Influence on Anion Transport on Glucose-induced Electrical Activity in the B-Cell

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SUMMARY

We have previously shown that the effect of glucose on electrical activity (EA) in islet B-cells is altered by modification of pH. The regulation of intracellular pH (pH_i) in nerve and muscle cells is coupled to anion exchange. In the present study we have examined the involvement of HCO_3:Cl exchange across the plasma membrane in the maintenance of glucose-induced EA in B-cells. 4,4′-diisothiocyanostilbene-2,2′ disulfonic acid (DISDS), an inhibitor of anion exchange, elicited a dose-related stimulation of EA in the presence of 11.1 mM glucose. The increase in the relative duration of the active phase (constant spike activity) was first observed at 20 μM DIDS, and a nearly maximal effect was obtained at 200 μM. The substitution of HCO_3^- by a Hepes buffer elicited constant spike activity. The application of 0.25 μM tributyltin, an electroneutral Cl:OH exchanger, also enhanced EA as indicated by an increase in the duration of the active phase. The influence of HCO_3^- withdrawal, DIDS, and tributyltin all elicited electrical events similar to that obtained by a decrease in pH_i. Our results suggest that anion exchange may be involved in the regulation of electrical events in the B-cell by influencing pH_i, as has been documented to occur in invertebrate nerve and muscle. DIABETES *31*:653–655, July 1982.

A fundamental event in stimulus secretion-coupling in the pancreatic islet B-cell is the induction of changes in ionic conductances of the plasma membrane and the attendant electrical activity (EA). One way in which glucose may invoke the changes in ionic events is by the generation of protons (H^+) by metabolism of this substrate.[1] In fact, we have recently reported that a decrease in pH by extracellular acidification or a permeable weak acid alters the cyclic nature of glucose-induced EA leading to depolarization and constant spike activity, whereas extracellular alkalinization or a permeable weak base partially or transiently inhibits EA.[2]

The inability to detect a decrease in intracellular pH (pH_i) in the presence of glucose that could account for the alterations in the electrical events may be due to the intracellular buffering power of the B-cell in addition to a pH_i regulating mechanism which responds to production of H^+ by transporting H^+ out of the cell. In accordance with this prediction, it has been shown that glucose produces a dose-related increase in the net output of H^+.[3] The regulation of pH_i in nerve and muscle cells apparently involves Na:H exchange in association with HCO_3:Cl exchange.[4] The evidence has stemmed from studies showing that acid extrusion is inhibited by the reduction of external [HCO_3^-] at constant extracellular pH (pH_o), by the application of anion transport inhibitors, or by the reduction of external [Na^+].[4] In an effort to determine if inhibition of anion exchange elicits changes in the electrical events similar to those induced by a decrease in pH_i,[2] we have determined the requirement of HCO_3^- or anion exchange for the maintenance of glucose-induced EA in B-cells.

METHODS

Islets of Langerhans were micro-dissected from fed CBA/J retired-breeder male mice (Jackson Labs, Bar Harbor, Maine). Microelectrode recordings from single B-cells were obtained using standard electrophysiologic techniques, as described in detail elsewhere.[2] The ionic content of the modified Krebs-Henseleit perifusion medium was (mM): Na^+, 136.2; K^+, 5; Ca^{2+}, 2.5; Mg^{2+}, 1.2; Cl^-, 120; HCO_3^-, 25; SO_4^{2-}, 1.2; $H_2PO_4^-$, 1.2. The medium was buffered with 16 mM Hepes, and the pH was adjusted to 7.4 by the addition of NaOH. Glucose was added to the above medium just before perifusion, and the medium was equilibrated with 95% O_2-5% CO_2 for a final pH of 7.4. DIDS (4,4′-diisothiocyanostilbene-2,2′ disulfonic acid) was added from a freshly-mixed stock solution. Older stocks seemed to lose their potency. Tributytin (TBT) was also added from a stock solution, but showed no loss of potency with time. For the studies

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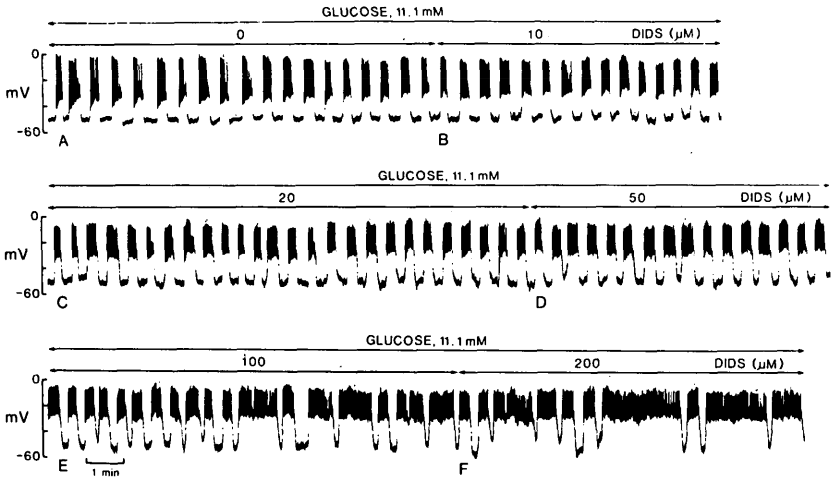

FIGURE 1. Effect of DIDS on glucose-induced electrical activity. (A) control activity in the presence of 11.1 mM glucose. (B–F) effects of the addition of 10, 20, 50, 100, and 200 μM DIDS to a medium containing 11.1 mM glucose. All records are continuous.

without HCO_3^-, the following medium was used (mM): Na^+, 130; K^+, 5.9; Ca^{2+}, 2.6; Mg^{2+}, 1.2; Cl^-, 139.8; SO_4^{2-}, 1.2; $H_2PO_4^-$, 1.2. The medium was buffered with 29 mM Hepes, and the pH was adjusted to 7.4 by the addition of NaOH. The solution was not gassed.

Chemicals: Hepes was obtained from Research Organics (Cleveland, Ohio); DIDS from Calbiochem-Behring Corp. (La Jolla, California); and TBT from Alfa Products (Danvers, Maine).

RESULTS

The disulfonic stilbene derivative, DIDS, inhibits anion exchange in erythrocytes[5] and inhibits the pH_i-regulating system in snail neurons, squid axons, and barnacle muscle.[4] DIDS was found to produce a dose-related stimulation of EA in the B-cell in the presence of 11.1 mM glucose (Figure 1). DIDS was found to influence the percent of time occupied by the active phase (constant spike activity) in relation to the total duration of the active plus silent phase. During the 10 min preceding the initial introduction of DIDS, the cell showed a level of 45.4 ± 1.5% activity (mean ± SE; N = 19: silent plus active phase pairs). This level of activity was increased by the addition of DIDS: 10 μM, 47.2 ± 1.0% (N = 13); 20 μM, 49.2 ± 1.3% (N = 24); 50 μM, 56.3 ± 1.8% (N = 13); 100 μM, 71 ± 3% (N = 15); and 200 μM, 80 ± 13% (N = 7). In all of the above calculations, the EA in the first minute following the change was not included. DIDS first significantly affected EA at 20 μM ($P < 0.05$). Above 100 μM DIDS, the EA became irregular with periods of constant spike activity separated by brief silent phases. Similar effects have been seen in 3 other cells with levels of DIDS greater than 100 μM.

The substitution of HCO_3^- by a Hepes buffer elicited constant spike activity and a subsequent oscillatory activity with greater duration of the active phase (Figure 2). Reintroduction of HCO_3^- elicited a reversible effect (Figure 2). The effect of HCO_3^- withdrawal on EA is similar to that induced by the application of a weak acid, glycodiazine, which also produces reversible depolarization and constant spike activity.[2]

To ascertain whether electroneutral Cl:OH exchange could directly stimulate EA similar to inhibition of HCO_3:Cl exchange, we used TBT. TBT mediates Cl:OH exchange [6] and has been used in gastric plasma membrane vesicles to dissipate either a H^+ or OH^- gradient, depending on the Cl^- gradient.[7] Application of 0.25 μM TBT enhanced EA due to 11.1 mM glucose (Figure 2). This effect was similar to that elicited by the addition of DIDS and the removal of HCO_3^-.

DISCUSSION

Information concerning the regulation of pH_i in B-cells is not available, nor is it clear to what extent metabolic generation of H^+ modulates glucose-induced EA. We have modeled this study after the extensive studies in the invertebrate squid axon, snail neuron, and giant barnacle muscle in which regulation of pH_i requires HCO_3^- and is sensitive to inhibitors of anion transport.[4]

We have found that inhibition of HCO_3^- influx by either omission of HCO_3^- or by the addition of DIDS induces an increase in EA, as illustrated by an increase in the percent of time occupied by the active phase of the oscillatory activity. This effect is similar to that invoked by a decrease in pH_o or by the application of a weak acid to decrease pH_i.[2] Although this is not conclusive evidence that inhibition of

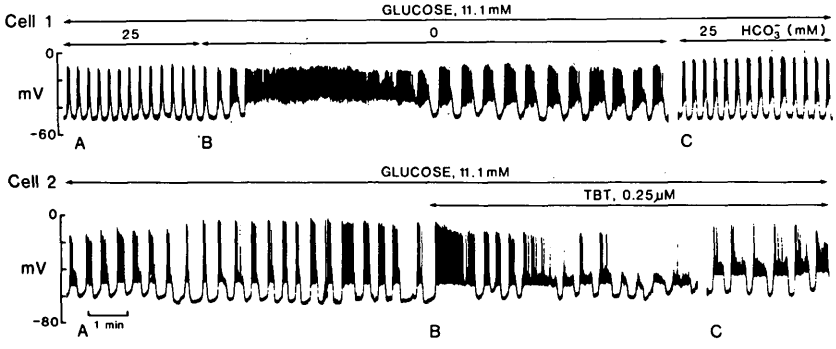

FIGURE 2. Effect of bicarbonate withdrawal and tributyltin addition on glucose-induced electrical activity. Cell 1. (A) control activity in the presence of 11.1 mM glucose and 25 mM HCO_3^-. (B) removal of HCO_3^-. (C) 10 min after readdition of 25 mM HCO_3^-. Cell 2. (A) control activity in the presence of 11.1 mM glucose. (B) effect of 0.25 μM tributyltin. (C) 10 min after addition of 0.25 μM tributyltin.

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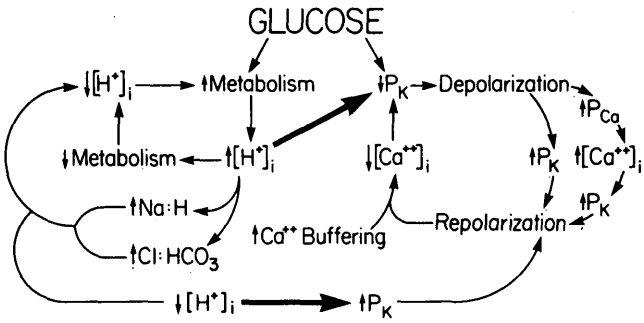

FIGURE 3. Model to account for the influence of H^+ and anion transport on glucose-induced electrical activity. Glucose, by decreasing P_K, initiates a cascade of events which, through the mediation of the concentration of intracellular Ca^{2+} and the membrane potential, results in a cyclic variation of depolarization and repolarization of the B-cell membrane potential. The metabolism of glucose results in an increased intracellular concentration of H^+ which, in turn, may have a negative feedback influence on the rate of metabolism (glycolysis), resulting in a decrease in the level of metabolically-generated H^+. The influence of glucose on P_K and metabolism may be coupled by $[H^+]_i$: an increase in $[H^+]_i$ may decrease P_K by competition for the K-channel. An increase in $[H^+]_i$ may also lead to an increased $Cl:HCO_3$ and/or Na:H exchange. This increased exchange will serve to decrease $[H^+]_i$ and, therefore, increase P_K. As a consequence of a decrease in $[H^+]_i$, the rate of metabolism may then increase. (See text for more details.)

anion transport leads to a decrease in pH_i, the application of the electroneutral Cl:OH exchange ionophore, TBT, suggests that acidification of the intracellular space by acceleration of OH^- efflux elicits an effect similar to that produced by inhibition of HCO_3:Cl exchange.

Figure 3 is a model which integrates the means by which metabolic production of H^+ and the regulation of pH_i by Na:H and HCO_3:Cl exchange, in concert with the well-documented involvement of changes in Ca^{2+} and K^+ permeabilities that regulate glucose-induced EA,[8] may modulate electrical events in the B-cell. The model indicates that the generation of H^+ via glucose metabolism is a feedback inhibitor of metabolism. The rate of glycolysis has been shown to be sensitive to small changes in pH. For example, glycolysis is decreased by a fall in pH_i in human red blood cells[9] and rat brain,[10] and is increased by small changes in pH_i in guinea pig leukocytes[11] and rat heart.[12] This is likely to be due to the sensitivity of phosphofructokinase (PFK), a rate-limiting enzyme of glycolysis. This has been particularly well illustrated in frog skeletal muscle in which a pH increase of about 0.1–0.2 U maximally activates PFK.[13] The rate of glucose-dependent glycolysis in B-cells exhibits a positive correlation with the influence of glucose on insulin release.[1] It is possible that H^+ generated via glycolysis are transported out of the B-cell via a Na:H exchange, as has been shown to occur in invertebrate preparations. The attendant increase in pH_i may then serve to increase the rate of glycolysis, as occurs in yeast and ascites cells.[14]

It seems likely that the failure to document small changes in pH_i due to glucose is a result of the transient nature of these pH changes. Such changes would be impossible to document with static ^{14}C-DMO (5,5′-dimethyloxazolidine -2,-4-dione) studies. However, the transient accumulation of H^+ in the cell may be sufficient to decrease K^+ conductance (P_K), as occurs in neural tissue.[15] The subsequent extrusion of H^+ via Na:H exchange would again increase P_K. We have previously shown that monensin, a Na:H electroneutral exchanger, elicits rapid inhibition of glucose-induced EA, thereby suggesting that an endogenous Na:H exchanger could be involved in the regulation of pH_i and EA.

The possibility that the proton can be used as currency for information transfer in the B-cell poses an interesting question, particularly with respect to its influence on metabolism and ion transport mechanisms that may allow the coupling of metabolic and ionic events.

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