Volume 234, number 1, 208–212 FEB 06021 July 1988

The ATP-sensitive potassium channel in pancreatic B-cells is inhibited in physiological bicarbonate buffer

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Received 9 May 1988

The effects of bicarbonate buffer (HCO_3^-/CO_2) on the activity of the two K^+ channels proposed by some to control the pancreatic B-cell membrane response to glucose were studied. Single K^+-channel records from membrane patches of cultured B-cells dissociated from adult rat islets exposed to a glucose- and bicarbonate-free medium (Na-Hepes in place of bicarbonate) exhibit the activity of both the ATP-sensitive as well as the $[Ca^{2+}]_i$-activated K^+ channels. However, in the presence of bicarbonate-buffered Krebs solution, the activity of the ATP-sensitive K^+ channel is inhibited leaving the activity of the K^+ channel activated by intracellular $[Ca^{2+}]_i$ unaffected. In the absence of bicarbonate (Hepes/NaOH in place of bicarbonate), lowering the external pH from 7.4 to 7.0 also has differential effects on the two K^+ channels. While the K^+ channel sensitive to ATP is inhibited, the K^+ channel activated by a rise in $[Ca^{2+}]_i$ is not affected. To determine whether the response of the B-cell in culture to bicarbonate is also present when the B-cell is functioning within the islet syncytium, the effects of bicarbonate removal on membrane potential of B-cells from intact mouse islets were compared. These studies showed that glucose-evoked electrical activity is also blocked in bicarbonate-free Krebs solution. Furthermore, in the absence of bicarbonate and presence of glucose (11 mM), electrical activity was recovered by lowering the pH_o from 7.4 to 7.0. The ATP-sensitive K^+-channel activity is greatly reduced by physiologically buffered solutions in pancreatic B-cells in culture. The most likely explanation for the bicarbonate effects is that they are mediated by cytosolic pH changes. Removal of bicarbonate (keeping the external pH at 7.4 with Hepes/NaOH as buffer) would increase the pH_i. Since the activity of the $[Ca^{2+}]_i$-dependent K^+ channels is not affected by the removal of the bicarbonate buffer, our patch-clamp data in cultured B-cells indicate an involment of $[Ca^{2+}]_i$-activated K^+ channels in the control of the membrane potential. For the B-cell in the islet, we propose that the burst pattern of electrical activity (Ca^{2+} entry) is controlled, at least in part, by the $[Ca^{2+}]_i$-activated K^+ channel.

Bicarbonate buffer; K^+-ATP channel; K^+-$[Ca^{2+}]_i$ channel; intracellular pH; Glucose sensing; (Pancreatic B-cell)

1. INTRODUCTION

Glucose-stimulated insulin release from islets of Langerhans is closely correlated with the electrical responses of B-cells to glucose [1]. These electrical responses depend on the fact that glucose modulates in some way the K^+ permeability of the B-cell [2,3] and include a cyclic 'burst' pattern of Ca^{2+} action potentials in response to a stimulatory range of glucose concentrations [4–6]. Two K^+ channels present in the membrane of the pancreatic B-cell may regulate this bursting electrical response. These are the ATP-sensitive, sulfonylurea blockable K^+ channel and the voltage, $[Ca^{2+}]_i$-activated K^+ channel [7]. Indeed, a number of recent attempts have been made to elucidate the mechanisms regulating these channels using single ion channel recording techniques (summarized in [8]). Some of these studies have emphasized the possible determinant role of the ATP-sensitive K^+ channel in glucose sensing [9–12]. However, other studies have shown that the activity of the $[Ca^{2+}]_i$-sensitive K^+ channel is also modulated by glucose, the physiologic secretagogue [13,14]. The important problem then is to determine whether either or

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Published by Elsevier Science Publishers B.V. (Biomedical Division)

both of these channels is a glucose sensor under physiological conditions.

In this paper we show that pH buffering conditions which support glucose-induced electrical activity and insulin secretion in islets, nonetheless inhibit the ATP-sensitive K^+ channel in cell-attached patches. The effects may be mediated through a change in intracellular pH_i which in turn diminishes the open probability of the ATP-sensitive K^+ channel. These data therefore indicate that caution must be exercised in attributing an exclusive role to the ATP-sensitive K^+ channel in glucose sensing.

2. MATERIALS AND METHODS

2.1. *Single channel recordings*

Patch pipets were prepared from micro-hematocrit capillary tubes (i.d. 1.1–1.2 mm) using a BB-CH microelectrode puller (Mecanex, Geneva). These pipets had an open tip resistance in the range 10–20 MΩ. When cell-attached patches were formed, the resistance rose to 10–20 GΩ.

Single K^+-channel currents (cell-attached configuration) were recorded under voltage-clamp conditions using a List EPC-5 amplifier (List-Electronics, Darmstadt-Eberstadt). The amplifier was used with the capacity null-bridge switch off. The amplitude and duration of single K^+-channel events were measured using a digital storage oscilloscope (model 4094, Nicolet Oscilloscope Division, Madison, WI).

2.2. *Cell cultures*

Pancreatic B-cells for culture were prepared from collagenase-isolated islets from adult rats [15]. Cell cultures were maintained for 2–10 days in a medium (CMRL 1066 from Gibco) supplemented with glucose (5.6 mM). After a few days, cells were firmly attached to the bottom of the tissue culture dishes (Corning 25000, Corning Glass Works, Corning, NY) and were ready for patch-clamp experiments. Patch-clamp experiments were carried out at room temperature (about 22°C). Cell-attached patches were formed on single cells or clumps of cells as described [16].

2.3. *Experimental solutions*

B-cell membrane potential recordings were made in intact mouse islets, as in [3]. The islets were superfused with either bicarbonate-buffered modified Krebs solution (mM: 120 NaCl, 25 $NaHCO_3$, 5 KCl, 2.5 $CaCl_2$, 1.1 $MgCl_2$, equilibrated with 95% O_2/5% CO_2 gas mixture to give a pH of 7.4 at 37°C) or with a bicarbonate-free medium (mM: 120 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.1 $MgCl_2$, 20 Hepes/NaOH equilibrated with 100% O_2, pH 7.4 at 37°C).

Single K^+-channel inward currents (cell-attached configuration) were recorded using a high K^+ solution in the patch-clamp pipet (mM: 140 KCl, 2.5 $CaCl_2$, 1.1 $MgCl_2$, 10 Hepes/KOH, pH 7.4). For single K^+-channel outward current recordings, the patch pipet was filled with a solution containing normal $[Na^+]_o$ and low $[Ca^{2+}]_o$ (mM: 135 NaCl, 5 KCl, 0.001 $CaCl_2$, 3.6 $MgCl_2$, 10 Hepes/NaOH, pH 7.4 at room temperature).

Pancreatic B-cells in the tissue culture dish were superfused with either a bicarbonate-free medium (mM: 135 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.1 $MgCl_2$, 10 Na-Hepes equilibrated with 100% O_2 to pH 7.4 or 7.0 at 22°C) or a bicarbonate-buffered Krebs solution (mM: 120 NaCl, 25 $NaHCO_3$, 5.0 KCl, 2.5 $CaCl_2$, 1.1 $MgCl_2$ equilibrated with 95% O_2/5% CO_2, pH 7.3–7.4 at room temperature). The solution in the tissue culture dish was changed at a rate of 1.5 cm^3/min (2 cm^3 medium in the dish). For single K^+-channel outward current recordings, cells were superfused with a low $[Ca^{2+}]_o$, high K^+ solution (mM: 125 KCl, 25 $NaHCO_3$, 0.001 $CaCl_2$, 3.6 $MgCl_2$) with or without bicarbonate as required.

3. RESULTS

3.1. *Effects of bicarbonate-free medium on glucose-evoked electrical activity*

The membrane potential recorded from an individual islet B-cell in the presence of glucose (>7 mM) spontaneously oscillates between two levels: one at about −55 mV and the other at about −40 mV during which action potentials occur. Upon removal of glucose the bursting pattern ceases, only to return when glucose is re-applied (fig.1A). However, replacement of HCO_3^-/CO_2 buffer by Hepes/NaOH buffer, keeping the pH constant at 7.4, resulted in loss of bursts of electrical activity (fig.1B). However, the electrical activity was restored to normal by returning to the physiological HCO_3^-/CO_2 buffer system (fig.1C; n = 9). In a few experiments (n = 2), occasional bursts of electrical activity were seen in the absence of bicarbonate. The inhibition of electrical activity persisted in the absence of bicarbonate for as long as 20 min. Lowering the pH_o of the bicarbonate-free medium from 7.4 to 7.0 induced a sustained recovery of glucose-evoked electrical activity (3 out of 5 cells).

3.2. *Blockade of the ATP-sensitive K^+ channel by bicarbonate*

Following the results with different buffers on electrical activity of B islet cells, we were, therefore, led to examine the effect of these different conditions on both K^+ channels. Fig.2 shows four segments of continuous recordings of inward K^+ currents through ATP-sensitive channels, in the absence of bicarbonate (A,C,D) and in its presence (B). Single K^+-channel events recorded in the absence of bicarbonate/CO_2 buffer at pH_o

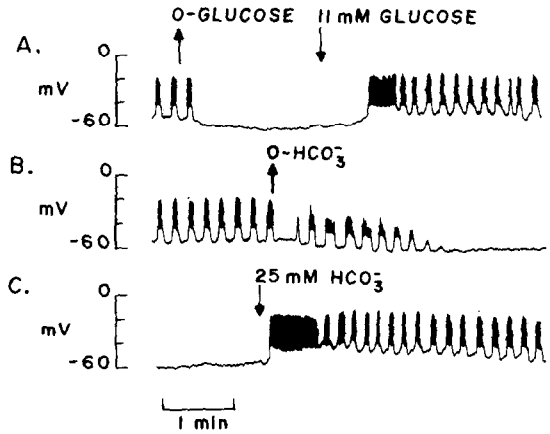

Fig.1. Comparison between the effect of glucose removal (A) and bicarbonate removal (B,C) on pancreatic B-cell electrical activity. Continuous chart record of the membrane potential fluctuations from the same B-cell. (A) Glucose was removed as indicated by the arrows and was maintained throughout parts B and C at 11 mM. (B) Perifusion of the islet with a bicarbonate-free solution (Na-Hepes in place of HCO_3^-/CO_2) at pH 7.4 started at the time indicated by the arrow. (C) Bicarbonate-buffered solution was applied at the time indicated by the arrow. Temperature was 37°C throughout.

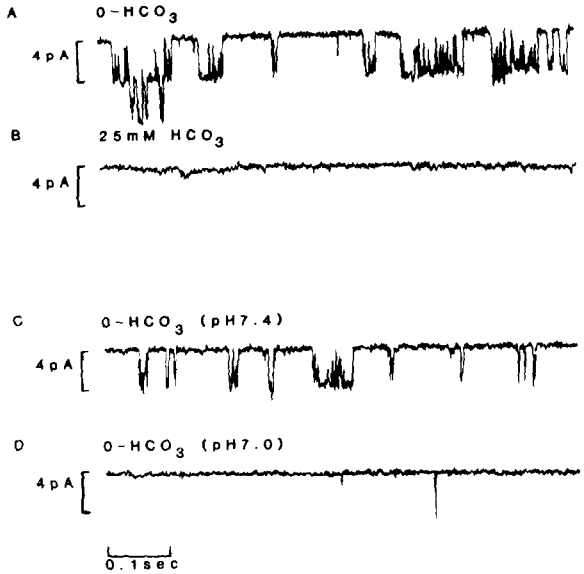

Fig.2. Blockade of the ATP-sensitive K^+ channel by bicarbonate buffer system (B) and by lowering the external pH from 7.4 to 7.0 (B). Four segments of continuous chart records of the ATP-sensitive K^+-channel activity in cell-attached patches. Pipet potential 0 mV throughout. In all the records downward deflections represent K^+-channel openings. (A) ATP-sensitive K^+-channel activity in the absence of bicarbonate buffer. There were two such channels in the patch. (B) Segment of the membrane current record from the same cell made towards the end of the exposure to a bicarbonate (HCO_3^-/CO_2) buffered solution. (C) Single K^+-channel currents in the absence of bicarbonate. 10 mM K-Hepes was used to control the pH_o at 7.4. (D) Segment of the current record made in the absence of bicarbonate towards the end of the exposure to the same medium used to make the record in part A but with the pH adjusted to 7.0.

7.4 (A–C) and at pH_o 7.0 (D) are shown. In the bicarbonate-free buffer, the ATP-sensitive K^+ channel was found to be active in all the patches examined (fig.2A,C; n = 12 cells). The mean value of the channel conductance estimated from records like those shown in fig.2 is 55.7 pS. Replacement of the bicarbonate-free medium by another solution of identical pH_o and ion composition but containing bicarbonate buffer induced a complete blockade of the ATP-sensitive K^+ channel (fig.2B; n = 12). In one of these experiments the inhibition was transient. In most cell-attached patches, subsequent removal of the bicarbonate buffer system only partially restored the activity of the ATP-sensitive K^+ channel, the recovery time being of the order of 10–20 min. K^+-channel activity was blocked within 3–5 min for as long as 20 min. Control experiments on excised B-cell patches showed that, while exposure of the inner aspect of the membrane patch to solutions containing ATP inhibited the channel activity, direct exposure to HCO_3^-/CO_2-buffered solutions keeping the pH constant at 7.4 did not affect activity of the ATP-sensitive K^+ channel.

3.3. *Blockade of the ATP-sensitive K^+ channel by lower pH in the absence of bicarbonate*

Of the two K^+ channels present in the B-cell membrane, only the $[Ca^{2+}]_i$-activated K^+ channel is inhibited by lowering pH on the cytoplasmic side [8]. In mouse islets of Langerhans the bicarbonate buffer system lowers pH_i and pH_i changes follow the changes in extracellular pH [17]. Contrary to our expectations, the ATP-sensitive K^+ channel was inhibited by lowering pH_o. In the majority (n = 10) of the cell-attached patches examined (n = 11), lowering the external $[pH]_o$ from 7.4 to 7.0 (Hepes/NaOH used in place of HCO_3^-/CO_2) significantly reduced the activity of the ATP-sensitive K^+ channel within 4 min (fig.1D). Only occasional brief openings were observed (fig.1D).

In most experiments, channel activity was not recovered for up to 15 min after returning to the control solution (pH 7.4).

3.4. *$[Ca^{2+}]_i$-dependent K^+ channel is insensitive to both bicarbonate removal and low pH_o (= 7)*

The activity of both K^+ channels is often seen in records made at positive membrane potentials (fig.3). This condition subsists so long as the cells are kept in a medium with no added bicarbonate and glucose. This is illustrated in fig.3A. Since the solution in the patch pipet contains low $[K^+]$ and the membrane patch is highly depolarized, the flow of K^+ current is now in the outward direction. Single channel events representing openings of the $[Ca^{2+}]_i$-dependent K^+ channel can be identified both by the brief duration of the openings and by the large amplitude of the current (mean conductance = 78 pS). While in all the cells examined (n = 9) replacement of the Hepes/NaOH buffer system by bicarbonate buffer had no significant effect on the $[Ca^{2+}]_i$-activated K^+ channel (fig.3B), the events from the ATP-sensitive K^+ channel were absent from the records (n = 8) or partially inhibited (n = 1). In all patches studied (n = 7), lowering pH_o of the bicarbonate-free solution from 7.4 to 7 had no effect on the $[Ca^{2+}]_i$-sensitive K^+-channel activity. Under these conditions the conductance

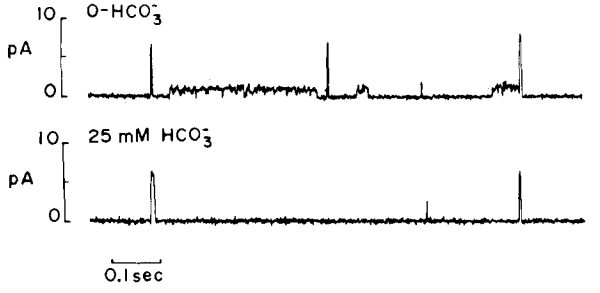

Fig.3. Differential blockade of the ATP-sensitive K^+ channel by a bicarbonate-buffered medium. Two segments of continuous chart records depicting the activity of the two K^+ channels in the same cell-attached patch (the ATP-sensitive K^+ channel is represented by the small-size single channel currents). Since $[K^+]_o$ in the external medium was 125 mM K^+ and the pipet potential was −20 mV, the membrane potential across the patch was close to 20 mV. (A) Record made in the absence of bicarbonate buffer in the medium. (B) Record made towards the end of the exposure to a bicarbonate-buffered medium. In the two records upward deflections represent K^+-channel openings.

of the ATP-sensitive channel was 17 pS (low $[K^+]$ in the pipet). However, the conductance of this K^+ channel in excised patches exposed to high $[K^+]$ on both sides was 55 pS for inward currents and 34 pS for outward currents, as reported by others [9–11]. The conductance of the $[Ca^{2+}]$-activated K^+ channel on excised patches exposed to symmetrical K^+ solutions was 200 pS for both inward as well as outward currents.

4. DISCUSSION

K^+-channel activity governs the B-membrane potential, which provides the link between metabolism and electrical activity (and thus Ca^{2+} entry) and allows the B-cell to sense glucose. We have seen that the ATP-sensitive K^+ channel, present in the plasmalemma of pancreatic B-cells, is active in the absence of both bicarbonate and glucose and inhibited in the presence of the physiological pH buffer HCO_3^-/CO_2 at room temperature. In the absence of bicarbonate the ATP-sensitive K^+ channels could be blocked by lowering the pH of the medium from 7.4 to 7.0. We propose that the blocking effect of the bicarbonate buffer is mediated by a decrease in intracellular pH. The simplest mechanism to explain a possible decrease in pH_i is to consider that the highly permeant CO_2 will cross the cell membrane of the B-cells exposed to a modified Krebs solution equilibrated with a gas mixture of 95% O_2/5% CO_2. Once CO_2 is inside the cell, HCO_3^- and H^+ will be formed. Thus, this reaction will probably cause a drop in pH_i.

In the absence of the bicarbonate buffer system (Hepes/NaOH in place of HCO_3^-/CO_2) glucose-evoked insulin secretion from rat islets is inhibited [18]. Removal of the physiological buffer system should increase pH_i restoring the activity of the ATP-sensitive K^+ channel. Our results on mouse islet cells showed that the removal of bicarbonate caused hyperpolarization of the B-cell membrane followed by a cessation of electrical activity, suggesting a direct involvement of the ATP-sensitive K^+ channel in these hyperpolarizing responses. The data also indicate that in physiological buffer the ATP-sensitive K^+ channel is less active than has been appreciated and this may explain the discrepancy between the estimated conductance of

a single B-cell (^{86}Rb flux in the presence of bicarbonate buffer) and observed K^+-channel activity (Na-Hepes in place of bicarbonate) [20].

There are a number of differences between the techniques used to study single channel currents in membrane patches and electrical activity in islet cells. This situation makes precise correlations difficult to establish with certainty. However, in this work an attempt was made to bring the cell culture system (as used for patch-clamp experiments) and the in vitro preparation of islets of Langerhans (as used for intracellular as well as insulin secretion protocols) closer together to be able to compare the results. It should be emphasized here that while patch-clamp experiments are carried out at room temperature, simultaneous measurements of membrane potentials and insulin release are carried out at 37°C [1]. Although glucose-induced electrical activity continues unchanged at 23°C, insulin secretion is blocked [23]. One might attempt to explain some of the differences in observations made in the two techniques by assuming that compensatory mechanisms, such as Na^+/H^+ and Na^+/Ca^{2+} exchanges and the Na^+/K^+ pump, might not operate with the same efficiency under the conditions used for patch-clamp recordings. Nonetheless, it remains clear that buffering conditions required for glucose-induced electrical activity and insulin secretion from islets of Langerhans inhibit the ATP-sensitive K^+ channel, yet the $[Ca^{2+}]_i$-activated K^+ channel is still operative.

Acknowledgements: We wish to thank Drs H. Pollard, R.M. Santos and A. Stutzin for many valuable comments. P.B.C. is supported by the E. Clarence Rice fellowship of the American Diabetes Association Washington DC affiliate.

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