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A metabolite-regulated potassium channel in rat pancreatic B cells

(patch clamp/K^+ permeability/stimulus-secretion coupling/islet of Langerhans/ATP-sensitive ion channel)

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ABSTRACT **In B cells from dispersed rat islet of Langerhans we have identified an inward rectifying voltage-independent K^+ channel whose behavior parallels the metabolically regulated potassium permeability (P_K) found in tracer flux and microelectrode recording studies. In cell-attached patches of membrane, the channel is closed when any one of several substrates (glucose, mannose, leucine, or glyceraldehyde) is added to the cell's bathing solution but is reopened on addition of an appropriate metabolic inhibitor, which prevents utilization of that substrate. In inside-out excised patches, a K^+ channel with nearly identical kinetic features is closed by addition of micromolar concentrations of ATP to the "cytoplasmic" solution. The ATP sensitivity of channel activity is modified by addition of ADP, suggesting competition at a nucleotide binding site. These results suggest the presence of a metabolically regulated K^+ channel gated by intracellular concentrations of ATP or the ratio of ATP/ADP concentrations.**

A popular hypothesis for stimulus–secretion coupling in pancreatic islet B cells is: nutrient metabolism by B cells → decreased K^+ permeability (P_K) → membrane depolarization → voltage-dependent Ca^{2+} entry → insulin granule exocytosis (1, 2). Using single-channel recording techniques, several groups have identified, in cell-attached patches of pancreatic islet cells, a potassium-selective channel whose activity is reduced by raising the glucose concentration of the medium bathing the rest of the cell (3–6). Others have reported the existence in inside-out excised patches of a potassium channel of similar conductance whose opening frequency is reduced by micromolar concentrations of ATP (4–8). Here we demonstrate that both channels represent the same "metabolite-regulated" K^+ channel in two different recording configurations and describe some regulatory effects of cytoplasmic nucleotides on channel gating.

METHODS AND MATERIALS

Rat pancreatic islets, isolated by collagenase digestion of chopped pancreases of adult male Sprague–Dawley rats were dispersed into small clumps of cells by incubation with the enzyme "dispase" (9). Cells prepared in this manner increase their insulin secretion nearly 3-fold above baseline in the presence of 10 mM glucose media (10). Clumps of cells were added to 35-mm tissue culture dishes "seeded" with appropriately sized glass coverslips and were cultured for 1–10 days at 37°C in modified CMRL medium (GIBCO) containing 10% heat-inactivated fetal bovine serum, 0.5% penicillin, and 0.5% streptomycin in 5% CO_2/95% air. Individual coverslip chips were then transferred to the recording chamber containing modified physiological saline solutions at 20–23°C. The basic zero-glucose extracellular saline solution (zero-glucose ES solution) consisted of 138 mM NaCl, 5.5 mM KCl, 0.5 mM $CaCl_2$, 2 mM $MgCl_2$, and 20 mM Hepes titrated with NaOH to pH 7.35. The solution used to fill patch pipettes as well as bathe inside-out excised patches (IS solution) consisted of 138 mM KCl, 2 mM Mg^{2+}, ≈5–20 μM $CaCl_2$, and 20 mM Hepes titrated with KOH to pH 7.0. IS solutions used to bathe inside-out patches often also contained 1 mM ethylene glycol bis(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid to reduce the frequency of Ca^{2+}-activated K^+ channels. Test metabolites and pharmacological agents obtained from Sigma were freshly prepared as isosmotic solutions, titrated where necessary to pH 7.0–7.2 and substituted for NaCl or KCl in the ES or IS solutions, respectively. Bath solutions were changed by flushing the 3-ml-capacity perfusion-recording chamber with 20–25 ml of test solution.

Single-channel recording was done with conventional patch-clamping techniques (11) using 3–5 MΩ fire-polished and wax-coated pipettes filled with appropriate saline solutions in contact with Ag/AgCl electrodes; 10–40 GΩ pipette-to-membrane seals were formed by suction. The signal was amplified with a List EPC-7 patch-clamp amplifier, filtered at 0.9 or 1.8 kHz through an eight-pole Bessel filter and then displayed on an oscilloscope for direct observation. The filtered signal was digitized and recorded at 2000 or 4000 samples per sec by a data-acquisition system that simultaneously generates and records voltage-clamp pulses. The acquisition system consists of a Midax analog I/O subsystem (Data Translation, Marlboro, MA), Sbx 275 graphics hardware (Intel, Hillsboro, OR), and a Bernoulli box cartridge disk drive (Iomega, Roy, UT)—all controlled by an Intel 286–310 microprocessor (Intel, Hillsboro, OR) running an RMX-86 operating system and software written by one of us (L.C.F.). In the kinetic analysis of single-channel currents, durations of open and closed events were determined by measuring the time between half-amplitude crossing with digitized data and an interactive graphics-based analysis system. We measured the average number of open channels in a similar manner, using level crossings to determine when 1, 2, 3, and more channels were open and averaging the results for a 10- to 60-sec segment of record. To standardize nomenclature, the clamping voltage, V_c, is in all experiments the negative of the potential of the interior of the pipette with respect to a bath ground.

In the rat, B cells are ≥ 85% of all islet cells and are the largest cells in the interior of the islet (12). We patched the largest cells in the strands and clumps of cells remaining after enzymatic digestion. About 95% of these cells displayed significant action current activity in 5–25 mM glucose-containing ES solution, which subsided over minutes after changing the bath to a zero-glucose ES solution, as well as

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Abbreviations: V_c, clamping voltage; γ_s, maximum-slope conductance; E_{rev}, zero-current potential; $\langle I \rangle / i$, average number of channels open.

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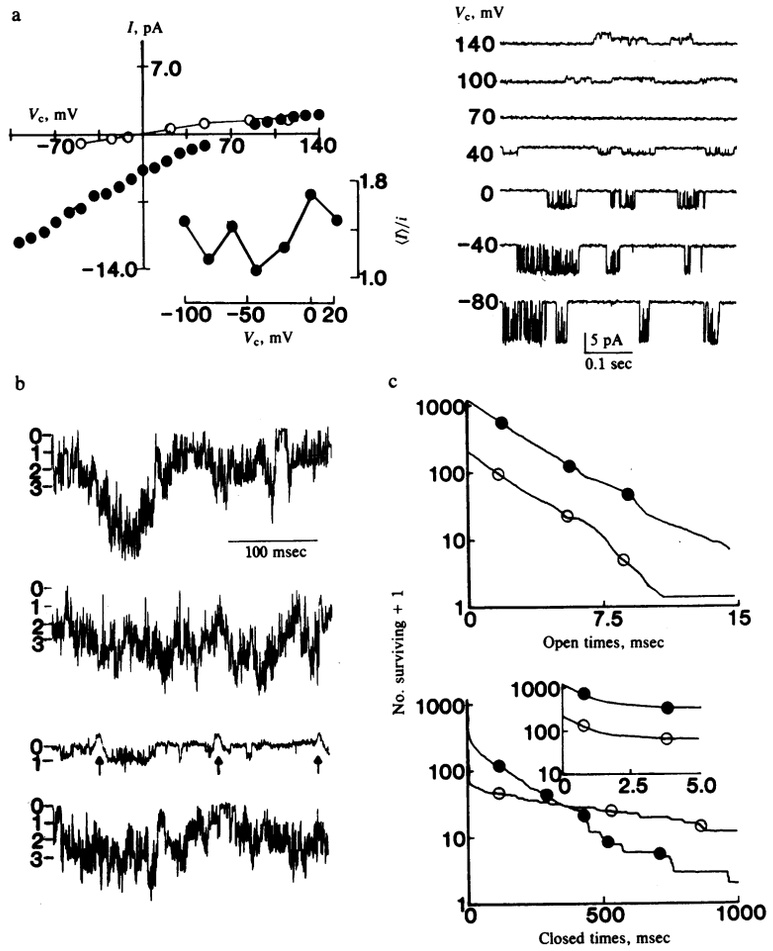

FIG. 1. (*a*) Data trace (*Right*) and current/voltage curves (*Left*) of an inwardly rectifying glucose-sensitive channel routinely seen in cell-attached B cell membrane patches. Cells were bathed in zero-glucose ES solution. ●, Pipette filled with IS solution; ○, patch pipette filled with zero-glucose ES solution. (*Inset*) Curve of average channel activity $\langle I\rangle/i$ vs. V_c in a patch containing at least four channels. (*b*) Time course of activity of population of such a channel during and after exposure of the remainder of the cell to 10 mM glucose. V_c = 0 mV. The pipette contained K^+-containing IS solution; the bath contained zero-glucose ES solution. The upper three traces were recorded 20, 85, and 105 sec after admission of 10 mM glucose; the bottom trace was recorded 140 sec after glucose washout, following 2.5 min of exposure. Individual spikes are marked with arrows. Open channel levels are indicated to the left. (*c*) Channel kinetics. Open-time survivor curves (*Upper*) and closed-time survivor curves (*Lower*) obtained both in the absence of glucose (●) and in 5 mM glucose (○). $\langle I\rangle/i$ was 0.074 in zero-glucose ES solution and 0.017 in 5 mM glucose-containing ES. While τ_{open} and $\tau_{closed,fast}$ were very similar, $\tau_{closed,\ slow}$ was 3.96-fold larger in 5 mM glucose. (*Lower Inset*) Survivor curve for short closures.

characteristic glucose-sensitive channels described below. These cells were assumed to be B cells.

RESULTS

Identification of a Glucose-Sensitive K^+ Channel in Cell-Attached Patches. In cell-attached membrane patches formed with IS-filled pipettes and the cell exposed to zero-glucose ES solution, the most consistently seen channel type at a V_c at or negative to 0 mV showed inward rectification of current, a maximum-slope conductance (γ_s) of 60–65 pS, and a zero-current potential (E_{rev}) of the $V_c \simeq +70$ mV (see Fig. 1*a*). This channel population closed within 2–5 min after perifusing the cells with 5–10 mM glucose-containing ES solution. The closure of this channel was coincident with the onset of spike activity of the cell. The channel reopened within 2–5 min after washout of glucose; this reopening was coincident with the disappearance of spike activity (Fig. 1*b*). This channel showed no significant voltage dependence, in that $\langle I\rangle/i$, the average number of channels open, changed little if at all with V_c over the range of ±100 mV (see Fig. 1*a Inset*). When patches were formed with pipettes filled with zero-glucose ES solution, which contained 5.5 mM K^+, current jumps with very similar kinetics and gating features were seen that now had a γ_s = 15 pS and an E_{rev} at V_c = 0 mV, suggesting that this channel is K^+ selective, rather than generally cation selective.

In several patches, channel activity was infrequent enough so that long time segments with nonoverlapping channel events were apparent even in zero-glucose ES. From these record segments (e.g., Fig. 1*a*), it was apparent that at a given V_c the channel activity consisted of clusters of openings to a single amplitude separated by longer pauses. Based on events from these records, open-time survivor curves were easily fit by a single time constant of decay, τ_{open}, while closed-time survivor curves were better fit by two time constants $\tau_{closed,\ fast}$ and $\tau_{closed,\ slow}$. Membrane potential had little effect on any of these parameters. Increases in extracellular glucose, which reduced average channel activity several times, increased $\tau_{closed,\ slow}$ without effecting τ_{open} or $\tau_{closed,\ fast}$ (see Fig. 1*c*), suggesting that the major effect of extracellular glucose is to lengthen the intervals between clusters of openings.§

Two other channels open at V_cs approximating the cell resting potential and having E_{rev}s at a V_c between +60 and +70 mV were seen in about half of the patches analyzed. One had a γ_s of 12 pS, and the other, 30 pS. Both channels showed inconsistent glucose sensitivity and often spontaneously disappeared.

Modulation of Gating of K^+ Channels in Cell-Attached Patches by Bath-Applied Metabolites, Metabolite Analogues, and Metabolite Inhibitors. Exposure of the remainder of the cell to glucose closed down channels in a patch of membrane unexposed to extracellular glucose. Hence, specific binding of glucose to the external surface of the channel is not crucial for channel closure. However, if a product of intracellular glucose metabolism leads to the closure of a population of channels, then the following predictions might be made. (*i*)

§Here we were not measuring single-channel event durations. However, given N independent and identical channels and segments of record with no overlapping channel openings, the ratio of $\tau_{closed,\ slow}$ in low glucose to that in high glucose in the N channel patch should equal that in the single-channel patch. The values of $\tau_{closed,\ fast}$ and τ_{open} should be equal to those of the single-channel patch, since $\tau_{closed,\ fast}$ is due to flickering.

Any other insulin secretogogue, which is used as a metabolic fuel by B cells (e.g., glyceraldehyde, leucine, or mannose) should mimic the effect of extracellular glucose in promoting channel closure. (*ii*) Specific inhibition of fuel metabolism by a glycolytic or mitochondrial inhibitor anywhere along the reaction

$$\text{Fuel} + O_2 \rightarrow CO_2 + H_2O\ (+NH_3) + \text{high energy bonds}$$

should prevent fuel-induced channel closure, as it has been shown to prevent fuel-induced membrane depolarization and insulin secretion (2, 14).

On a molar basis of comparison, channel closure was almost equivalently accomplished by D-glyceraldehyde, L-leucine, and D-glucose, with ≈2–3 mM of each roughly halving channel activity (Fig. 2). D-Mannose, another secretogogue, was about half as effective as those above, while D-galactose and 3-*O*-methylglucose, two nonmetabolized sugars, hardly altered channel opening frequency even up to 25 mM.

Fig. 3 shows the effects of representative metabolic inhibitors on metabolite-stimulated type I channel closure. Addition of 5–20 mM mannoheptulose, an inhibitor of hexokinase, reopened channels closed down by 5–10 mM glucose but not by glyceraldehyde, which enters glycolysis at the level of glyceraldehyde 3-phosphate. (Similarly, addition of 5 mM iodoacetate, a competitive inhibitor of glyceraldehyde 3-phosphate dehydrogenase, produced a reopening of channels when these channels were previously closed by glucose or glyceraldehyde but not when the channels were closed by leucine, which enters the central metabolic pathway as acetyl CoA.) Addition of sodium azide, a mitochondrial electron transport inhibitor at cytochrome a_3, reopened channels closed in the presence of glucose (or glyceraldehyde or leucine, not shown) within 1–2 min. Interestingly, average channel activity in the presence of Na^+ azide was usually severalfold higher than that seen in zero-glucose ES solution. The latter effect also occurred within 1–2 min after addition of either the protonophore mitochondrial uncoupler carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) (3 μM) or KCN (3 mM). A similar effect was seen with sodium arsenate (3 mM), although more slowly (over 5–10 min). KCN and sodium arsenate were poorly reversible.

Identification of ATP-Sensitive K^+ Channel in Inside-Out Patches and Characterization of Its Gating Function. Patches of membrane formed at the tip of pipettes filled with IS

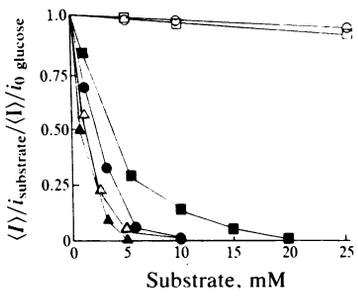

FIG. 2. Average number of channels open in the presence of various substrates as a function of substrate concentration. In each experiment, several concentrations of glucose were tested followed by several concentrations of one or more other substances. ○, 3-*O*-methylglucose; □, galactose; ■, mannose; ●, leucine; △, glucose; ▲, glyceraldehyde. The glucose curve represents an average of six experiments. Other substrates are represented by single representative curves from one of at least three experiments. Average channel activity at zero substrate ranges from 0.38 to 5.4. Measurements were done at a fixed $V_c = -60$ or -80 mV. No significant voltage dependence of channel activity was seen with any of these substances.

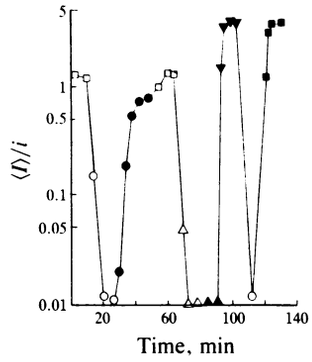

FIG. 3. Average number of channels open as a function of time in an experiment where multiple variable substrates and metabolic inhibitors were added. □, 0 mM glucose; ○, 5 mM glucose; ●, 5 mM glucose/20 mM mannoheptulose; △, 5 mM glyceraldehyde; ▲, 5 mM glyceraldehyde/20 mM mannoheptulose; ▼, 5 mM glucose/3 mM azide; ■, no glucose and 3 mM azide.

solution and then excised into IS showed a 55-pS voltage-independent outward-rectifying channel with τ_{open} and $\tau_{closed,fast}$ nearly identical to that of the 60-pS metabolite-sensitive K^+ channel recorded in cell-attached patches (see Fig. 4*a*). Replacing KCl of the IS solution with NaCl shifted E_{rev} to an extrapolated V_c of -55 to -60 mV, permitting calculation of $P_{K^+}/P_{Na^+} \approx 20$, from the Goldman–Hodgkin–Katz equation. Adding 1 mM EGTA to the IS solution or changing the pH of IS over the range 6.5–7.3 did not noticeably affect $\langle I\rangle/i$. Addition to the IS bath of micromolar concentrations of either ATP or a nonhydrolyzable analog such as adenosine 5′-[β,γ-methylene]triphosphate or 5-adenylyl imidodiphosphate reduced $\langle I\rangle/i$ with nearly equal effectiveness. Note that the major effect of ATP on channel kinetics was to increase $\tau_{closed,\ slow}$ and that the change in this parameter was sufficient to account for the change in $\langle I\rangle/i$ (Fig. 4*c*).

In 10 experiments, the concentration of ATP or ATP analogs required to reduce channel activity by half ranged from 20 to 200 μM. ADP closed down channel activity, but less effectively than ATP by a factor of ≈10–40. The effects of 1–2 mM AMP or adenosine were hardly detectable. Addition of up to 25 mM glucose, glucose 6-phosphate, glyceraldehyde, or 5 mM sodium azide also had no effect.

In about half of the inside-out excised patches examined, another effect of ATP was noted (for example, see Fig. 5). When a patch was excised into IS solution, channel activity was not vigorous. Upon addition of 0.2–1.0 mM ATP, channel activity further declined to nearly undetectable levels. On washout of ATP, the channel activity was severalfold higher than immediately after excision. This "refreshment effect," which waned over the next 10–20 min in the absence of ATP, was seen over several cycles of ATP introduction and washout. No "refreshment effect" was seen after exposure of the patch to ATP in the absence of Mg^{2+}; to adenosine 5′-[β,γ-methylene]triphosphate or 5′-adenylyl imidodiphosphate, even in the presence of Mg^{2+}; or to ADP or AMP in the presence of Mg^{2+}. In three experiments, $\langle I\rangle/i$ of the "refreshed" excised patch was ≥ 50% of $\langle I\rangle/i$ seen before excision in the cell-attached patch after exposure of the cell to sodium azide, suggesting that channels could be maintained after excision.

The ability of a variety of metabolites and metabolite/metabolic inhibitor pairs to predictably alter channel activity makes intracellular ATP concentration a good candidate for physiological channel gating. The exquisite ATP sensitivity of the channel suggests that some endogenous inhibitor of ATP action was lost in our excision experiments. Cells often show reciprocal changes in ATP and ADP

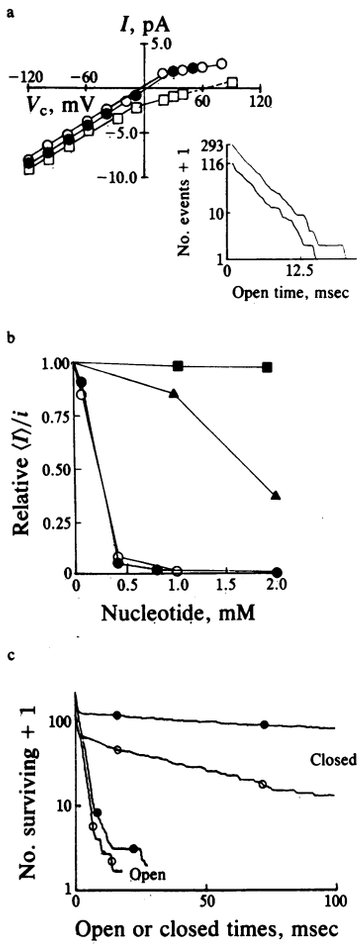

FIG. 4. (*a*) Current/voltage curves for inward-rectifying glucose-sensitive channel in cell-attached patch with cell bathed in IS solution (●), after inside-out excision into IS (○) and again after flushing the bath with a modified ES solution with no added Ca^{2+} (□). The pipette contained IS solution with 20 μM Ca^{2+}. (*Inset*) Open-channel survivor curves for channels in cell-attached (lower curve) and excised (upper curve) patch configurations. (*b*) Average number of channels open in a single inside-out excised patch as a function of "cytoplasmic" nucleotide concentration applied to the cytoplasmic bath. This patch showed little of the ATP refreshment effect described in the text and Fig. 5. Pipette solution and bath contained symmetric IS solution buffered at pH 7.0. ATP (●), adenosine 5′-[β,γ-methylene]triphosphate (○), and ADP (▲) data are from a single patch; AMP data (■) are from another patch with similar ATP calibration. (*c*) Open- and closed-time survivor curves of channels in inside-out excised patches where "cytoplasmic" bathing solution (i.e., IS solution) contained no added ATP (○) or 50 μM ATP (●). $\langle I\rangle/i$ was 0.08 in IS with no ATP and 0.017 in IS with 50 μM ATP. Neither $\tau_{closed,\ fast}$ nor τ_{open} were altered by addition of ATP, but $\tau_{closed,\ slow}$ increased from 56.7 to 255 msec.

concentrations after various metabolic maneuvers. Since ADP itself has weak channel-closing activity, the interaction of the weak partial agonist, ADP, with the strong agonist, ATP, might shift the ATP sensitivity of the channel into the physiological range. Fig. 6*b* shows a representative experiment of a total of five experiments in which progressive addition of ADP (80–1200 μM) in the presence of fixed ATP (400 μM) resulted in channel reopening. Fig. 6*a* shows a patch where nearly 1 mM ATP was needed to reduce $\langle I\rangle/i$ to <0.001, in the absence of ADP. Reversing the "cytoplasmic" ATP/ADP ratio from 2.5 mM ATP/0.5 mM ADP to 0.5 mM ATP/2.5 mM ADP, resulted in a 20-fold increase in channel activity $\langle I\rangle/i$. This change in $\langle I\rangle/i$ is comparable to that seen

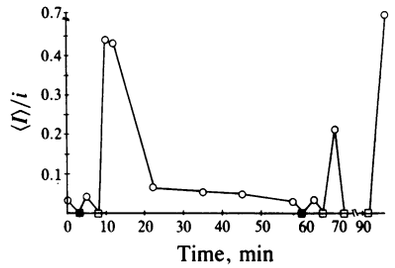

FIG. 5. Time course of an average number of channels open in a single inside-out excised patch during exposure to solutions containing no ATP (○), 200 μM adenosine 5′-[β,γ-methylene]triphosphate (■), and 200 μM ATP (□). Time 0 is represented immediately after excision. The pipette solution and bath were both IS solution buffered to pH 7.2.

in cell-attached patches on adding 3 mM azide to an ES solution supplemented with 5 mM glucose.

DISCUSSION

In this study we demonstrate an inward-rectifying potassium channel present in cell-attached patches of pancreatic beta-cell plasma membranes which is open at the resting potential and physiological extracellular K^+ concentrations. Several features make it a likely candidate for the channel type whose closure underlies the metabolite-stimulated decrease in plasma membrane K^+ permeability. These are the rapid closure of this channel on exposure of the cell to a variety of metabolic fuels (including glucose, leucine, and glyceraldehyde); the reopening of the channel on addition of an appropriate metabolic inhibitor in the face of the fuel substrate (e.g., mannoheptulose, iodoacetic acid, or sodium azide in the face of glucose); and the abundance of this channel (up to 8 channels per ≈1-μm membrane patch). The resultant plasma membrane depolarization would then set off

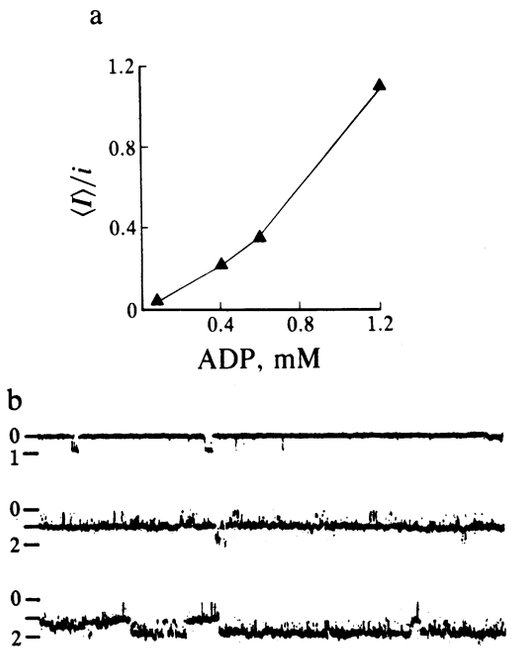

FIG. 6. (*a*) Effect of ADP concentration on $\langle I\rangle/i$ of ATP-sensitive channels in inside-out excised patch; ATP was fixed at 400 μM. V_c = −60 mV. (*b*) Effect on $\langle I\rangle/i$ of altering ATP/ADP concentration ratio at constant additive concentrations of ATP and ADP. Traces: top, 2.5 mM ATP/0.5 mM ADP; middle, 1.5 mM ATP/1.5 mM ADP; bottom, 0.5 mM ATP/2.5 mM ADP. V_c = −60 mV. Open channel levels are indicated to the left.

cycles of electrical activity and Ca_0^{2+}-dependent insulin release. We also demonstrate that after excision of the membrane patch there is an inward-rectifying K^+ channel with nearly identical conductance and kinetic features whose average activity is inhibited by the application to its cytoplasmic surface of micromolar concentrations of ATP and millimolar concentrations of ADP but not by application of near micromolar concentrations of Ca^{2+} or H^+ or millimolar concentrations of various substrates (e.g., glucose), phosphorylated metabolic intermediates (e.g., glucose 6-phosphate), or AMP. Application of either the extracellular metabolite to the intact cell or ATP to the cytoplasmic surface of the excised patch gates these channels in an identical manner (i.e., by lengthening the intervals between bursts of channel openings). Thus, our results confirm and extend recently reported results (3–7) and further suggest that the substrate-sensitive K^+ channels in cell-attached patches and the ATP-sensitive K^+ channels in inside-out excised patches are probably the same "metabolically-regulated" K^+ channel. They further demonstrate that these ATP-sensitive channels are similar to those in cardiac myocytes (13).

Our experiments further point out two novel and interesting ways in which the activity of the ATP-sensitive channels may be modulated. (*i*) ATP appears to have a dual effect on the channel: ATP gating of channel activity, which is mimicked by a nonhydrolyzable ATP analog, and ATP "refreshment" of channel activity, which is not mimicked by a nonhydrolyzable ATP analog and does not occur in the absence of Mg^{2+}. This raises the question of whether ATP might both bind to a specific receptor site on a channel and serve as a substrate for channel phosphorylation that helps to maintain the channel in a "gateable" state. (*ii*) Addition of ADP in micromolar to millimolar concentrations significantly reduces the inhibitory effect of ATP on channel activity, so that near millimolar concentrations of ATP are required to produce the same inhibition of $\langle I \rangle / i$ as that previously produced by micromolar concentrations of ATP alone.

An appealing possibility is that gating of the metabolically regulated K^+ channel might actually be controlled by the relative cytoplasmic ATP/ADP concentrations. An increase in intracellular ATP concentration or the ATP/ADP concentration ratio resulting from exposure to metabolites might close channels, while a decrease in ATP concentration or ATP/ADP concentration ratio from exposure to metabolite inhibitors might reopen channels. ATP is the cell's major energy currency; its intracellular concentration rapidly and significantly increases with metabolite utilization and decreases with exposure to metabolic inhibitors (12). Average channel activity changes most noticeably over a range of metabolite and metabolic inhibitor concentrations, which appear to have maximal effects on intracellular ATP concentrations (e.g., glucose over the range of 0–3 mM). There is some evidence for reciprocal changes in intracellular ADP concentrations during similar maneuvers.

Currently, the major obstacles to testing quantitatively the latter speculation are: (*i*) lack of knowledge of the free ATP and ADP concentrations in the region of the plasma membrane in beta cells under bath metabolite concentrations similar to those used in our experiments and (*ii*) lack of understanding of whether important channel-gating modulators, perhaps loosely attached to the channel protein, are lost on patch excision and perifusion. Average ATP concentrations, though quantitatively similar from spectrofluorometric or ^{31}P NMR measurements, do not reflect organelle compartmentalization. Average ADP concentrations measured spectrofluorometrically and by ^{31}P NMR are grossly discordant (15), ADP being barely detectable by the latter method. Additionally ATP-sensitive channels in close proximity to active membrane pump ATPases may actually see significantly less ATP and more ADP than the average cytoplasmic concentration.

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