed with a mixture of 95 per cent O_2 and 5 per cent CO_2 and maintained at 37°C. When changing solutions, three minutes were allowed for the solution to pass through the tubing dead space and to reach the de-

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sired concentration in the chamber (volume 5 ml.).

Electrical activity was recorded intracellularly with a glass microelectrode of 30-100 megohms resistance, filled with 3 M potassium acetate. The input was led into a Mentor (N-950) high-input impedance amplifier via a Ag:AgCl wire inserted into the microelectrode. The amplifier provided means for calibrating the system by applying a standard signal through the reference lead. Electrical responses were monitored on a DC-coupled Tektronix oscilloscope (5031), and permanent records were obtained from a Honeywell (906C) oscillograph.

Rat pancreatic islets consist largely of centrally located β-cells which constitute 60 to 80 per cent of the total tissue mass compared to 10 to 30 per cent for the peripherally located α-cells.[10,11] Hence, in these studies the electrode was placed deeply into the islet for penetration of cells and intracellular recording. Within a ten-minute period usually three to ten cells were successfully impaled as seen by a sudden drop in potential from baseline or zero (see figure 1). For cells not showing spike discharges the transmembrane potential remained steady without any fluctuation until withdrawal, whereas in active cells spike discharges were immediately visualized on the oscilloscope or from the oscillograph record (figure 1).

Perfusion of isolated rat pancreas

Studies of insulin release were performed with the isolated perfused rat pancreas as described previously in detail.[12] The perfusate consisted of a bicarbonate buffered salt solution[12] to which were added 7 to 8 per cent dextran, 0.5 mM pyruvate and 2.5 mM lactate. Dextran proved as efficient as albumin in preventing loss of insulin through adsorption. Insulin was measured by the double antibody radioimmunoassay technic as described by Hales and Randle[13] using a porcine insulin standard.

Special reagents and chemicals

Iodoacetate and all amino acids were obtained from Sigma Chemical Company, St. Louis. In the amino acid mixture the individual acids were present in proportions found in normal rat serum.[14]

RESULTS

Electrical responses to glucose, L-isoleucine and an amino acid mixture

The electrical activity of rat islet cells was a function of the glucose level in the bathing fluid (table 1). The values for the incidence of firing are comparable to those reported for microdissected mouse islets, but the membrane potentials for these rat islets

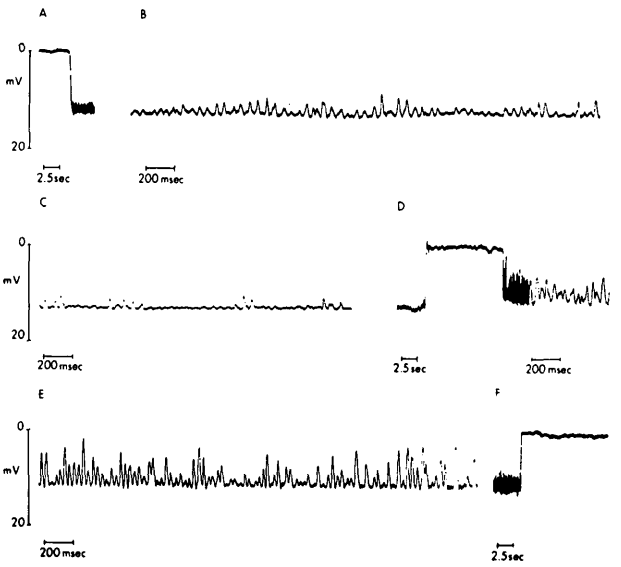

FIG. 1. Patterns of spike activity recorded from two different rat islet cells in the presence of an amino acid mixture (10 mM) plus basal glucose (4.2 mM). The amino acid mixture contained 20 natural amino acids in proportions found in normal rat serum.[14] The records illustrate how spike activity and membrane potential are measured in each individual cell. In A a sudden downward deflection from zero potential or baseline is seen as the electrode enters the first cell. B and C illustrate the spike activity of the same cell obtained at a faster record speed during relatively active and quiet periods of discharge, respectively. D shows the rapid return of the potential to baseline after the electrode is withdrawn from this cell. After several seconds the other cell is impaled. E illustrates the pattern of the spike discharge of this relatively more active cell, and F shows the return to baseline potential after the electrode is withdrawn. Note the changes in record speed.

harvested with the collagenase method are somewhat lower (21 and 17 mV in low and high glucose, respectively, for cells of microdissected mouse islets[1]). This difference may well be due to the aggressive treatment with crude collagenase. Nevertheless, these results show that islets isolated by the widely used collagenase method closely resemble the microdissected islets in their electrical responses to low and high glucose.

TABLE 1

The effect of glucose on the electrical response of islet cells

Glucose (mM)	Membrane Potential (—mV)	% of Cells Exhibiting Spike Activity (%)	Number of Experiments
5.5	15.1 ± 1.2	18 ± 3	4
16.6	12.0 ± 0.5*	69 ± 3†	11

The islets were superfused for a period of thirty to sixty minutes during which the electrical responses of a sufficiently large number of cells (fifty or more per hour) to low or high glucose were recorded. The means and S.E.M. of the electrical responses are shown.
*$p \leq 0.05$, †$p \leq 0.01$.

It was found that the permissive role of glucose which allows isoleucine to stimulate insulin release and glycolysis is also reflected by the electrical activity. Three examples of the spike activity recorded intracellularly from rat islet cells in the presence of isoleucine (8 mM) and glucose (4.2 mM) are given to illustrate how variable the responses are from cell to cell (figure 2). This variability seems to be an important characteristic of the electrical activity of the β-cells, which must now be categorized as excitable tissue though less specialized than nerve and skeletal muscle. Indeed, the variability of the patterns of electrical activity seen in rat and also mouse[3] islet cells is a phenomenon also routinely observed in smooth muscle.[15] The spike discharges ranged from 1-8 mV in amplitude and from 25-75 msec in duration. In addition, the pattern of firing varied from irregular (figure 2B), to nearly regular (figure 2C). Similar patterns of activity were observed when islets were exposed to high glucose without the amino acid (not shown) or to a mixture of amino acids with low glucose (see below and figure 1).

The permissive action of glucose on the induction of spike activity by isoleucine is shown in table 2. In

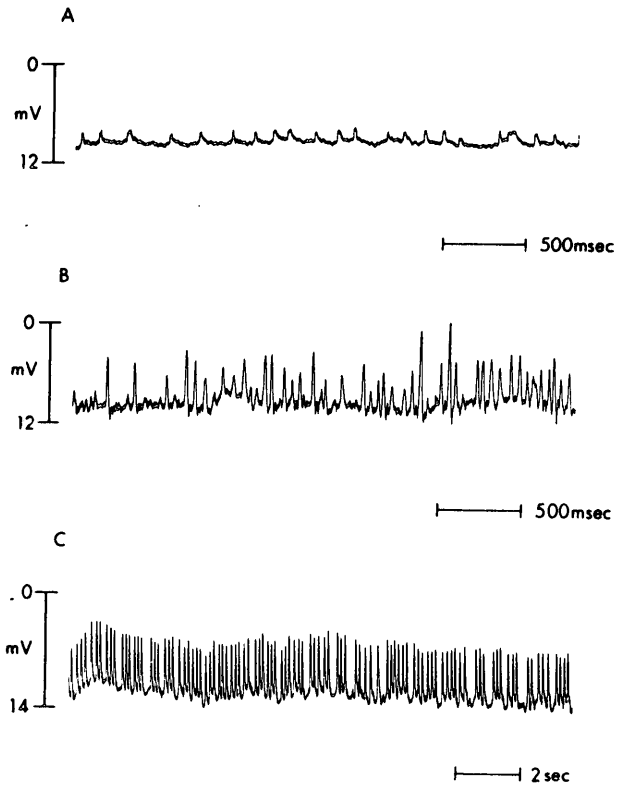

FIG. 2. Patterns of spike activity recorded from three different rat islet cells in the presence of L-isoleucine (8 mM) and glucose (4.2 mM).

TABLE 2

The effect of basal glucose on the induction of spike activity by L-isoleucine in islet cells

	Conditions	Time (min.)	% of Cells Exhibiting Spike Activity
a.	Isoleucine	20-60	7 ± 4
	Glucose	63-120	20 ± 5
b.	Isoleucine + glucose	20-60	51 ± 5
	Glucose	63-120	17 ± 4*
c.	Glucose	20-60	19 ± 4
	Isoleucine + glucose	63-120	56 ± 3*

In each of the three experimental conditions (a-c) the islets were exposed to a given substance or combination of substances for two consecutive periods of sixty minutes, during which time a sufficiently large number of cells (fifty or more) was impaled to measure the membrane potential and to record the presence or absence of spike activity. The islets in each experiment were superfused for twenty minutes before impalement was begun. The level of glucose used was 4.2 mM and that of L-isoleucine 8 mM. The medium also contained 5 mM lactate and 0.5 mM pyruvate. Each experiment was performed three times. The means and S.E.M. of the percentage of cells showing spike activity are recorded (* $p \leq 0.05$, as compared to the corresponding control.) The corresponding membrane potentials are the basis of figure 3.

each experiment with basal glucose alone, about 20 per cent of the cells exhibited spike activity and with isoleucine alone only 7 per cent of the cells fired. On addition of isoleucine to basal glucose, the percentage of cells with spike activity increased threefold. The sequence of additions was immaterial (Exp. b vs. Exp. c). It was also found that isoleucine depolarized the cell membrane only in the presence of glucose (figure 3): the membrane potential with isoleucine alone was essentially the same as with low glucose alone. In the

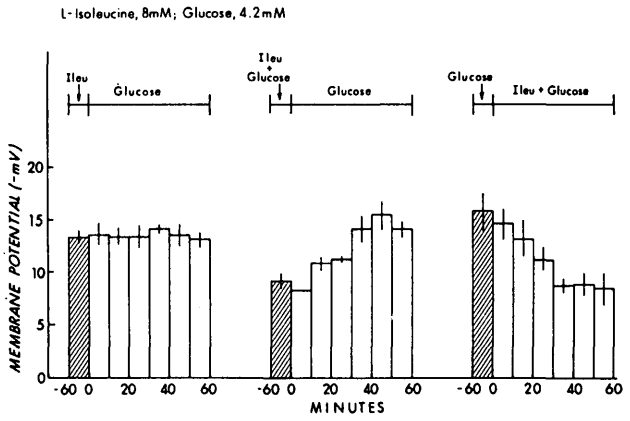

FIG. 3. Effect of L-isoleucine (8 mM) plus glucose (4.2 mM) on islet cell membrane potentials. The cross-hatched bar in each of the three experimental protocols indicates the time period during which the islets were exposed to the solution; the mean value of the membrane potential was obtained during impalement from t^{-40} to t^{0}. After switching solutions the mean values of the membrane potentials are shown for each ensuing ten minute period up to sixty minutes. The line on each bar represents two standard errors of the mean of three experiments.

presence of glucose, isoleucine caused depolarization of the membrane potential from 14-15 mV to about 9 mV. After changing to a medium containing only glucose, the potential slowly increased and stabilized at 15 mV by thirty minutes. The cross experiment in the right portion of figure 3 shows that the time course of depolarization of the islet cells was essentially the same as that obtained for repolarization.

In contrast to the changes in membrane potential, which occurred slowly, there was no resolvable delay for the appearance or disappearance of the spike activity recorded from these cells (figure 4). It is tempting to attach importance to the changes with time, but this should be done with caution since each point represents an average of only eighteen cells impaled.

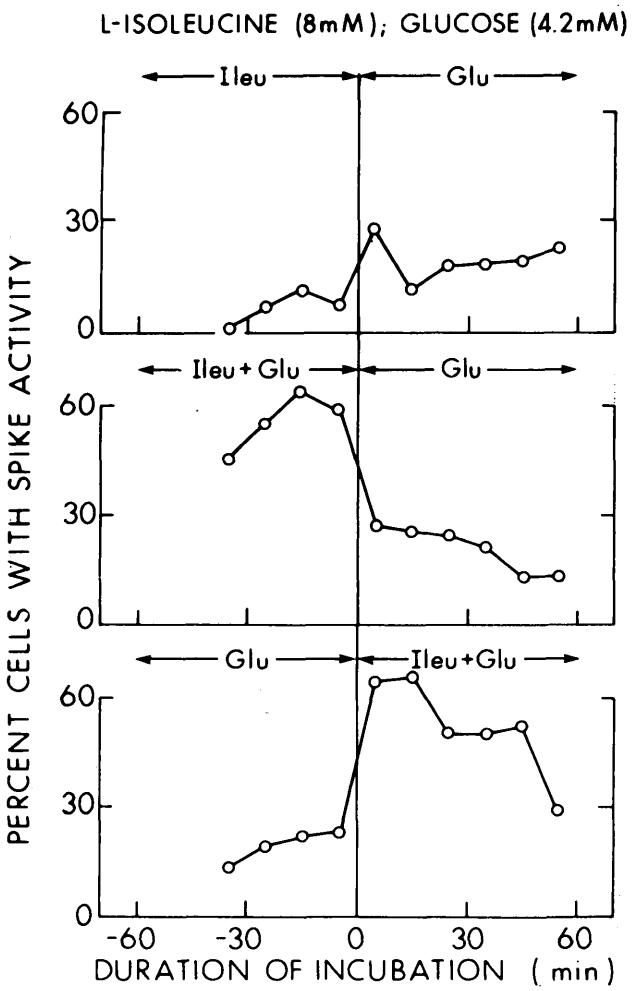

FIG. 4. Time course of the change in spike activity on addition or withdrawal of isoleucine. The islet was superfused for twenty minutes (t^{-60} to t^{-40}) before impalement of cells was begun. When the solutions were changed, three minutes were allowed for the new solution to reach the chamber and achieve the desired concentration before determining the electrical response of the islet cells. Each point represents on the average eighteen cells impaled.

A mixture of twenty amino acids in proportions found in normal rat serum, but at levels about three times those of animals fasted overnight,[14] was a highly effective stimulus in the presence of basal glucose (table 2). There seemed to be a small effect in the absence of glucose (Exp. a, table 3). In contrast to high glucose, and the combination of isoleucine and basal glucose, the mixture of amino acids did not decrease the average membrane potential of the islet cells, whether basal glucose was included or not. As found in the measurements with other β-cell stimuli, electrical responses to the amino acid mixture plus glucose varied from cell to cell (figure 1).

TABLE 3

The effect of basal glucose on the electrical response to an amino acid mixture

	Amino Acids (10 mM)	Glucose (4.2 mM)	Time (min)	Membrane Potential (—mV)	% of Cells Exhibiting Spike Activity
a.	—	—	0-30	14.7 ± 0.5	20 ± 3
	+	—	33-63	14.1 ± 0.3	31 ± 3
	—	—	66-96	15.0 ± 0.4	16 ± 2
b.	—	+	0-30	14.5 ± 0.4	23 ± 4
	+	+	33-63	13.9 ± 0.7	77 ± 3
	—	+	66-96	13.7 ± 0.6	28 ± 2

In addition to lactate (5 mM) and pyruvate (0.5 mM) the medium contained the mixture of twenty amino acids [14] in the presence or absence of basal glucose as indicated. In each of the two kinds of experiments stimulation by the amino acids (thirty-three to sixty-six minutes) was bracketed by control periods in the absence of the stimulus (zero to thirty and sixty-six to ninety-six minutes). Each experiment was performed four times. The means ± S.E.M. are recorded. In each of the two experiments spike activity was increased significantly by the amino acid mixture above the control periods. ($p \leq 0.05$).

The effects of iodoacetate (IOA) on the electrical responses to glucose and isoleucine

It was shown previously that inhibition of lactate formation[6] and also glucose utilization (formation of T_2O from 5-T-glucose)[7] by 0.2 mM IOA occurs within thirty to forty-five minutes of exposure but does not affect the secretory response of the β-cell to glucose. The increase in glycolysis by the addition of basal glucose to isoleucine was similarly inhibited by IOA.[7] The corresponding electrophysiological studies now show that this inhibitor has no effect on the responses evoked either by high glucose or by isoleucine plus basal glucose (table 4). The values of the membrane potentials and also the percentages of cells with spike activity were the same whether iodoacetate was present or not, even in the period after blockade of glycolysis (forty to eighty minutes).

Corresponding studies of the effect of IOA on the

TABLE 4

Effect of iodoacetate on electrical response induced by high glucose or by L-isoleucine plus basal glucose

Condition	Time (min.)	Membrane Potential (—mV)	% of Cells Exhibiting Spike Activity	No. of Experiments
a. *High glucose*				
Control	0-80	12.4 ± 0.7	65 ± 2	3
+ iodoacetate	0-40	11.7 ± 0.4	69 ± 5	3
	43-80	11.2 ± 0.5	62 ± 2	3
b. *Basal glucose* + isoleucine				
Control	20-60	9.2 ± 0.7	51 ± 5	3
+ iodoacetate	20-40	9.6 ± 0.6	55 ± 5	6
	43-80	9.7 ± 0.5	58 ± 7	6

As in all electrophysiological experiments, pyruvate (0.5 mM) and lactate (5.0 mM) were present in the superfusate, which contained either (a) high glucose (16.6 mM) or (b) L-isoleucine (8 mM) plus basal glucose (4.2 mM). When present the iodoacetate level was 0.2 mM. The data with iodoacetate are presented separately for two time periods because of the lag in iodoacetate action.[6,7] The means and S.E.M. of the indicated number of experiments are recorded. The values for the electrical parameters in the presence of iodoacetate are not different from the controls ($p \leq 0.2$). The difference of the membrane potentials for the controls in a and b is statistically significant ($p \leq 0.05$).

electrical responses provoked by the amino acid mixture plus basal glucose were not done. It should be noted in this context that in parallel metabolic studies[7] amino acids served as a relatively weak stimulant of lactate production from basal glucose.

In these electrical studies phosphate-buffered saline was used instead of bicarbonate-buffered saline employed in the corresponding metabolic studies.[7] This change was necessary since it proved difficult to maintain the physiological pH in the superfusion apparatus designed for electrical studies. It was, however, found in preliminary perifusion experiments that isolated islets exhibited a typical biphasic release of insulin when exposed to high glucose in phosphate-buffered saline. Other investigations have also shown that islets respond in a typical manner in phosphate-buffered solution.[16] It was also assessed whether the efficacy of iodoacetate was altered in phosphate-buffered saline, and it was found that the lactate formation from high glucose was completely blocked by 0.2 mM iodoacetate as observed in bicarbonate-buffered saline.[6]

Effect of iodoacetate on the secretory responses to isoleucine and an amino acid mixture

In these functional studies (figure 5) the isolated rat pancreas was perfused for a total of seventy-five minutes, consisting of a forty-five minute prestimulatory and a thirty-minute stimulatory period. Secretion of insulin was low in the presence of basal glucose alone (5 mM) and almost nonexistent in the presence of isoleucine alone (10 mM) or the amino acid mixture (10 mM). When isoleucine or the amino acid mixture was added to basal glucose the biphasic insulin release profiles elicited were qualitatively similar except that the amino acid mixture-induced release occurred more promptly than did isoleucine. In the two profiles the first phase peaked between thirty and sixty seconds after adding the amino acid mixture and between four and five minutes after addition of isoleucine. For the remainder of the thirty-minute stimulatory period following this first phase a slowly increasing second phase was observed with isoleucine and a damped oscillatory response with the amino acid mixture.

As with the electrical responses, IOA at 0.2 mM did not alter the secretory response to isoleucine plus glucose nor did it have any effect on the insulin release profile induced by the amino acid mixture plus basal glucose.

DISCUSSION

The pancreatic islets are not unique among a group of secretory tissues in which chemical stimulation of secretion is accompanied by changes in the transmembrane potential of the cell. Such an electrical response has been observed in endocrine glands (adrenal medulla,[17] adrenal cortex,[18] thyroid[19]) and also exocrine glands (salivary,[20] exocrine pancreas[21]). But the islet cells have the exceptional characteristic that physiological secretagogues elicit spike discharges, a bioelectrical response which in the case of glucose shows a concentration dependency similar to the secretory response.[3]

The present data and related results in the literature[1,3,22,23] are consistent with the concept that electrical activity of the β-cells is coupled to the insulin releasing function of these cells. This holds true when high glucose levels alone or when combinations of isoleucine or amino acids plus basal glucose are used as β-cell stimuli. Under these conditions spike

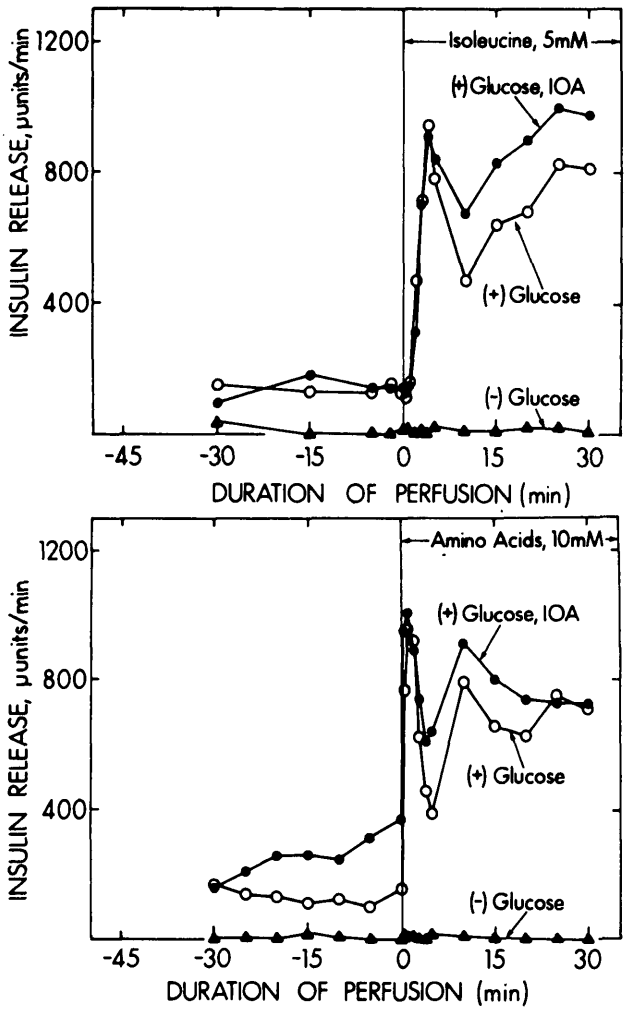

FIG. 5. Iodoacetate and the permissive action of glucose for insulin release due to isoleucine (A) (upper), and an amino acid mixture (B) (lower). The pancreases were perfused with a dextran-salt solution containing 0.5 mM pyruvate and 2.5 mM lactate in all three protocols. Basal glucose, when included, was 5 mM and iodoacetate (IOA) was 0.2 mM. When present, these were added at -45 minutes. At the end of the preperfusion period of forty-five minutes, either isoleucine or a physiologic mixture of twenty amino acids was added. Samples were taken at the intervals indicated in the figure. In A the means of three experiments are shown; in B the mean values represent five experiments performed with glucose and three in its absence. The results of experiments without glucose but containing IOA were indistinguishable from the corresponding data without the SH reagent and are not shown for reasons of clarity of the graph. Also not shown are the results of control experiments (n = 3) containing basal glucose and IOA throughout but lacking amino acids, since iodoacetate had no significant effect on insulin release in the presence of basal glucose alone.

activity and insulin release changed concomitantly. But the decrease of the average membrane potential seen with high glucose and with isoleucine plus basal glucose does not seem to be an absolute prerequisite for increased insulin release, since this depolarization was absent with the amino acid mixture plus basal glucose. Since the changes of spike activity and changes in membrane potential have very different time constants when stimulated with isoleucine and basal glucose it might be suggested that the two electrical events may very well be associated with separate cellular functions at the plasma membrane. It should also be noted in this connection that in mouse islets KCl depolarizes the islet cell membrane without inducing spike activity[22] and leucine elicits spike activity without accompanying depolarization.[23]

It was previously observed that exposure of the pancreas for thirty minutes to high glucose or to L-isoleucine plus basal glucose lowers the threshold for glucose stimulation of insulin release,[8] an effect not seen after exposure to a physiological mixture of twenty amino acids plus basal glucose.[24] Therefore, this capacity of stimulants to induce poststimulatory hyperresponsiveness might be causally related to the ability of such stimulants to depolarize the islet cells. Since it has been found that certain α-ketomonocarboxylic acids and acetoacetate, as well as β-hydroxybutyrate, similarly increase responsiveness of the islets to basal glucose,[8] it would seem desirable to study the actions of these substances on islet cells with electrophysiological means.

From previous data on the permissive action of glucose[8,24] it was not possible to decide whether glucose increased the affinity or efficacy of the amino acids or, conversely, whether the amino acids increased the efficacy of glucose. The present results make the first alternative more likely, since the depolarization, which seems to be characteristic of glucose action (references 1-3 and this study), was not seen with the amino acid mixture plus basal glucose.

It must not be overlooked, however, that the association between electrical activity and releasing function does not seem to be absolute, since we have observed that cytochalasin B, which potentiates glucose-induced insulin release,[25] causes a decrease or even a complete cessation of the electrical firing of β-cells exposed to 16.6 mM glucose.[26] Although spike activity was inhibited by cytochalasin B, glucose still depolarized the β-cells as in the control condition.

It had been rather generally accepted that the metabolism of glucose and its insulin releasing function parallel each other.[4] However, exposure to a level of IOA for a period sufficient to block glycolysis does not affect either the secretory function or the electrical behavior of the β-cells. In the face of this evidence it is almost impossible to defend the earlier concept that changes in the level of certain specific glucose metabo-

lites or cofactors of glucose metabolism trigger insulin release[4,27] and electrical activity.[1,2] Perhaps some caution must be exercised in accepting this interpretation as final since (in the case of amino acid-stimulated release) it is based on the assumption that the islet cells of the isolated perfused pancreas behave qualitatively and quantitatively like isolated islets obtained by the collagenase procedure. However, no compelling evidence speaks against this assumption.

Findings that appeared to support the earlier view can be interpreted in other ways. For example, it has been found that pretreatment of mouse islets with phloridzin and mannoheptulose, substances that inhibit the uptake[28] and metabolism[27] of glucose, respectively, completely block glucose-induced electrical activity,[2] as well as glucose-provoked insulin release.[27,28] Although this suggested that increased metabolism of glucose is necessary for induction of electrical activity and the secretory responses, it has been pointed out that blockade by mannoheptulose or phloridzin of glucoreceptors may instead be responsible for inhibition of these two functions.[2,28-30]

In view of the present study and other published data discussed, we have adopted a working hypothesis that the direct and indirect stimulatory actions of glucose involve interaction with the β-cell membrane inducing a primary biophysical change leading to altered ionic fluxes and initiation of spike activity and hormone release, without the primary involvement of glycolytic metabolites and cofactors.

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