J. Membrane Biol. 109, 135–143 (1989)

The Journal of
Membrane Biology
© Springer-Verlag New York Inc. 1989

# Modulation of Gating of a Metabolically Regulated, ATP-Dependent $K^+$ Channel by Intracellular pH in B Cells of the Pancreatic Islet

Stanley Misler, Kevin Gillis, and Joseph Tabcharani
The Departments of Medicine and Cell Biology/Physiology and the Program in Biomedical Engineering, Washington University, St. Louis, Missouri 63110

**Summary.** Membrane-permeant weak acids and bases, when applied to the bath, modulate the resting membrane potential and the glucose-induced electrical activity of pancreatic B cells, as well as their insulin secretion. These substances alter the activity of a metabolite-regulated, ATP-sensitive $K^+$ channel which underlies the B-cell resting potential. We now present several lines of evidence indicating that the channel may be directly gated by $pH_i$. (1) The time course of $K^+$(ATP) channel activity during exposure to and washout of $NH_4Cl$ under a variety of experimental conditions, including alteration of the electrochemical gradient for $NH_4Cl$ entry and inhibition of the $Na_o^+/H_i^+$ exchanger, resembles the time course of $pH_i$ measured in other cell types that have been similarly treated. (2) Increasing $pH_o$ over the range 6.25–7.9 increases $K^+$(ATP) channel activity in cell-attached patches where the cell surface exposed to the bath has been permeabilized to $H^+$ by the application of the $K^+/H^+$ exchanger nigericin. (3) Increasing $pH_i$ over a similar range produces similar effects on $K^+$(ATP) channels in inside-out excised patches exposed to small concentrations of $ATP_i$. The physiological role of $\Delta pH_i$ in the metabolic gating of this channel remains to be explored.

**Key Words** ATP-dependent $K^+$ channel · intracellular pH · ammonium chloride · nigericin

## Introduction

In B cells of the pancreatic islet, intracellular pH has often been considered as a possible link in the transduction of glucose metabolism to insulin secretion (for review *see* Pace, 1984*a*). This idea stems from evidence that (i) cellular metabolism can generate protons; (ii) intracellular pH can alter cell potassium permeability; and (iii) bath-applied membrane-permeant weak acids and bases can modulate metabolite-induced changes in $P_{K^+}$, electrical activity and insulin secretion, perhaps by acidifying or alkalinizing the cell (Eddlestone & Beigelman, 1983; Smith & Pace, 1983; Pace, 1984*b*; Rosario & Rojas, 1986*b*). Measurements of changes in intracellular pH during glucose metabolism has been attempted in several insulin secreting cells; results vary, however, from a small increase, to small decreases, to no change.

There is now a growing concensus that $ATP_i$-inhibited $K^+$ (or $K^+$(ATP)) channels underlie the metabolically regulated resting $K^+$ permeability ($P_{K^+}$) of B cells (for review *see*, e.g., Ashcroft, 1988). Closure of these channel by bath-applied metabolites depolarizes these cells and triggers cycles of electrical activity. Recently we found that bath application of 20 mM ammonium chloride, which transiently alkalinizes many other cells by ~0.5 pH units, transiently increases the mean activity ($I/i$) of $K^+$(ATP) channels several-fold; bath application of sodium propionate, which transiently acidifies many other cells by 0.5 pH units, transiently decreases $I/i$. (Misler et al., 1987). These data, however, are in apparent conflict with previous reports that $K^+$(ATP) channel activity in inside-out excised patches is virtually insensitive to $pH_i$ over the range pH 7.3 to 6.6 in the absence of ATP (*see* Cook & Hales, 1984; Misler et al., 1986). To resolve this discrepancy, we have examined the time course of $K^+$(ATP) channel activity during exposure to and washout of $NH_4Cl$ under a variety of experimental conditions, including alteration of the electrochemical gradient for cation entry and inhibition of the $Na_o^+/H_i^+$ exchanger. We have also examined the effect of $pH_i$ on $K^+$(ATP) channel activity in inside-out excised patches in the presence of ATP and the effect of $pH_o$ on $K^+$(ATP) activity in cell-attached patches where most of the cell has been rendered permeable to $H^+$ via the application of the $K^+/H^+$ exchanger nigericin. These new data suggest that, in the presence of $ATP_i$, $K^+$(ATP) channel activity is affected by $pH_i$ over a range of $pH_i$'s assumed to bracket the extremes of cell pH produced by exposure to and removal of bath-applied weak acids and bases. We suggest that this occurs through an alter-

ation in the interaction of ATP with the $K^+$(ATP) channel. This work has been reported previously in abstract form (Gillis et al., 1988; Gillis & Misler, 1988).

## Materials and Methods

Specific methods for preparation and culture of normal rat islet cells were previously detailed elsewhere (*see* Misler et al., 1986). Single channel recording was done with conventional patch-clamping techniques standard for our lab, using a digital data acquisition and analysis system. Currents were filtered at 0.5–0.9 kHz and sampled at 1.0–2.0 kHz. To standardize nomenclature, the clamping potential, $V_c$, is defined as the negative of the potential of the interior of the pipette with respect to ground. The average number of channels open in a patch is denoted (by convention) as $I/i$; it is equal to the number of channels in the patch times the open probability of the individual channel ($N \cdot p_o$). We measured this quantity with our interactive graphics-based analysis system using level crossings to determine when 1, 2, 3 and more channels were open, and averaging the results for a 20-sec to 2-min segment of record. In experiments where the time course of channel activity was required, channel currents were usually recorded continuously.

The standard extracellular-like solution (ES) contained (in mM): NaCl, 140; KCl, 5.5; $CaCl_2$, 2; $MgCl_2$, 1; HEPES · NaOH, 20, titrated to pH 7.3. The usual intracellular-like solution (IS) contained (in mM): KCl, 144; $MgCl_2$, 2; HEPES · KOH, 20, titrated to pH 7.35 with KOH. This solution was modified for use as the cytoplasmic solution for inside-out excised patch (e.g., Fig. 6) by addition of 1 mM EGTA, hence making it similar to an intracellular-like solution. The pH of the IS or ES was modified over the pH range 6.8–7.8 by titrating HEPES buffer with HCl or KOH (NaOH) to near the desired pH, and over the range 6.6–6.25 by using 20 mM PIPES instead of HEPES as the buffer and titrating this solution to the required pH.

## Results

### Time Course of Effect of Bath Application of $NH_4Cl$ on $K^+$(ATP) Channel Activity in the Cell-Attached Patch

In our earlier studies with cell-attached patches of B-cell membrane, we found that brief addition at constant $pH_o$ of 15–20 mM $NH_4Cl$ to the bath, a maneuver which transiently alkalinizes cells, resulted in a rapid increase in $K^+$(ATP) channel activity and cessation of glucose-induced action current activity. Conversely, brief addition of sodium propionate (15–20 mM), a maneuver which rapidly acidifies cells, resulted in a rapid decrease in $K^+$(ATP) channel activity and, occasionally, the onset of action currents, even in the absence of glucose. Cells are known to handle "loads" of membrane-permeant acids and bases in typical ways. For instance, in the case of $NH_4Cl$ exposure, typically, the uncharged permeant weak base $NH_3$ rapidly enters the cell by diffusion and associates with $H^+$ ions to produce $NH_4^+$, thus alkalinizing the cell (phase A). Subsequently, slower entry, over minutes, of the cationic conjugate acid, $NH_4^+$, through $K^+$ and/or $Na^+$ channels, reduces the net alkalinization (phase B, or "plateau phase acidification"). Removal of $NH_4Cl$ from the bath results in a rapid efflux of $NH_3$ from the cell, hence liberating $H^+$ ions and resulting in "washout acidification" (phase C). The magnitude of "washout acidification" is directly related to the magnitude of "plateau phase acidification." The baseline $pH_i$ is slowly restored, in part by the operation of a $Na_o^+/H_i^+$ exchanger (phase D) (*see* Boron, 1985). Assuming that $K^+$(ATP) channel is directly affected by $pH_i$, or that the concentration of a gating substance is rapidly affected by $pH_i$, the time course of $K^+$(ATP) channel activity during $NH_4Cl$ exposure and its modification by certain ionic alterations and pharmacological maneuvers should be predictable, at least in broad outline.

Figure 1 depicts the time course of B cell $K^+$(ATP) channel activity in the cell-attached patch during prolonged addition and subsequent washout of 20 mM $NH_4Cl$ from a physiological saline (or ES) bath. Four distinct phases of channel activity are clearly seen, each corresponding to a phase of $pH_i$, outlined above, assuming that cell acidification reduces channel activity, while cell alkalinization increases it. This pattern was seen in six similar experiments. The time course of the final phase of channel activity seemed most variable; recovery of channel activity ($I/i$) occurred over 3–10 min in different cells.

Figure 2 demonstrates that cell exposure to $NH_4Cl$ in the presence of an isotonic KCl bath (IS) results in a slow, progressive rise in $I/i$, rather than the biphasic increase in $I/i$ (phases *A* and *B*) seen with an ES bath. Correspondingly, $I/i$ falls monotonically on washout of $NH_4Cl$, rather than displaying the undershoot and subsequent recovery (phases *C* and *D*) seen with the ES bath. Exposure of the cell to isotonic KCl should abolish the membrane potential and, hence, the electrical driving force for passive $NH_4^+$ entry into the cell. Therefore, this altered time course of $I/i$ is consistent with the reduction in $NH_4^+$ entry, leaving the alkalinizing action of $NH_3$ entry unopposed.

Figures 3 and 4 demonstrate that, at the time of removal of $NH_4Cl$ from an ES bath, addition of a $Na_o^+/H_i^+$ exchange blocker, amiloride (200 $\mu$M), or isosomotic substitution of N-methyl-glucamine · HCl for NaCl (at constant $pH_o$) results in a near abolition or, at least, very profound slowing of the recovery of $I/i$ (phase D). Phase D returns after

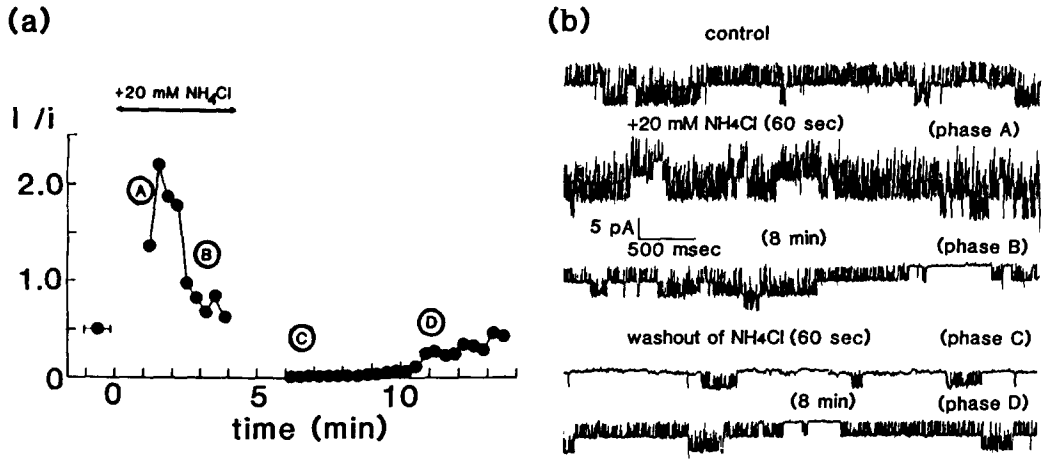



**Fig. 1.** Time course of activity of the $K^+$(ATP) channel in the cell-attached patch in response to addition and removal of $NH_4Cl$ in the presence of an ES bath. (IS pipette, ES ± $NH_4Cl$ bath, $V_c$ = 0 mV). (*a*) Each point in the time course represents a 20-sec average. (*b*) Representative traces of each of the phases. Note that the amplitude of the $K^+$(ATP) channel current is decreased by nearly twofold during $NH_4Cl$ exposure and recovers slowly after $NH_4Cl$ washout

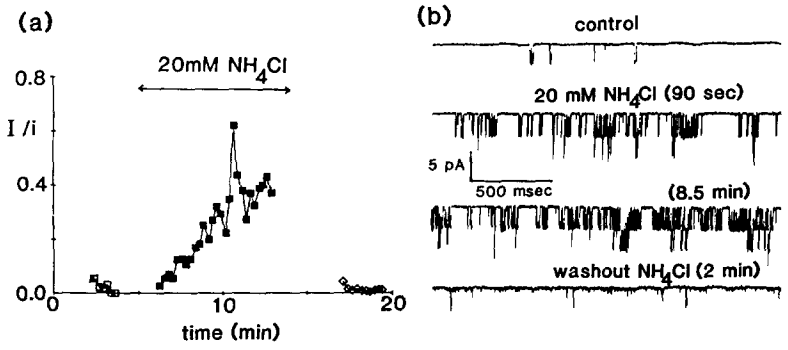



**Fig. 2.** Response of the $K^+$(ATP) channel to addition and removal of $NH_4Cl$ in the presence of an IS bath. (Cell-attached, IS pipette). As compared with Fig. 1, note that in the IS bath phases *B* and *C* were nearly abolished. (*a*) Points in the time course are 10-sec averages. (*b*) Representative traces are given ($V_c$ = −60 mV)

washout of amiloride or readmission of NaCl. As addition of amiloride or replacement of $Na^+$ with N-methylglucamine$^+$ are recognized maneuvers for blocking recovery from cell acidification; these results are also consistent with the alteration of $K^+$(ATP) channel activity by changing $pH_i$. Addition of amiloride to a normal ES bath results in a reversible two- to threefold reduction in $K^+$(ATP) channel activity over 5–10 min (*see* Fig. 3*c*).

### Approaches to Examine More Direct Effects of $pH_i$ on $K^+$(ATP) Channel Gating in Cell-Attached and Inside-Out Excised Patches

The results presented above are consistent with the notion that $K^+$(ATP) channel activity is directly or indirectly gated by changes in $pH_i$. In earlier experiments, however, changes in $pH_i$, over a moderate range (pH 7.3–6.6), did not appear to consistently alter $K^+$(ATP) channel activity in the inside-out excised patch in the absence of ATP. The discrepancy between the cell-attached and excised patch results might have arisen for several reasons: (i) $\Delta pH_i$ was unable to alter ATP-channel interaction, as ATP was not present in the bath; (ii) dissociable modulators of channel pH sensitivity might have been lost during patch excision; or (iii) $\Delta pH_i$ may be exerting an indirect effect in the intact cell by altering the concentration of substrates (e.g., the ATP/ADP ratio) that gate the channel.

Figure 5 presents an approach to examining the $pH_i$ sensitivity of the $K^+$(ATP) channel in the cell-attached patch. Here, the cell, "patched" with an ES-filled pipette, was bathed in IS containing 5 μg/ml nigericin. This protonophore serves as a plasma membrane $K^+/H^+$ exchanger; in the face of a small or absent $K^+$ gradient, it can be used to equilibrate $pH_i$ with ambient $pH_o$. By a similar mechanism, it reduces or collapses the $H^+$ gradient across mito-

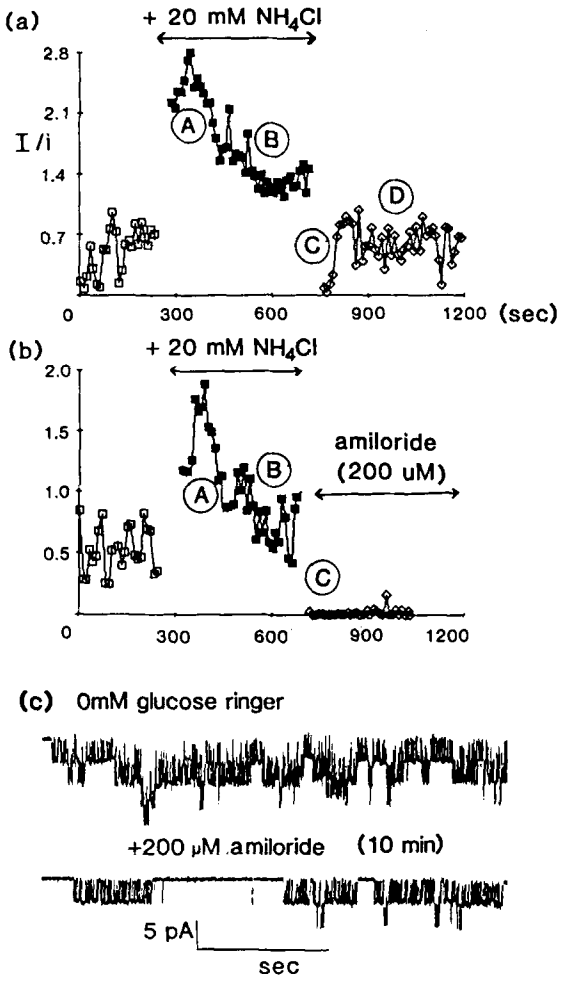



**Fig. 3.** Response of the $K^+$(ATP) channel to addition and removal of $NH_4Cl$ is altered by the presence of amiloride. (ES bath, IS pipette, cell-attached patch held at $V_c$ = 0 mV). Each point represents a 10-sec average of channel activity. (*a*) Control experiment demonstrating the normal quadriphasic response to addition and washout of $NH_4Cl$. (*b*) Subsequent experiment on the same patch showing that addition of 200 $\mu$M amiloride blocks the recovery from cell acidification (phase *D*) presumably by blocking $Na_o^+/H_i^+$ exchange. (*c*) Traces of channel currents indicating a reduction in *I/i* caused by addition of amiloride (200 $\mu$M) to the ES bath

chondrial inner membranes and uncouples mitochondrial oxidative phosphorylation. Addition of nigericin increased *I/i* to a new steady-state level over several minutes (*see* inset). This effect resembles those produced by the protonophore uncoupler, dinitrophenol, or the cytochrome $a_3$ inhibitor, sodium azide, and might be expected to result from a reduction in cytosolic ATP levels. When $pH_o$ was varied over the range 7.9 to 6.25 in the presence of nigericin, by exchanging the bath with IS solutions variously buffered by HEPES or PIPES titrated to different pH's, *I/i* decreased monotonically with decreasing $pH_o$. Little variation in *I/i* with $pH_o$ was seen in the absence of nigericin when $pH_o$ was varied over a similar range in physiological saline (ES) (*see* Fig. 5*b*) or in the presence of 3 mM sodium azide. Exchanging PIPES buffer for HEPES buffer at $pH_o$ of 6.9 or 7.3 produced no detectable effect on *I/i* (three experiments). These data support the hypothesis that the activity of the $K^+$(ATP) channel is substantially influenced by changes in $pH_i$.

Figure 6 re-examines the effects of varying $pH_i$ on $K^+$(ATP) channel activity in the inside-out excised patch. In the two experiments depicted here, $pH_i$ sensitivity was determined in the presence and absence of small concentrations of ATP. The concentrations of ATP chosen for these experiments were sufficient to maintain channel activity at steady-state values (i.e., prevent time-dependent "rundown" of channel activity), while reducing mean channel activity at least two- to threefold below that recorded in the absence of ATP. From these two complete experiments performed over a wide range of $pH_i$ and five less complete ones, it was apparent that, in the absence of ATP, changes in $pH_i$ had little consistent effect on $K^+$(ATP) channel activity until $pH_i$ was reduced to below pH 6.5, where channels abruptly closed. On the contrary, in the presence of ATP, *I/i* decreased smoothly with decreasing $pH_i$. These results suggest that $pH_i$ alters the interaction of ATP with the $K^+$(ATP) channel over a wide range of $pH_i$.

## Lack of Effect of Alteration of $pH_i$ on other $K^+$ Channels in Cell-Attached B-Cell Patches

In several experiments, such as that depicted in Fig. 7, we examined the effect of $NH_4Cl$ exposure on the gating of a voltage- and $Ca^{2+}$-activated $K^+$, or $K^+(Ca^{2+})$ channel. In inside-out excised patches, these channels have a maximum slope conductance of 200–250 pS with symmetric IS (pipette and bath) and ~100 pS with ES in the pipette and IS in the bath. Their activity maximally increases *e*-fold per 10–12 mV incremental depolarization, and they are maximally $Ca^{2+}$ sensitive at their cytoplasmic surface at a range of free [$Ca^{2+}$] between 0.1 and 5 $\mu$M. These channels are seen to open at high depolarizing voltages in the cell-attached patch (*see* Cook, Ikeuchi & Fujimoto, 1984; Findlay, Dunne & Petersen, 1985; Tabcharani & Misler, 1989). Gating of these channels by pH has previously been considered as the source of cell depolarization induced by weak acids and cell hyperpolarization induced by

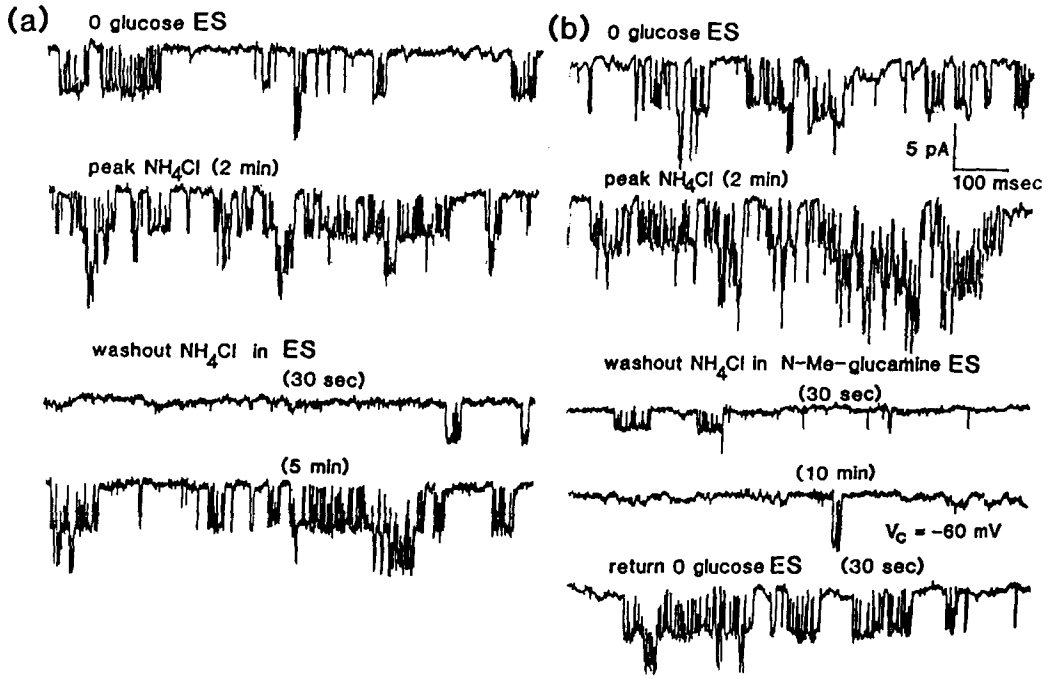



**Fig. 4.** The recovery in $I/i$ of the $K^+$(ATP) channel from acidification (phase *D*) can also be blocked by $Na_o^+$ removal. (Cell-attached, IS pipette, $V_c = 0$ mV except where indicated). (*a*) Traces from a control experiment with normal ES bath. (*b*) Traces from a subsequent experiment on the same patch with N-methyl-glucamine · HCl (N-Me-glucamine) replacing the NaCl in the ES bath at the time of washout of $NH_4Cl$. The reduction in single channel amplitude with acidification is believed to be due to membrane depolarization, because *I-V* curves under such conditions show no reduction in maximum slope conductance

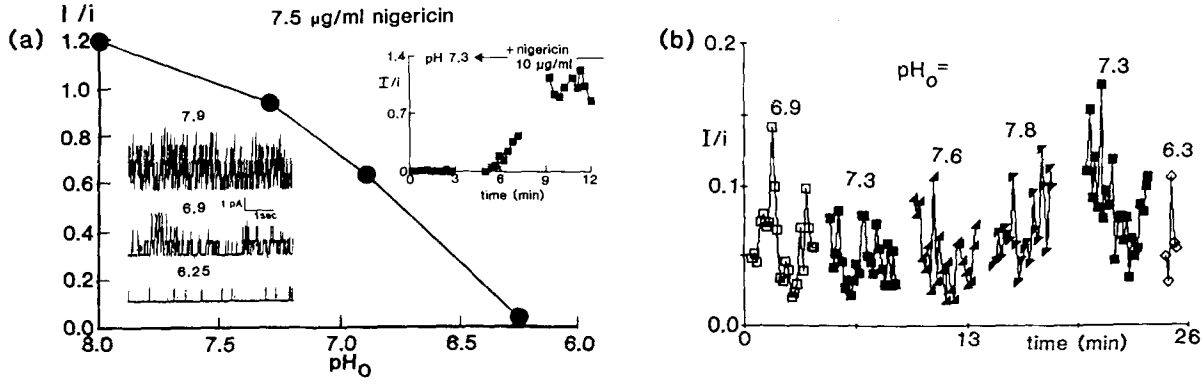



**Fig. 5.** $pH_o$ affects the activity of the $K^+$(ATP) channel in the presence of the protonophore nigericin. (*a*) Representative cell-attached patch experiment (ES pipette, IS bath) demonstrating that reducing $pH_o$ decreases $I/i$ of the $K^+$(ATP) channel in the presence of 7.5 µg/ml nigericin (each point is a 1–3 min average). *Left insert:* sample traces. *Right insert:* time course of initial increase in $I/i$ following addition of nigericin. (*b*) Lack of effect of $pH_o$ on $K^+$(ATP) channel activity in the absence of nigericin (each point is a 10-sec average)

weak bases (Rosario & Rojas, 1986*b*). $K^+(Ca^{2+})$ channels in inside-out excised patches from neonatal mouse islet cells have been reported to be activated by elevated $pH_i$ and suppressed by reduced $pH_i$ over the range of pH 8.0 to 6.0 (Cook et al., 1984). In the experiment shown here, which was typical of a series of three, the activity of the $K^+(Ca^{2+})$ channel was determined as a function of membrane clamping voltage ($V_c$) immediately before and again 10 min after addition of 20 mM $NH_4Cl$ to an IS bath. At the later time, mean activity of $K^+$(ATP) had reached a new and higher steady

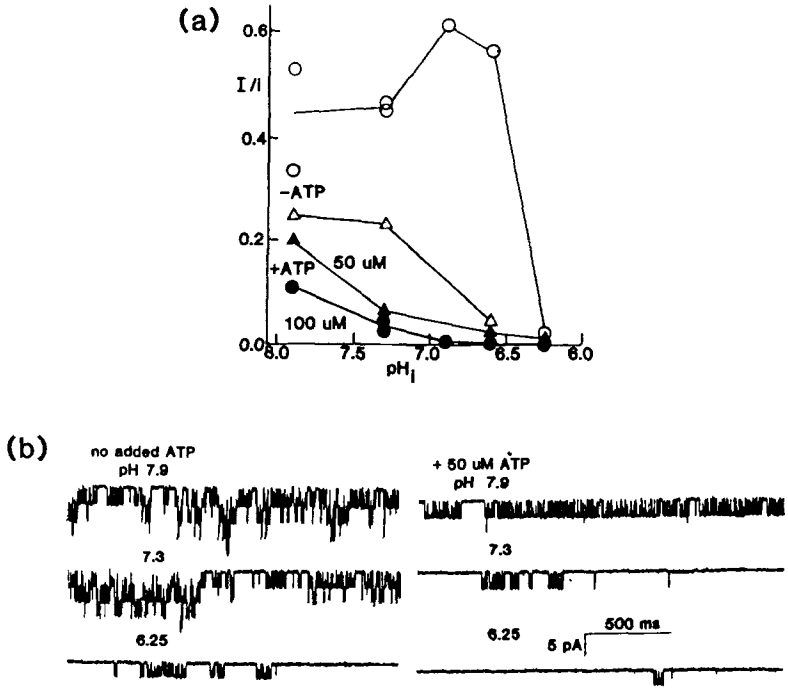



**Fig. 6.** Effect of $pH_i$ on $K^+$(ATP) channel activity in the inside-out excised patch. (*a*) Titration curve of $K^+$(ATP) activity from two experiments, demonstrating that alkalinization above physiological pH acts by disinhibiting ATP-induced closure. Open circles: 0 ATP; filled circles: 100 $\mu$M ATP; open triangles: 0 ATP; closed triangles: 50 $\mu$M ATP. Each point is a 2 min average. (*b*) Representative traces from the experiment symbolized by triangles in (*a*)

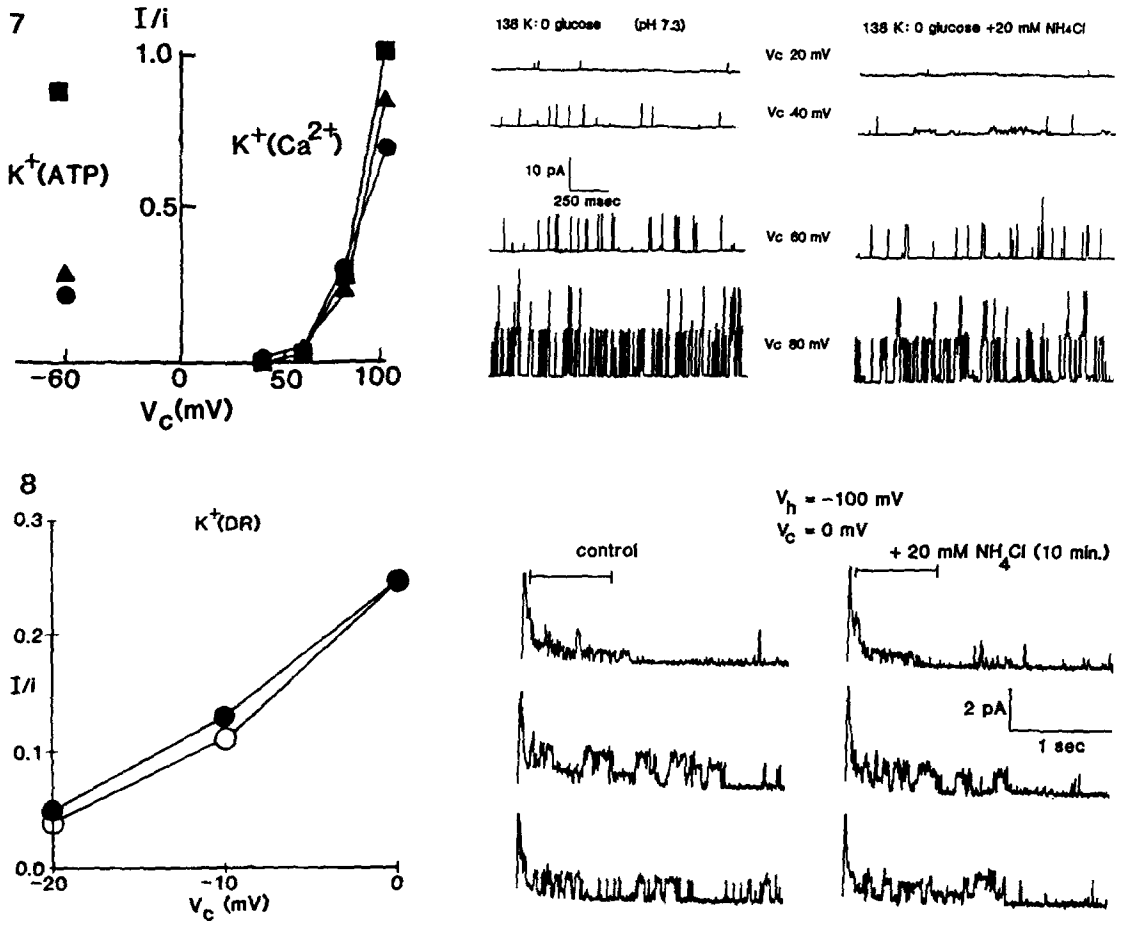
state, suggesting that $pH_i$ had stabilized at a more alkaline level. $K^+(Ca^{2+})$ channel, however, was not altered.

Another pair of voltage-dependent $K^+$ channels, often seen to open in response to membrane depolarization in many cell-attached patches, also appears to be insensitive to elevated $pH_i$. These "delayed rectifier" type $K^+$ channels (*see* Rorsman & Trube, 1986; Misler et al., 1988; Zuenkler, Trube & Ohno-Shosaku, 1988) have conductances of 5–7 and 15–17 pS (ES pipette). They are activated by depolarization >20–30 mV from rest, where they display an *e*-fold increase in initial activity with each 7–8 mV incremental depolarization and then inactivate over seconds. Figure 8 demonstrates the lack of effect on the activity of the 15-pS channel in a cell-attached patch after application of $NH_4Cl$ to an IS bath containing 10 mM glucose.

## Discussion

We have further examined the effects of a bath-applied, membrane-permeant weak base on the activity of $K^+$(ATP) channels in cell-attached patches of rat and human pancreatic islet B cells. This maneuver is known to reduce metabolite-induced electrical activity and insulin secretion by B cells. A variety of evidence on the metabolic gating, selectivity and pharmacology of the $K^+$(ATP) channel, coupled with its being the predominant $K^+$ channel open at rest, strongly suggests that it is the major contributor to resting $K^+$ permeability (*see*, e.g., Misler et al., 1987). We now present evidence that the time course of $K^+$(ATP) channel activity in rat islet B cells, during exposure to and washout of $NH_4Cl$, is consistent with the channel's being directly or indirectly gated by changes in intracellular pH.

In B cells exposed to physiological saline, $K^+$(ATP) channel activity (i) increases over 60–90 sec after application of 20 mM $NH_4Cl$; (ii) recovers over several minutes of continuous exposure; (iii) rapidly undershoots the baseline values on washout of $NH_4Cl$, and (iv) finally recovers to baseline values over a variable time course. The time course of these events resembles the phases of changes in internal pH measured in many cells during exposure and washout of $NH_4Cl$, namely, (i) immediate alkalinization, (ii) plateau phase acidification, (iii) washout acidification and (iv) recovery alkalinization from acidification which restores baseline pH. Application of $NH_4Cl$ in a near isotonic KCL solution, which slows passive entry of $NH_4^+$ and prevents plateau acidification, blocks the slow reduction in $K^+$(ATP) channel activity during prolonged $NH_4Cl$ exposure. Blockade of $Na_o^+/H_i^+$ exchange by addition of amiloride or substitution of N-methylglucamine$^+$ for $Na_o^+$ during $NH_4Cl$ washout, maneuvers which block recovery alkalinization in most cells, greatly slow the recovery of channel activity after $NH_4Cl$ washout. The role of $Cl^-/HCO_3^-$ ex-




**Figs. 7 and 8.** Lack of effect of $pH_i$ on two voltage-gated channels in pancreatic B cells. In each case, the cells were bathed in IS containing 10 mM glucose. This concentration of glucose was sufficient to close $K^+$(ATP) channels. $NH_4Cl$ was then added to the IS bath, which should result in a prolonged alkalinization (*see* Fig. 2 and text). The IS bath also clamps the cell's resting potential to near zero, so the $V_c$ always represents the true potential across the patch of membrane. (Cell-attached patches.) **Fig. 7.** *Left:* Tabulated voltage-dependence of the activity of the "maxi-$K^+$" $Ca^{2+}$-activated $K^+$ channel before (triangles), during exposure to (squares), and again after washout of (circles) 15 mM $NH_4Cl$. (IS pipette) Each point is the average current recorded for at least 30 sec at the indicated potential. Right: Sample traces obtained at a range of $V_c$'s. **Fig. 8.** *Left:* Tabulated voltage dependence of the activity of the 15-pS "delayed rectifier" type $K^+$ channel before (open circles) and 10 min after (filled circles) addition of 20 mM $NH_4Cl$ to IS bath. (ES pipette) Each point is the average number of channels open measured between 64–864 sec after 10 depolarizing pulses from −90 mV to the indicated potentials. *Right:* Sample traces obtained at $V_c = 0$ mV. Section of record included in analysis shown on left is indicated by time bracket in panel on right. Note presence of small outward currents corresponding to 5–7 pS $K^+$(DR) channels. Their activity is likewise unaffected by the test maneuver

changer in pH restoration was not evaluated, as we used $HCO_3^-$-free buffers. In addition, we now demonstrate that, in the presence of $ATP_i$, $K^+$(ATP) channel activity in inside-out patches changes monotonically with bath pH over the range of 7.9 to 6.2, with increasing pH increasing mean channel activity ($I/i$) over that range. Under conditions where bath application of $NH_4Cl$ increases $I/i$ of the $K^+$(ATP) channel many-fold, the activities of the large conductance voltage-dependent, $Ca^{2+}$-activated $K^+$ (or $K^+(Ca^{2+})$) channels and delayed rectifier-type $K^+$ (or $K^+$(DR)) channels are unaffected.

While we have concentrated on the time course of effects of cell loading with a weak base ($NH_3$) on $K^+$(ATP) channel gating, cell loading with a membrane-permeant weak acid should predictably alter $K^+$(ATP) channel activity as well. Cell exposure to the salt of a weak organic acid ($C^+A^-$) initially results in diffusional entry of the uncharged weak acid ($AH^o$) with prompt cellular acidification. The subsequent time course of $pH_i$ recovery, or even further slow acidification, depends on the balance between the operations of (i) an acid extrusion system, which tends to raise $pH_i$, and (ii) diffusional

efflux system for $A^-$, which tends to reduce $pH_i$. Washout of $C^+A^-$ results in a variable time course of recovery of $pH_i$, which may include significant overshoot, depending on the degree of $pH_i$ recovery during acid loading. In experiments in which we continuously monitored $K^+$(ATP) channel activity in resting rat B cells or $NaN_3$-treated RINm5F insulinoma cells, we noted an abrupt, immediate drop on exposure to 20 mM sodium propionate. In most B cells, $K^+$(ATP) activity rapidly returned to normal; in others, it remained depressed for minutes. In two experiments where $K^+$(ATP) activity rapidly recovered during sodium propionate exposure, a second application of propionate in the presence of 200 $\mu$M amiloride resulted in slowed or absent recovery. Overshoot of $I/i$ on washout was variably seen; it was most often in cells that showed recovery of $I/i$ during propionate exposure. Often, in these cases, the overshoot was quite prolonged; however, channel activity could then be reduced by addition of glucose (Gillis et al., 1988). Hence, qualitatively, these time courses seemed related to changes in $pH_i$, though not as clearly as with $NH_4Cl$. The membrane permeant weak acid $CO_2$ is present in the physiological buffer pair $CO_2/HCO_3$. Recent evidence suggests that replacement of HEPES buffer with $CO_2/HCO_3$, which might result in at least transient acidification, produced B-cell depolarization and closure of $K^+$(ATP) channels (Carroll et al., 1988).

In this series of experiments, we had hoped (i) to examine the range of change in $K^+$(ATP) channel activity during $NH_4Cl$ exposure and washout, and then (ii) to attempt to estimate the range of $pH_i$ achieved by calibrating the $pH_i$ sensitivity of that cell-attached patch with the alteration of $pH_i$ in the presence of nigericin. Unfortunately, to date, no patch has survived a full range of maneuvers. A more definitive approach may be to perform a set of companion experiments following the course of $pH_i$ spectrofluorometrically in cells loaded with a membrane-permeant carboxyfluorescein derivative (e.g., BCECF), which is directly sensitive to $pH_i$. Judging from the pH response of a host of other cells to similar maneuvers, $pH_i$ might rise as high as 7.6–7.8 with application of $NH_4Cl$ and fall as low as 6.5 with washout.

An effect which we cannot as yet explain is the slow reduction in $K^+$(ATP) channel amplitude during prolonged exposure of the cell to $NH_4Cl$ in a modified extracellular solution. In cell-attached patches formed with near isotonic KCl pipettes, single channel conductance measured from inward currents (IS pipette and ES bath) is not affected by this maneuver, but a sizeable shift in the reversal (or zero current) potential of the channel is seen (often as much as 30 mV). This suggests a steady-state cell depolarization. The $K^+$(ATP) channel may be somewhat permeable to $NH_4Cl$ in that, at rest, the B-cell membrane is estimated to be roughly 1/3 as permeable to $NH_4^+$ as to $K^+$ (Rosario & Rojas, 1986*a*). Assuming (i) that $NH_4^+$ acts as a permeant blocker of the $K^+$(ATP) channel from the cytoplasmic surface of the membrane, as it does with the $K^+(Ca^{2+})$ channel (*see* Tabcharani & Misler, 1989), and (ii) the $K^+$(ATP) channel of the B cell, like the $K^+(Ca^{2+})$ channel, behaves like a multi-ion channel where mutual repulsion between cations augments their traversal of the membrane, two predictions seem reasonable. (i) Partial replacement of $NH_4^+$ for bath $Na^+$ should decrease $E_{rev}$ of the channel. (ii) Accumulation of $NH_4^+$ intracellularly in the presence of low extracellular $K^+$ might reduce channel conductance. The net effect would be to reduce the contribution of $K^+$(ATP) channel to the resting potential. In the face of other resting conductances (e.g., a nonselective cation channel), this would result in cell depolarization.

Three major physiological questions remain unanswered by this study:

1) Do changes in fuel metabolism by the B cell alter $pH_i$ sufficiently to significantly contribute to channel gating? Unfortunately, there is no consistent set of calibrated data on $pH_i$ in the normal adult rat B cell for comparison. This situation should be remediable using BCECF-loaded cells. It is interesting to note that in situations where the opposing effects of lowering both $pH_i$ and ATP are made to compete (e.g., addition of mitochondrial inhibitors) $K^+$(ATP) channel activity rises very dramatically.

2) How does a change in $pH_i$ actually alter ATP gating of the channel? Reducing $pH_i$ 6.5–6.25 appears to block the channel even in the absence of ATP, perhaps by titrating an amino acid residue and affecting a conformational change that deforms the channel. The reduction in activity is not expected to be due to an acceleration of channel "rundown," because the effect can be at least partially reversed by returning to pH 7.3 without "refreshing" the channels in an ATP-containing solution. Increasing $pH_i$, on the other hand, modulates the gating of the channel by $ATP_i$. Such an effect could be due to a change in the affinity of the binding site for ATP or a shift in the ionization of ATP. Recently, it has been suggested that $ATP^{4-}$ may be the species that binds to channel (Ashcroft & Kakei, 1987; Dunne, Illot & Petersen, 1987). Given that the $pK_a$ of the phosphate group of ATP is 6.5 (Alberty, Smith & Bock, 1951), it is possible that $ATP^{3-}$ is more effective than $ATP^{4-}$ in closing the $K^+$(ATP) channel. This effect should be directly testable.

3) Does a change in intracellular pH, provoked

by bath-applied weak acids and bases, also alter $K^+$(ATP) channel gating by altering ATP production or ATP/ADP ratio? Neutral weak acids and bases should partition across the mitochondrial inner membrane where they might alter compartmental pH, thereby changing the proton-motive force which drives ATP production. Experiments to measure ATP levels under comparable conditions are in progress.

We thank Michael McDaniel (Department of Pathology, Washington University) for generously providing the rat islets used in these studies, Abed Hammoud for his participation in the performance and analysis of experiments depicted in Figs. 2 and 3*a*, Lee Falke for continuing encouragement, stimulating discussion and programming help, and Betty Ytzaina for preparing the typescript. This work was supported by the National Institutes of Health (grant no. DK-37380).

## References

Alberty, R.A., Smith, R.M., Bock, R.M. 1951. Apparent ionization constants of the adenosine phosphates and related compounds. *J. Biol. Chem.* **193:**425–434

Ashcroft, F. 1988. Adenosine 5'-triphosphate-sensitive potassium channels. *Annu. Rev. Neurosci.* **11:**97–118

Ashcroft, F.M., Kakei, M. 1987. Effects of external $Mg^{2+}$ on ATP-sensitive K channels in isolated rat pancreatic B cells. *J. Physiol. (London)* **390:**72P

Boron, W.F. 1985. Control of intracellular pH. *In:* The Kidney: Physiology and Pathophysiology. D.W. Seldin and G. Giebisch, editors. pp. 1417–1439. Raven, New York

Carroll, P.B., Li, M.X., Rojas, E., Atwater, I. 1988. The ATP-sensitive $K^+$ channel in pancreatic B cells is inhibited in physiological bicarbonate buffer. *FEBS Lett.* **234:**208–212

Cook, D.L., Hales, N. 1984. Intracellular ATP directly blocks $K^+$ channels in pancreatic B cells. *Nature (London)* **311:**271–273

Cook, D.L., Ikeuchi, M., Fujimoto, W.Y. 1984. Lowering of $pH_i$ inhibits $Ca^{2+}$-activated $K^+$ channels in pancreatic B cells. *Nature (London)* **311:**269–271

Dunne, M.J., Illot, M.C., Petersen, O.H. 1987. Interaction of diazoxide, tolbutamide and $ATP^{4-}$ on nucleotide-dependent $K^+$ channels in an insulin-secreting cell line. *J. Membrane Biol.* **99:**215–224

Eddlestone, G.T., Beigelman, P.M. 1983. Pancreatic $\beta$ cell electrical activity: The role of anions and control of pH. *Am. J. Physiol.* **244:**C188–C197

Findlay, I., Dunne, M.J., Petersen, O.H. 1985. High-conductance $K^+$ channel in pancreatic islet cells can be activated and inactivated by internal calcium. *J. Membrane Biol.* **83:**169–175

Gillis, K., Misler, S. 1988. Effects of $pH_i$ on the activity of a metabolite regulated, $ATP_i$-sensitive $K^+$ channel in rat pancreatic islet cells. *J. Gen. Physiol.* **92:**6–7*a*

Gillis, K., Tabcharani, J., Hammoud, A., Misler, S. 1988. Effects of ammonium chloride and sodium propionate on the activity of a metabolite regulated $K^+$ channel in rat pancreatic islet and RIN insulinoma cells. *Biophys. J.* **53:**550*a*

Misler, S., Falke, L.C., Gillis, K., McDaniel, M.L. 1986. A metabolite-regulated potassium channel in rat pancreatic B cells. *Proc. Natl. Acad. Sci. USA* **83:**7119–7123

Misler, S., Gillis, K., Tabcharani, J., Falke, L. 1987. Gating and selectivity of a metabolite regulated $K^+$ channel in rat pancreatic islet B cells. *J. Gen. Physiol.* **90:**19*a*

Misler, S., Tabcharani, J., Gillis, K., Hammoud, A., Falke, L. 1988. Voltage gated single $K^+$ and $Ca^{2+}$ channels in rat pancreatic islet B cells. *J. Gen. Physiol.* **92:**7*a*–8*a*

Pace, C.S. 1984*a*. Role of pH as a transduction device in triggering electrical and secretory responses in islet B cells. *Fed. Proc.* **43:**2379–2384

Pace, C.S. 1984*b*. Amiloride and organic weak acids enhance the electrical response of B cells to glucose. *Mol. Physiol.* **6:**339–348

Rorsman, P., Trube, G. 1986. Calcium and delayed potassium currents in mouse pancreatic $\beta$ cell under voltage clamp conditions. *J. Physiol. (London)* **374:**531–550

Rosario, L.M., Rojas, E. 1986*a*. Potassium channel selectivity in mouse pancreatic B cells. *Am. J. Physiol.* **250:**C90–C94

Rosario, L.M., Rojas, E. 1986*b*. Modulation of $K^+$ conductance by intracellular pH in pancreatic beta cells. *FEBS Lett.* **200:**203–209

Smith, J.S., Pace, C.S. 1983. Modification of glucose induced insulin release by alteration of pH. *Diabetes* **32:**61–66

Tabcharani, J.A., Misler, S. 1989. $Ca^{2+}$ activated $K^+$ channel in rat pancreatic islet B cells: Permeation gating and blockade by cations. *Biochim. Biophys. Acta (in press)*

Zuenkler, B.J., Trube, G., Ohno-Shosaku, T. 1988. Forskolin-induced block of delayed rectifier $K^+$ channels in pancreatic $\beta$-cells is not mediated by cAMP. *Pfluegers Arch.* **411:**613–619

Received 8 November 1988; revised 4 January 1989