Modification of Glucose-induced Insulin Release by Alteration of pH

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SUMMARY

Protons (H^+) generated by glucose metabolism have been proposed to serve as a coupling factor between cationic and secretory events in the B-cell. We have examined the influence of alteration of extracellular or intracellular pH (pH_o or pH_i) on dynamic secretory responses of perifused rat islets to 4.2, 8.4, or 16.7 mM glucose. Reduction of pH_o from 7.4 to 7.0 inhibited the secretory response to 16.7, but not 8.4 mM glucose, by 47% during the 30-min period following medium change. Increase of pH_o from 7.4 to 7.8 had no influence on the secretory response to glucose. Alteration of pH_o had no influence on basal insulin release in the presence of 4.2 mM glucose. Sulfamerazine (5 mM), a permeable weak acid, augmented the secretory response to 8.4 mM glucose by 60% but had no influence on the response to 16.7 mM glucose. In contrast, imidazole (10 mM), a permeable weak base, inhibited the secretory response to both 8.4 (62%) and 16.7 mM (72%) glucose. Another weak base, NH_4Cl (20 mM), also inhibited the secretory response to 8.4 (61%) and 16.7 mM (68%) glucose. Alteration of pH_i by sulfamerazine and imidazole did not alter basal insulin release in the presence of 4.2 mM glucose. A comparison of the present findings to those obtained for the influence of pH on glucose-induced electrical activity indicates that alteration of pH_i, and not pH_o, induces parallel effects on glucose-induced electrical and secretory events.

DIABETES 32:61–66, January 1983.

Numerous investigations have been made to reveal which agents generated by glucose metabolism can account for metabolic regulation of cationic events that occur in stimulus-secretion coupling in B-cells. Consequently, it has been found that glucose at low concentrations (<4.0 mM) influences several key cationic events, namely, the efflux of $^{86}Rb^+$ and $^{45}Ca^{++}$ (first phase).[1] These functional changes have been postulated to be coupled to an increased generation of protons (H^+).[2] In fact, it has been found that glucose increases H^+ output from islets with a dose-response curve that is similar to that for $^{86}Rb^+$ and $^{45}Ca^{++}$ effluxes.[1,3] However, based on data obtained by the use of ^{14}C-DMO (5,5′-dimethyloxazolidine-2,4-dione), there is apparently no detectable change in intracellular pH (pH_i) accompanying glucose stimulation of insulin release.[3] Nevertheless, it has been calculated, taking into consideration the buffering capacity of the islets, that there is about a 0.09-U decrease in pH_i when islets are exposed to stimulatory concentrations of glucose.[4] It is conceivable that small changes in pH_i may reduce K^+ permeability (P_K) in B-cells, as has been found to occur in nerve fibers.[5,6] In the absence of glucose, a decrease of extracellular pH (pH_o) reduces P_K in a manner consistent with P_K changes observed in nerve.[7] Electrophysiological studies have revealed that changes in pH_o or pH_i profoundly influence glucose-induced electrical activity.[8] A decrease in pH_o or pH_i induces depolarization and continuous spike activity, whereas an increase in pH_o or pH_i leads to a reduction or complete cessation of the electrical activity in the presence of a stimulatory concentration of glucose.

Thus far, the influence of changes in pH_o on insulin release due to glucose has only been determined using statically incubated islets.[4] The results of these studies revealed that the optimal pH for insulin release increased as the concentration of glucose was raised. To assess to what extent changes in pH_o or pH_i influence glucose-induced insulin release, and to what extent these changes are coupled to pH-induced changes in electrical activity of B-cells, we have examined the influence of changes in pH_o or pH_i on the dynamic secretory response of perifused islets by using a permeable weak acid (sulfamerazine) or base (imidazole or NH_4Cl). Our results indicate that alterations in pH_i, but not pH_o, more closely maintain the positive correlation between electrical and secretory responses.

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Received for publication 24 June 1982.

MATERIALS AND METHODS

Pancreatic islets were isolated from male Sprague-Dawley rats (250–300 g) by a modified collagenase technique.[9] After washing, the mixture of islets and acinar tissue was diluted with 50 ml of Hanks' solution in a 30-×-200-mm test tube and allowed to settle for 30 s. The intact islets at the bottom of the cylinder were removed with a siliconized Pasteur pipette. This procedure was repeated as many times as necessary to obtain a sufficient quantity of islets. The clean islets were then placed in a petri dish and transferred via a pipette into perifusion chambers.

The dynamic release of insulin from islets was studied in a perifusion system. The setup consists of a LKB 2132 Microperpex double-headed peristaltic pump with silastic tubing, i.d. 1.3 mm, connected to a 13-mm Swinnex filter chamber via polyethylene tubing, 1.19 mm i.d. A 12-mm Nitex filter cloth with 10-μm pores was placed on the surface of the plastic mesh in the chamber. The chamber, filled with medium containing 100 islets and attached tubing, was placed in a water bath at 37°C, and the perifusate was collected via a fraction collector. The medium used was Krebs-Ringer containing 0.5% bovine serum albumin. Changes in the pH were achieved by equilibrating the medium with different concentrations of $NaHCO_3$ against the same gas mixture of 95% O_2 and 5% CO_2. The NaCl concentration was modified to maintain isoosmolarity and total Na^+ concentration. Changes in the medium were accomplished by means of two three-way stopcocks aligned in series. Switching controls were performed for each set of experiments without changing the test media. The medium was drawn by the peristaltic action of the pump through the perifusion chamber at a flow rate of 1 ml/min. Lag time for the system (the time necessary for the medium to reach the fraction collector after switching solutions) was 1 min as determined by passing trypan blue through the system. The islets were initially exposed to 2.8 mM glucose and samples were collected at 5-min intervals for 30-min to establish a baseline of insulin release. The medium was then switched to one containing 4.2–16.7 mM glucose, and samples were collected at 1-min intervals for the next 30–40 min, 2-min intervals for 40–50 min, and 5-min intervals for 50–60 min, to determine if the islets were glucose-sensitive. The medium was then switched to one containing glucose plus the test substance, and samples were collected in a manner identical to that described previously. Samples were stored at −20°C until assayed for insulin content over the range of 0–25 μU/0.1 ml using the alcohol precipitation and single antibody method.[10] Porcine insulin was used as the standard (courtesy of Eli Lilly and Company, Indianapolis, Indiana). Pork insulin labeled with ^{125}I was obtained from New England Nuclear (Boston, Massachusetts); sulfamerazine, imidazole, and NH_4Cl were obtained from Sigma Chemical Company (St. Louis, Missouri); monensin was obtained from Calbiochem (La Jolla, California).

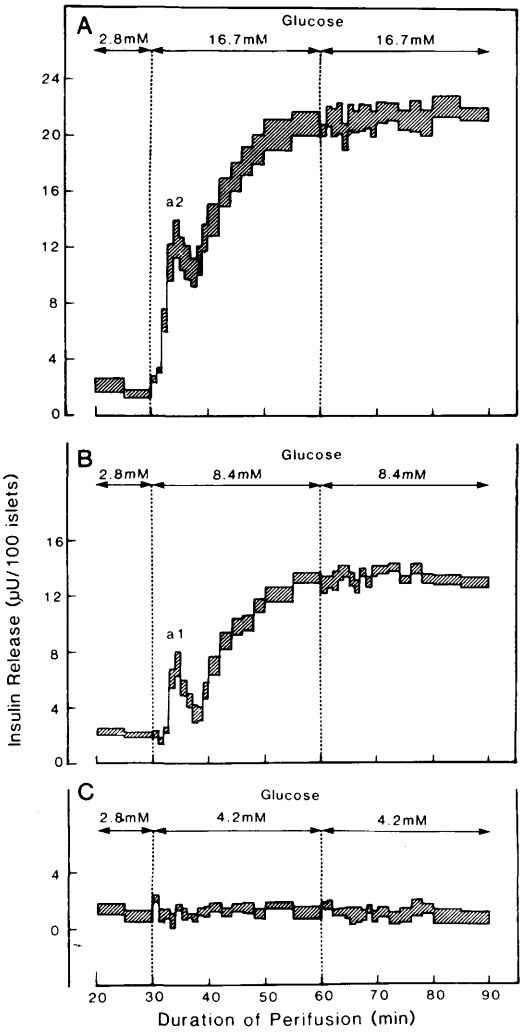

FIGURE 1. Influence of switching the medium on basal and glucose-induced insulin release from perifused islets. Islets are perifused in basal (2.8 mM) glucose for 30 min before "challenge" with (A) 16.7, (B) 8.4, or (C) 4.2 mM D-glucose at pH 7.4. After an additional 30-min period, the medium was switched to one of identical content. These results serve as switching controls. The secretory response is the mean of at least four experiments. The shaded area under the curve is the standard error of the mean. Abbreviations used in all figures: a1 represents the significant difference ($P < 0.05$) between the basal secretory response for 25–30 min at 2.8 mM glucose and the total insulin released 2–7 min after changing the solution; a2 represents a significant difference ($P < 0.01$) and a3 ($P < 0.001$); b1 represents the significant difference ($P < 0.05$) for the average total insulin released for the time periods 10–30 min after changing the solution to higher glucose and 10–30 min after changing to the test solution; b2 represents a significant difference ($P < 0.01$) and b3 ($P < 0.001$); c1 represents a significant difference ($P < 0.05$) between 2–7 min after switching to the test substance and 9–30 min after switching to the test substance; d1 represents the significant difference ($P < 0.05$) between 10–30 min after exposure to high glucose and 2–7 min after exposure to the test solution.

RESULTS

Secretory response to glucose. Increasing the concentration of glucose in the perifusate from 2.8 to 16.7 mM resulted in a dose-response in which increasing levels of glucose (4.2, 8.4, and 16.7 mM) yielded increasing quantities of insulin released (1.0 ± 0.8, 13.8 ± 0.8, and 22.8 ± 1.4 μU/100 islets/min, respectively). Switching from one concentration of glucose to identical media had no effect on insulin release and thus served as a control for possible switching artifacts for the length of time the islets were perifused (Figures 1A, B, and C).

Secretory response to changes in pH_o. Alteration of the medium pH from 7.4 to 7.0 in the presence of 16.7 mM glucose elicited no change in the amount of insulin released

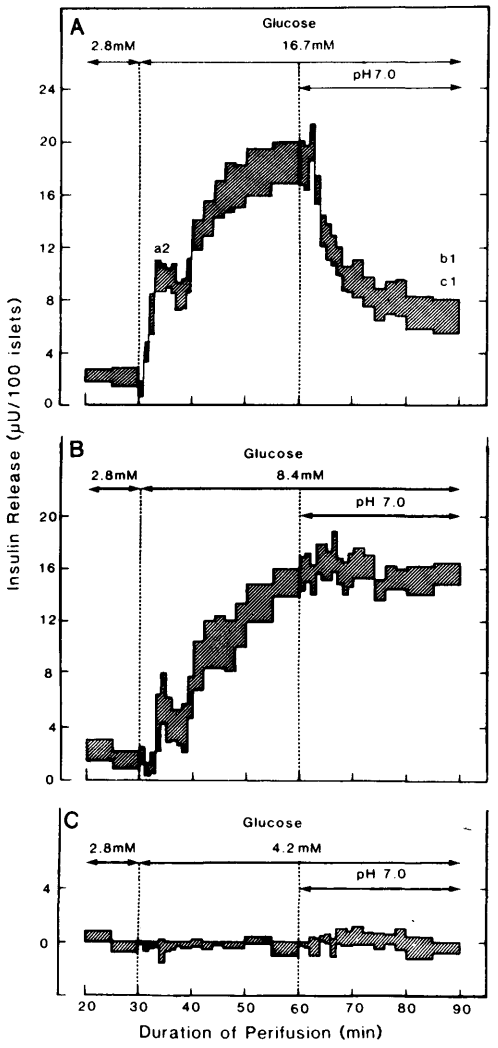

FIGURE 2. Influence of medium acidification on basal and glucose-induced insulin release from perifused islets. The experimental protocol was identical to that of Figure 1 for the first 60 min. At that time, the pH of the medium was lowered to 7.0 in the presence of (A) 16.7, (B) 8.4, or (C) 4.2 mM glucose. The values for insulin release are the means of at least three (five at 16.7 mM) experiments. The shaded area under the curve is the standard error of the mean.

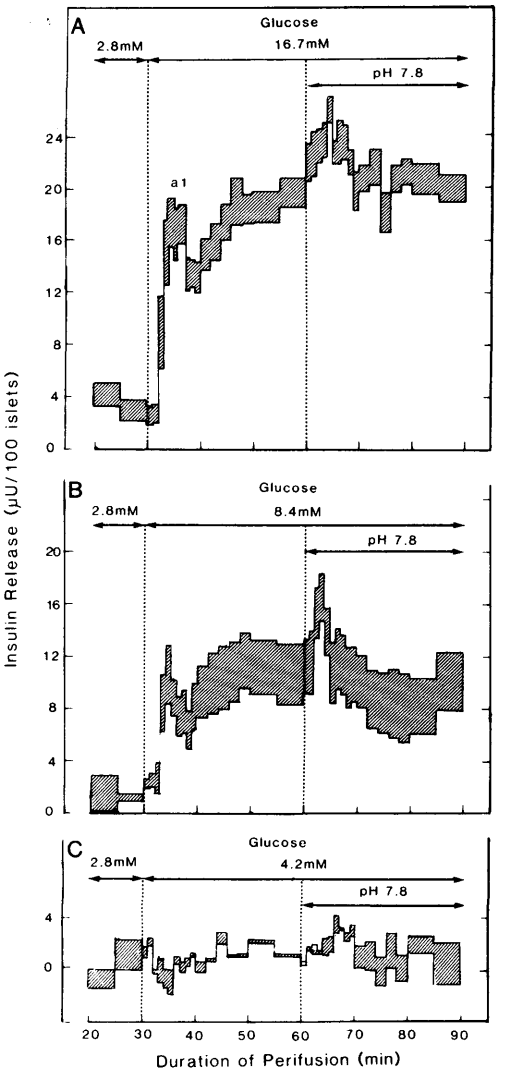

FIGURE 3. Influence of medium alkalinization on basal and glucose-induced insulin release from perifused islets. The experimental protocol was identical to that for acidification, but the pH was changed from 7.4 to 7.8 in the presence of (A) 16.7, (B) 8.4, or (C) 4.2 mM glucose. The values for insulin release are the means of at least three experiments. The shaded area under the curve is the standard error of the mean.

between 2 and 7 min after changing solutions but inhibited insulin release by 47% 10–30 min after changing solutions (Figure 2A). The acidification of the medium had no effect on insulin release at glucose concentrations of 4.2 or 8.4 mM (Figures 2B and C).

Alteration of the perifusate pH from 7.4 to 7.8 did not significantly alter the amount of insulin release elicited by 4.2, 8.4, or 16.7 mM glucose (Figure 3).

Secretory response to changes in pH_i. In another series of experiments, the effects of altering pH_i of the B-cell using a permeable weak acid or base, or the Na:H ionophore, monensin, was examined. The permeable weak acid, sulfamerazine[11] (5 mM), had no effect on insulin release due to 16.7 mM glucose (Figure 4A). In contrast, sulfamerazine in the presence of 8.4 mM glucose evoked a substantial increase in insulin release (Figure 4B). There was an initial increase for 1 min (from 11.8 ± 1.6 to 17.2 ± 2.0 μU/100 islets/min, $P < 0.05$), then a decline for 3 min (from 17.2 ± 2.0 to 8.0 ± 1.2 μU/100 islets/min, $P < 0.05$), followed by a continuous increase in insulin release until the quantity of release was comparable to that achieved with 16.7 mM glucose (compare Figure 4B with Figure 4A). Sulfamerazine did not influence basal insulin release obtained with 4.2 mM glucose (Figure 4C).

Imidazole and NH_4Cl, permeable weak bases, were used to increase pH_i. When 10 mM imidazole was added in the presence of 16.7 mM glucose, there was an initial decline in insulin release for 2 min (from 20.2 ± 1.8 to 10.4 ± 2.6 μU/100 islets/min, $P < 0.05$) (Figure 5A). Within an additional 2-min period, there was a partial recovery (from 10.4 ± 2.6 to 14.4 ± 3.6, μU/100 islets/min, NS) of the amount of insulin released after which there was a gradual decline. Similar results were obtained using 20 mM NH_4Cl. The initial decline and recovery in insulin release (from 26.6 ± 1 to 11.8 ± 1.8, $P < 0.01$ and then to 20.8 ± 2.0 μU/100 islets/min, $P < 0.05$)

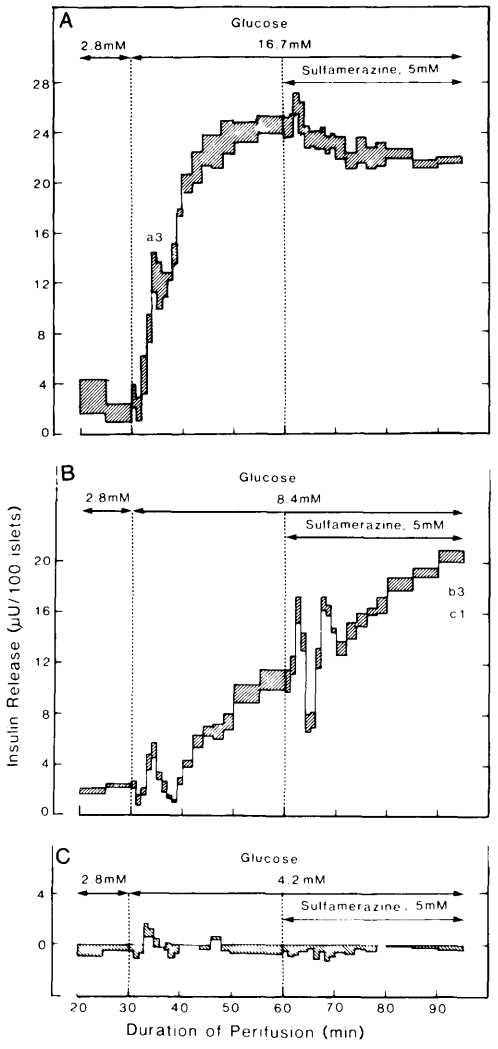

FIGURE 4. Influence of the permeable weak acid sulfamerazine on basal and glucose-induced insulin release from perifused islets. The secretory response is the mean of four experiments for (A) 16.7 and (B) 8.4 mM glucose, and of two experiments for (C) 4.2 mM glucose. The shaded area under the curve is the standard error of the mean for A and B, and one-half of the range for C.

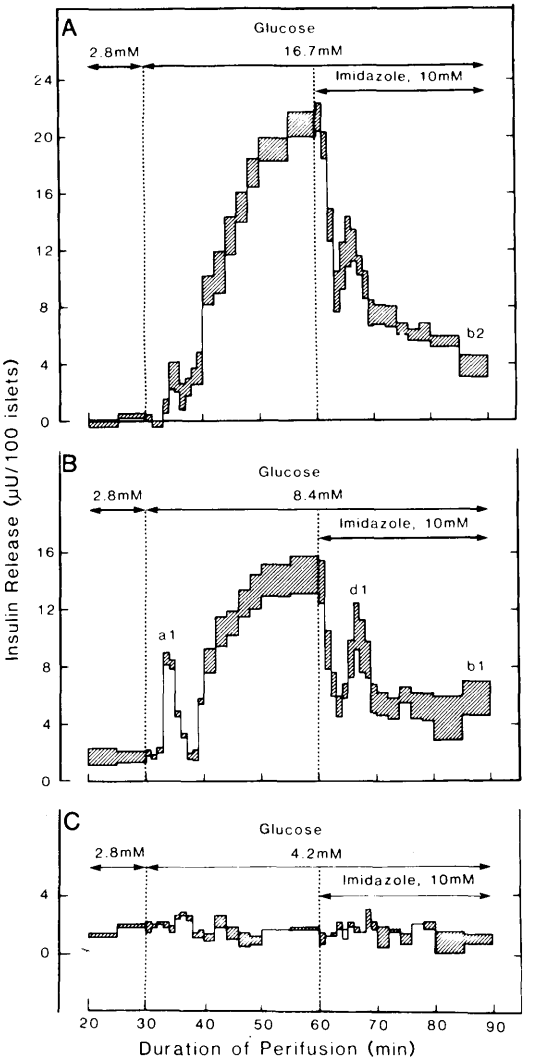

FIGURE 5. Influence of the permeable weak base imidazole on basal and glucose-induced insulin release from perifused islets. The secretory responses represent the mean of six experiments at (A) 16.7 and (B) 8.4 mM glucose, and of two experiments at (C) 4.2 mM glucose. The shaded area under the curve is the standard error of the mean for A and B, and one-half of the range for C.

was followed by a decline to 8 ± 1.5 μU, $P < 0.1$ (Figure 6A). A similar pattern of inhibition was found when imidazole or NH_4Cl was added to medium containing 8.4 mM glucose (Figures 5B and 6B). Imidazole had no influence on basal insulin release obtained in the presence of 4.2 mM glucose (Figure 5C).

Monensin (15 μM), an electroneutral Na:H antiporter, exerted an immediate and sustained inhibition of insulin release. This was evident in the presence of either 16.7 or 11.1 mM glucose (Figures 7A and B).

DISCUSSION

Changes in medium pH have been found to have no effect on basal insulin release and a varying influence on insulin release depending on the concentration of glucose added to statically incubated islets.[4] At low or 6.7 mM glucose, an extracellular pH of 7.0 elicited a maximal secretory response. The optimal pH for obtaining a maximum secretory response shifted to alkaline values as the concentration of glucose was increased.[4] Although the same investigators have indicated that changes in pH_o elicit parallel changes in pH_i,[4] this finding was not supported by determinations of pH_i using ^{14}C-DMO. A decrease in pH_o from 7.4 to 6.3 was found to decrease pH_i from 7.2 to 6.8, suggesting that pH_i changes only slightly with changes in pH_o.[12] In fact, the permeability of most membranes to H^+ is low.[6] Hence, the use of a permeable weak acid or base is more likely to alter pH_i, allowing one to determine its influence on cationic and secretory events.

The addition of a permeable weak base to the medium will result in cellular entry of the unprotonated form until equal concentrations of this form exist on both sides of the membrane. Depending on the cellular pH, a variable quantity of the unprotonated base will protonate, resulting in further entry of the neutral form until the ratio of charged to neutral form reflects the pH of the cellular compartment. The effect of this process will be to transiently alkalinize the cell interior. Imidazole, with a pK_a of 6.9, is quite suitable for such an effect since a large fraction of the base will be in neutral

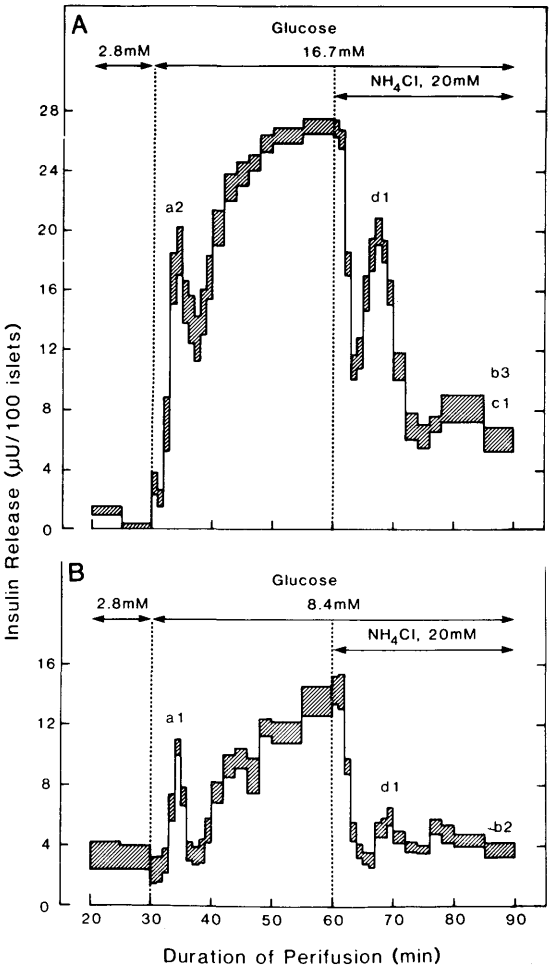

FIGURE 6. Influence of the weak base NH_4Cl on glucose-induced insulin release from perifused islets. Values are for eight experiments at (A) 16.7 mM and for six experiments at (B) 8.4 mM glucose. The shaded area under the curve is the standard error of the mean.

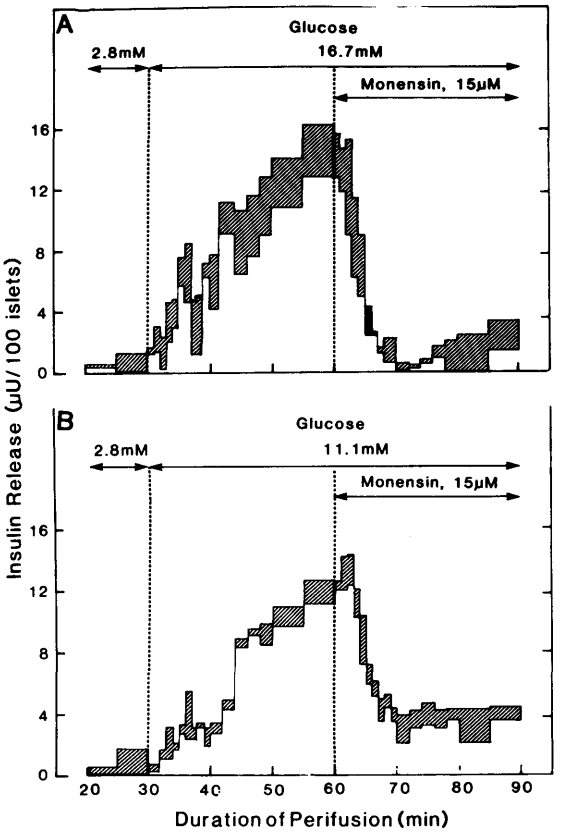

FIGURE 7. Influence of monensin on glucose-induced insulin release from perifused islets. The secretory response is the mean of two experiments at (A) 16.7 mM and (B) 11.1 mM glucose. The shaded area represents one-half the range of the mean.

form at pH 7.4, resulting in rapid entry and rapid alkalinization. In the mouse B-cell, imidazole elicits a change in the electrical activity in less than 1 min,[8] suggesting that the change in pH_i is quite rapid. Conversely, the addition of a permeable weak acid will result in equilibration of the neutral form, with release of protons intracellularly until the ratio of neutral to charged form of the weak acid reflects the cell pH.[8]

One should remember that imidazole has other effects in addition to altering the pH_i. At concentrations of 26–28 mM, imidazole increases cyclic AMP phosphodiesterase activity by 12–24%.[13,14] It is doubtful, though, that imidazole would totally inhibit insulin release merely as a result of a decline in islet cAMP content. Further evidence to support the argument that imidazole inhibition of insulin release is due to pH_i alkalinization comes from our study with NH_4Cl. NH_4Cl, the action of which presumably only increases pH_i as directly measured by a pH sensitive electrode in barnacle muscle and squid axon,[6] produced an identical kinetic inhibitory pattern to that obtained with imidazole (compare Figures 5 and 6).

In other studies, it was found that exposure of islets to 30% pCO_2 elicited a decrease in pH_i from 7.1 to 6.4.[15] However, there was no influence of CO_2-induced intracellular acidification on basal or glucose-stimulated (16.7 mM) insulin release. These results compare favorably with what we have found using sulfamerazine to decrease pH_i. This drug had no influence on insulin release in the presence of 4.2 mM (basal) or 16.7 mM glucose, but elicited a substantial increase in the rate of insulin release in the presence of 8.4 mM glucose.

Alteration of pH_o from 7.4 to 7.8 did not influence the secretory response to 4.2, 8.4, or 16.7 mM glucose, although alkalinization of pH_i by the use of imidazole or NH_4Cl led to an impressive inhibition of insulin release due to 8.4 or 16.7 mM glucose. Immediately after addition of the permeable weak bases to the medium, there was an inhibition of insulin release followed by a brief increment of insulin release. This transient escape from the inhibitory influence of alkalinization of the intracellular space may be due to the ability of the native cellular buffering systems to briefly counteract the influence of the weak bases.

Either a decrease in pH_o or the presence of the permeable weak acid glycodiazine has been found to alter the cyclic nature of glucose-induced electrical activity, leading to depolarization and constant spike activity.[8] Conversely, extracellular alkalinization or the presence of imidazole or monensin has been found to inhibit electrical activity, leading to a decrease or cessation of spike activity and hyperpolarization.[8] The influence of sulfamerazine (a weak acid with

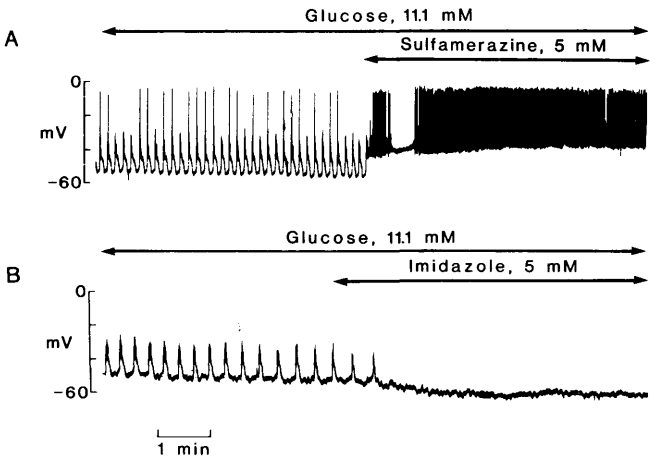

FIGURE 8. Effect of the permeable weak acid, sulfamerazine, or base, imidazole, on glucose-induced electrical activity recorded from a single B-cell of a microdissected mouse islet. Normal oscillatory electrical patterns are seen at 11.1 mM glucose. Addition of 5 mM sulfamerazine results in constant spike activity (A). Addition of 5 mM imidazole results in cessation of electrical burst activity (B). The imidazole recording is by courtesy of Dr. John Tarvin and Dr. Caroline Pace.

actions identical to glycodiazine) and imidazole on the electrical activity of a single B-cell is shown in Figure 8. It is generally accepted that glucose induces parallel changes in electrical and secretory responses.[16] In view of this, it is surprising that an increase in pH_o has no influence on the secretory response and that a decrease in pH_o inhibits insulin release due to high glucose.

In conclusion, it appears that alteration of pH_i, and not pH_o, provokes parallel changes in electrical and secretory events in glucose-stimulated islets. It is difficult, however, to interpret the results of our experiments as evidence that the metabolic generation of H^+ is the primary cellular control system for triggering changes in both cationic fluxes and insulin release. There is no influence of a change in pH_o or pH_i on basal insulin release in the presence of 4.2 mM glucose. But according to other studies, there are substantial changes in the efflux of H^+, K^+, or Ca^{++} within the noninsulinotropic range of glucose concentrations (2–5 mM).[2,4] Therefore, it remains to be determined to what extent the effect of experimental alteration of pH_i on ionic and secretory responses can be related to the control of these events via metabolic production of H^+.

ACKNOWLEDGMENTS

We gratefully acknowledge Doug Swain for his help in preparing the illustrations, Ray Boshell for his excellent technical help, Donna Motes for her patient assistance in typing the manuscript, and Dr. J. T. Tarvin for his collaboration and aid in the preparation of this manuscript.

This work was supported by a grant from the National Institutes of Health (AM21973). Dr. C. S. Pace is a recipient of a Research Career Development Award (AM00499). Dr. J. S. Smith is supported by the Diabetes Trust Fund.

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