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Characterization of ATP-Sensitive Potassium Channels Functionally Expressed in Pituitary GH_3 Cells

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Abstract. ATP-sensitive K^+ (K_{ATP}) channels have been characterized in pituitary GH_3 cells with the aid of the patch-clamp technique. In the cell-attached configuration, the presence of diazoxide (100 μM) revealed the presence of glibenclamide-sensitive K_{ATP} channel exhibiting a unitary conductance of 74 pS. Metabolic inhibition induced by 2,4-dinitrophenol (1 mM) or sodium cyanide (300 μM) increased K_{ATP} channel activity, while nicorandil (100 μM) had no effect on it. In the inside-out configuration, Mg-ATP applied intracellularly suppressed the activity of K_{ATP} channels in a concentration-dependent manner with an IC_{50} value of 30 μM. The activation of phospholipase A_2 caused by mellitin (1 μM) was found to enhance K_{ATP} channel activity and further application of aristolochic acid (30 μM) reduced the mellitin-induced increase in channel activity. The challenging of cells with 4,4′-dithiodipyridine (100 μM) also induced K_{ATP} channel activity. Diazoxide, mellitin and 4,4′-dithiodipyridine activated the K_{ATP} channels that exhibited similar channel-opening kinetics. In addition, under current-clamp conditions, the application of diazoxide (100 μM) hyperpolarized the membrane potential and reduced the firing rate of spontaneous action potentials. The present study clearly indicates that K_{ATP} channels similar to those seen in pancreatic β cells are functionally expressed in GH_3 cells. In addition to the presence of Ca^{2+}-activated K^+ channels, K_{ATP} channels found in these cells could thus play an important role in controlling hormonal release by regulating the membrane potential.

Key words: GH_3 cells — ATP-sensitive K^+ channels — Diazoxide — Glibenclamide — Phospholipase A_2 — 4,4′-dithiodipyridine

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Introduction

ATP-sensitive K^+ (K_{ATP}) channels are ubiquitously distributed in a variety of cell types, including pancreatic β cells, cardiac myocytes, skeletal muscle cells, neurons and pituitary cells (Bernardi et al., 1993; Inagaki et al., 1995, Ashcroft & Gribble, 1998; Liu, Seino & Kirchgessner, 1999). These channels are well known to provide a link between the electrical activity of cell membrane and cellular metabolism (Ashcroft & Gribble, 1998). Recently, it has been demonstrated that the K_{ATP} channels are heteromers composed of sulphonylurea receptors (SURs) and Kir6.X subunits (Babenko, Aguilar-Bryan & Bryan, 1998; Seino et al., 1999). In addition to their regulation by intracellular ATP, various pharmacological agents can modulate K_{ATP} channel activity. For example, K^+ channel openers such as diazoxide can enhance channel activity while sulfonylureas, such as glibenclamide, can interact with the SUR1 subunit of the K_{ATP} channel in pancreatic β cells and stimulate insulin release (Ashcroft & Gribble, 1998). Previous studies have shown that adenohypophysis cells exhibit K_{ATP} channel activity and that the secretion of growth hormone derived from pituitary cells of diabetic rats was affected (Bernardi et al., 1993). A sulfonylurea receptor has also been cloned from pituitary microadenoma that secretes growth hormone (Zhu et al., 1998*b*). However, to our knowledge, electrical properties of K_{ATP} channels in pituitary cells appear to be rarely described as compared with those of Ca^{2+}-activated K^+ channels (Wu, Li & Chiang, 2000).

How the change in the activity of phospholipase A_2 causes any effects on K^+ channel activity remains controversial. For example, the increase of phospholipase A_2 activity by mellitin was found to suppress the opening probability of K_{ATP} channels in insulin-secreting HIT cells (Eddlestone, 1995). Mellitin is a 26-amino acid

polypeptide contained in bee venom and known to be an activator of phospholipase A_2 (Clark et al., 1991). However, in GH_3 cells, mellitin was found to increase the activity of large-conductance Ca^{2+}-activated K^+ channels (Denson, Worrell & Eaton, 1996). Moreover, several reports have demonstrated that the activation of phospholipase A_2 can act to enhance K^+ outward currents in neurons (Zhu et al., 1998*a;* van Tol-Steye et al., 1999).

There also appears to be little consistency among cells with regard to the effect of reactive oxygen species on K_{ATP} channel activity. The increased production of reactive oxygen species with hydrogen peroxide was found to increase the opening probability of K_{ATP} channels in ventricular myocytes (Goldhaber et al., 1989; Ichinari et al., 1996) and in pancreatic β cells (Krippeit-Drews et al., 1994; Nakazaki et al., 1995; Krippeit-Drews et al., 1999). In contrast, the presence of the sulfhydryl-oxidizing agents was reported to suppress the activity of K_{ATP} channels in skeletal muscle cells (Weik & Neumcke, 1989). Little is known whether the production of reactive oxygen species caused by sulfhydryl-oxidizing agents affects the K_{ATP} channel activity in neuroendocrine cells.

Therefore, the objective of the present study was to (i) characterize functional K_{ATP} channels in pituitary GH_3 cells, (ii) determine whether substances that are known to affect K_{ATP} channel activity also open K_{ATP} channels in GH_3 cells, (iii) find out whether the challenging of cells with mellitin or sulfhydryl-oxidizing agent (i.e., 4,4′-dithiodipyridine) affects channel activity, and (iv) examine whether this opening will result in a hyperpolarization that would be required if a K_{ATP} channel opener should decrease hormonal secretion. Previous observations at our laboratory showed that large-conductance Ca^{2+}-activated K^+ channels, which are sensitive to voltage and Ca^{2+}, are expressed in these cells (Wu et al., 2000). The present study provides evidence that K_{ATP} channels are functionally expressed in GH_3 cells.

Materials and Methods

Cell Preparation

GH_3 (a cell line from a rat anterior pituitary adenoma) cells were obtained from the Culture Collection and Research Center (CCRC-60015, Hsinchu, Taiwan). Cells were routinely cultured in 50-ml Ham's F-12 medium (Life Technologies, Grand Island, NY) that was supplemented with 15% horse serum (v/v), 2.5% fetal calf serum (v/v), and 2 mM *L*-glutamate (Life Technologies) in a 5% CO_2 atmosphere. Cells were subcultured once a week, and a new stock line was generated from frozen cells (frozen in 10% glycol in medium plus serum) every 3 months. The experiments were performed after 5 or 6 days of subcultivation (60 to 80% confluence).

Electrophysiological Measurements

Immediately before each experiment, GH_3 cells were dissociated and an aliquot of cell suspension was placed into a recording chamber affixed to the stage of an inverted phase-contrast microscope (Diaphot-200; Nikon, Tokyo, Japan). The microscope was coupled to a video-camera system with a magnification of up to 1500× to continually monitor cell size during the experiments. Cells were bathed at room temperature (20–25°C) in normal Tyrode's solution containing 1.8 mM $CaCl_2$. Ionic currents were recorded in the cell-attached or inside-out configuration of the patch-clamp technique, using a patch-clamp amplifier (RK-400; Bio-Logic, Claix, France) (Hamill et al., 1981). Patch pipettes (3 to 5 MΩ in bathing solution) were made from borosilicated glass capillary tubes (Kimble Products, Vineland, NJ) using a two-step pipette puller (PB-7; Narishige Scientific, Tokyo, Japan), and the tips were heat-polished with a microforge (MF-83; Narishige). A programmable stimulator (SMP-311; Bio-Logic) was used for the digitial generation of voltage pulses. Tested drugs were applied by perfusion or added to the bath to obtain the final concentration indicated.

The signals, consisting of voltage and current tracings, were displayed on a digitial storage oscilloscope (model 1602; Gould, Valley View, OH) and online recorded in a digital audiotape recorder (model 1204; Bio-Logic). After the experiments, the data were fed back and stored in a Pentium III-grade computer (Lemel, Taipei, Taiwan) at 10 kHz through an analog/digital interface (Digidata 1320A; Axon Instruments, Foster City, CA) using the Clampex subroutine of the pCLAMP 8.0 software (Axon Instruments).

Single Channel Analysis

Single channel currents were analyzed using Fetchan and Pstat subroutines in the pCLAMP software (Axon Instruments). Multi-Gaussian adjustments of the amplitude distributions between channels were used to determine unitary currents. The functional independence among channels was verified by comparing the observed stationary probability with the values calculated according to the binomial law. The number of active channels in a patch, *N*, was taken as the maximum number of channels simultaneously open under conditions of maximum opening probability. When there were a sufficiently large number of independent observations, the opening probabilities ($N \cdot P_o$) of unitary current were evaluated by an iterative process that was continued until the χ^2 value was no longer changed. The single channel conductance was calculated by a linear regression using mean values of the current amplitudes measured at different voltages.

The open lifetime distributions of K_{ATP} channels were fit with a logarithmically scaled bin width using the method of McManus, Blatz & Magelby (1987). When the square root of the number of events in a bin is plotted against the open lifetime, each component of the open lifetime distribution will appear as a clear peak and the respective time constant falls in the vicinity of this peak. The amplitude histograms were obtained from a continuous recording of 30 sec and fitted with the Gaussian distribution function.

The concentration-dependent relation to Mg-ATP on the inhibition of K_{ATP} channel activity was fitted to the Hill equation by using a nonlinear regression analysis (Origin 6.0, Microcal Software, Northampton, MA). That is,

$$\text{Percentage inhibition} = E_{max} \times [C]^n/(IC_{50}{}^n + [C]^n)$$

where $[C]$ represents the concentration of Mg-ATP; IC_{50} and n are the concentrations required for a 50% inhibition and the Hill coefficient, respectively; and E_{max} is Mg-ATP-induced maximal inhibition of K_{ATP} channels.

All values are reported as means ± SEM. The paired or unpaired

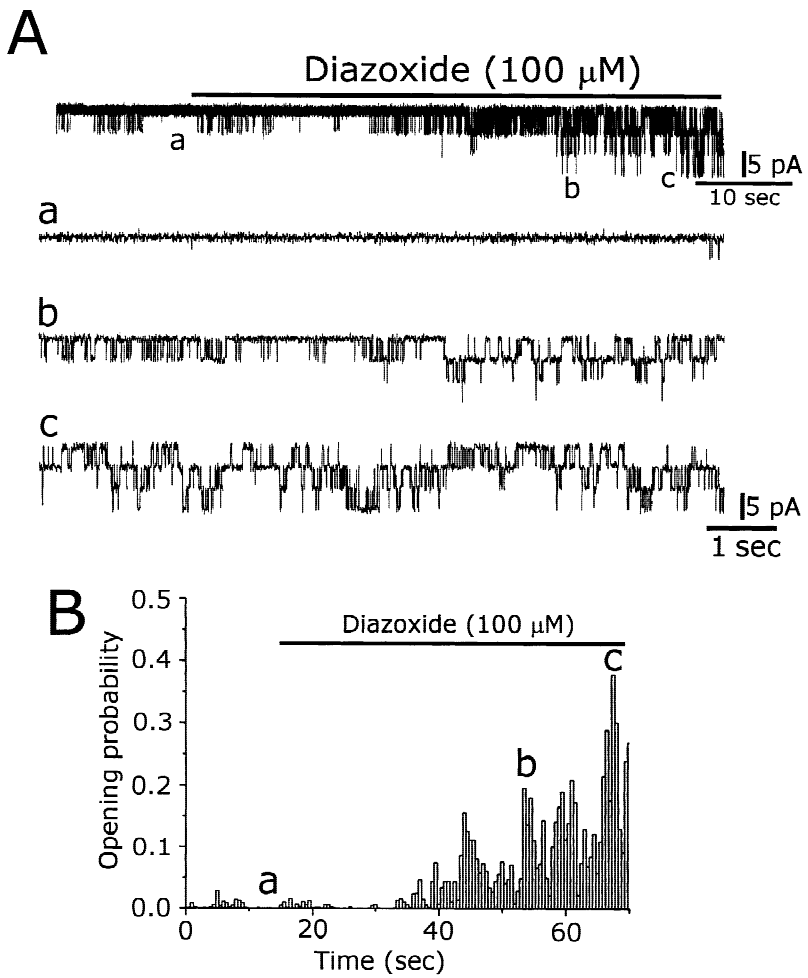

Fig. 1. Stimulatory effect of diazoxide on K_{ATP} channels in GH_3 cells. The experiments were conducted with symmetrical K^+ concentration (145 mM) on each side of the membrane. Under the cell-attached configuration, the cell was held at −60 mV. (*Panel A*) The original current trace showing the change in the behavior of K_{ATP} channels after addition of diazoxide (100 μM). In this and the following figures, channel openings are shown as a downward deflection. The lower part in *panel A* shows current traces obtained in an expanded time scale corresponding to those labeled a, b and c in upper part. (*Panel B*) Opening probability for the activity of K_{ATP} channels shown in A plotted against time of recording. Bin width is 0.5 sec. The protocol for the addition of diazoxide (100 μM) to the bath is denoted by horizontal bar shown in each panel.

Student's *t* test and ANOVA with a least-significance difference method for multiple comparison were used for the statistical evaluation of differences among means. Differences between the values were considered statistically significant when P was <0.05.

Drugs and Solutions

Diazoxide, glibenclamide, sodium cyanide, 2,4-dinitrophenol, 4,4′-dithiodipyridine (DTDP) and reduced glutathione (GSH) were purchased from Sigma (St Louis, MO). Mellitin and aristolochic acid (8-methoxy-6-nitrophenanthro(3,4-d)-1,3-dioxole-5-carboxylic acid) were obtained from Biomol (Plymouth Meeting, PA). Nicorandil was kindly provided by Chugai Pharmaceutical (Tokyo, Japan). All other chemicals were of analytical grade. The composition of normal Tyrode's solution was as follows (in mM): NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5, and HEPES-NaOH buffer 5.5 (pH 7.4). To record membrane potential, the patch pipettes were filled with solution (in mM): KCl 140, KH_2PO_4 1, $MgCl_2$ 1, EGTA 0.1, Na_2ATP_3, Na_2GTP 0.1, and HEPES-KOH buffer 5 (pH 7.2). In the cell-attached or inside-out configuration of single-channel recordings, high K^+-bathing solution contained (mM): KCl 145, $MgCl_2$ 0.53 and HEPES-KOH buffer 5 (pH 7.4), and the pipette solution contained (mM): KCl 145, $MgCl_2$ 2, and HEPES-KOH buffer 5 (pH 7.2).

Results

Effect of Diazoxide on the Activity of K_{ATP} Channels in GH_3 Cells

In these experiments, GH_3 cells were bathed in symmetrical K^+ concentration (145 mM). In the cell-attached configuration, each cell was held at the level of −60 mV. When diazoxide (100 μM) was applied to the bath, channel activity was greatly increased (Fig. 1). Diazoxide is a potent opener of SUR1/Kir6.2 K_{ATP} channels expressed in pancreatic β cells (D'hahan et al., 1999). The K^+ channel currents occurred in rapid open-close transitions and in brief bursts with a unitary current amplitude of 4.28 ± 0.12 pA (n = 7) at −60 mV. The presence of diazoxide (100 μM) significantly increased opening probability from 0.013 ± 0.015 to 0.28 ± 0.031 (n = 8). However, there was no significant difference in the amplitude of unitary inward current between the absence and presence of diazoxide (4.28 ± 0.12 pA [n = 7] *vs.* 4.31 ± 0.11 pA [n = 8]). The effect of diazoxide on

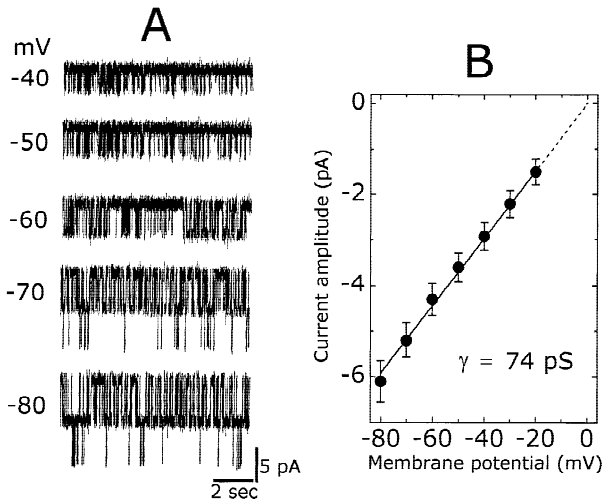

Fig. 2. Effect of diazoxide on the current-voltage relation of K_{ATP} channels in GH_3 cells. The cells were bathed in symmetrical K^+ solution and experiments were conducted under cell-attached configuration. (*Panel A*) Examples of K_{ATP} channels in the presence of diazoxide (100 μM) measured from cells at various membrane potentials. Diazoxide was applied to the bathing solution. The numbers at the beginning of each current trace mark the voltage applied to the patch pipette. (*Panel B*) Current-voltage relation of K_{ATP} channels in the presence of diazoxide (100 μM). Each point represents mean ± SEM (n = 5–9). The single channel conductance of unitary inward current was 74 pS with a reversal potential of 0 mV.

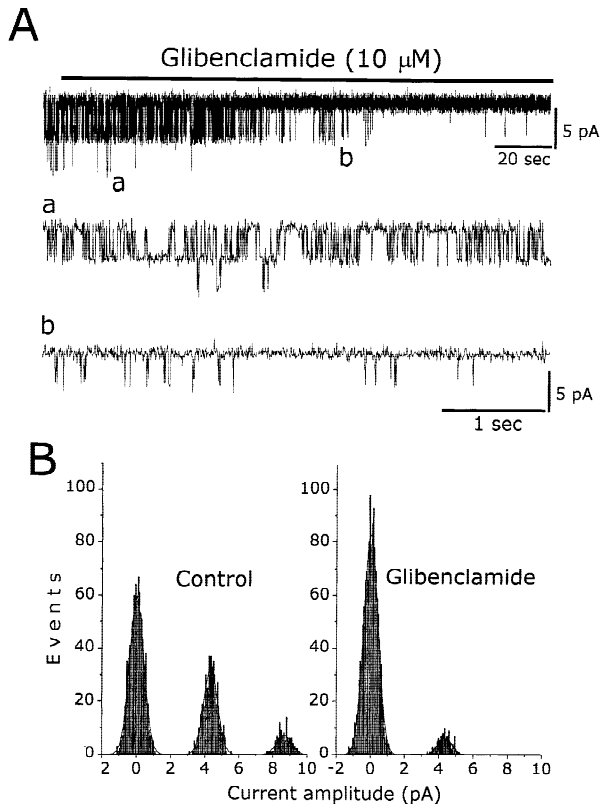

Fig. 3. Effect of glibenclamide on the activity of K_{ATP} channels recorded from an inside-out patch of GH_3 cells. (*Panel A*) Original current traces showing the change in channel activity after addition of glibenclamide. The experiments were conducted with symmetrical K^+ concentration (145 mM). The holding potential was −60 mV and bath medium contained 10 μM Mg-ATP. The horizontal bar indicates the application of glibenclamide (10 μM). The lower parts in *panel A* show the current traces obtained in an expanded time scale. The original current traces (a and b) shown in upper part correspond to those labeled a and b in lower part. (*Panel B*) Amplitude histograms measured in the control and after addition of glibenclamide (10 μM). All points shown in the amplitude histograms were fitted by one or more Gaussian distributions using the method of maximum likelihood. The closed state corresponds to the peak at 0 pA.

channel activity at various membrane potentials was also examined. The plot of current amplitude as a function of holding potential was constructed. Figure 2 illustrates the averaged current-voltage relation of single channel currents in the presence of diazoxide (100 μM). The opening probability of these channels appeared to be insensitive to the change in the level of holding potentials. The single channel conductance of these channels calculated from a linear current-voltage relationship was 74 ± 2 pS (n = 8) with a reversal potential of 0 ± 1 mV (n = 8). The value of unitary conductance for these channels was found to be lower than that of large-conductance Ca^{2+}-activated K^+ channels (Wu et al., 2000), but similar to that of K_{ATP} channels reported in pancreatic β cells (Ashcroft & Gribble, 1998; Mukai et al., 1998).

Effect of Glibenclamide on the Activity of K_{ATP} Channels in GH_3 Cells

The effect of glibenclamide on K_{ATP} channel activity was also examined. As shown in Fig. 3, under symmetrical K^+ (145 mM) condition in which bath medium contained 10 μM Mg-ATP and the potential was held at −60 mV, the activity of K_{ATP} channels could be observed in a detached patch. When glibenclamide (10 μM) was added to the bath, the activity of channel opening was greatly decreased (Fig. 3*A*). The opening probability of the channel at −60 mV in the absence of glibenclamide was found to be 0.35 ± 0.04 (n = 9). The addition of glibenclamide (10 μM) significantly decreased the activity to 0.015 ± 0.008 (n = 8). However, the amplitude of unitary inward current was unaffected during exposure to glibenclamide (4.34 ± 0.12 pA [n = 9] *vs.* 4.31 ± 0.11 pA [n = 8]) (Fig. 3*B*). When glibenclamide was washed out, channel activity was returned to the control level. Thus, it is clear that the activity of K_{ATP} channels expressed in GH_3 cells can be suppressed by the presence of glibenclamide.

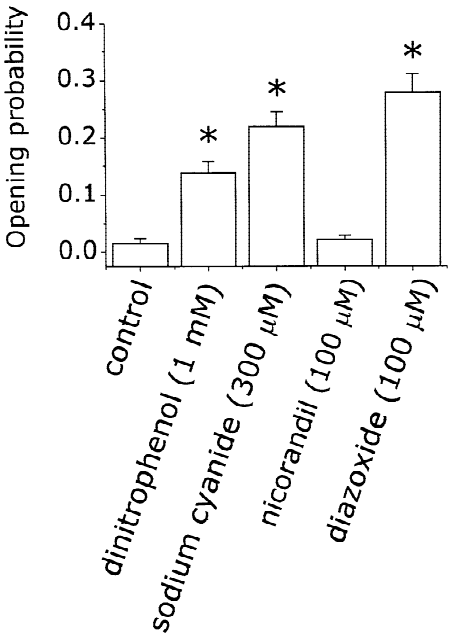

Fig. 4. Comparison between the effect of diazoxide (100 μM) and those of 2,4-dinitrophenol (1 mM), sodium cyanide (300 μM), and nicorandil (100 μM) on the activity of K_{ATP} in GH_3 cells bathed in symmetrical K^+ solution (145 mM). Cell-attached configuration was performed in these experiments. The potential held at each patch was −60 mV. The opening probability after application of each agent was plotted. Each point represents mean ± SEM (n = 6–8). *Significantly different from controls.

Comparison of The Effects of Diazoxide and Those of 2,4-Dinitrophenol, Sodium Cyanide, and Nicorandil on the Activity of K_{ATP} Channels

To investigate whether metabolic inhibition influences K_{ATP} channel activity, the effects of 2,4-dinitrophenol, sodium cyanide, and nicorandil on the activity of K_{ATP} channels present in GH_3 cells were also examined and compared. These experiments were conducted in the cell-attached configuration. As summarized in Fig. 4, both 2,4-dinitrophenol (1 mM) and sodium cyanide (300 μM) significantly produced a stimulatory effect on channel activity. However, nicorandil (100 μM) was not found to induce channel activity. These results showed that metabolic inhibition induced by either 2,4-dinitrophenol or sodium cyanide may reduce the level of intracellular ATP, thus producing an increase in K_{ATP} channel activity in these cells (Ashcroft & Gribble, 1998).

Concentration-Response Relation for the Inhibitory Effect of Mg-ATP on K_{ATP} Channels

The relationship between the concentration of Mg-ATP and the opening probability of K_{ATP} channels was further determined. Another series of experiments was conducted with symmetrical K^+ concentration (145 mM), the inside-out configuration was performed and the holding potential was −60 mV. As illustrated in Fig. 5, Mg-ATP (1 μM–1 mM) suppressed channel activity in a concentration-dependent manner. The half-maximal concentration (IC_{50}) required for the inhibitory effect of Mg-ATP on the activity of K_{ATP} channels was 30 μM, 1 mM Mg-ATP almost completely suppressed channel activity. The results are compatible with the notion that the opening of K_{ATP} channels in GH_3 cells is associated with a decrease in the concentration of intracellular ATP.

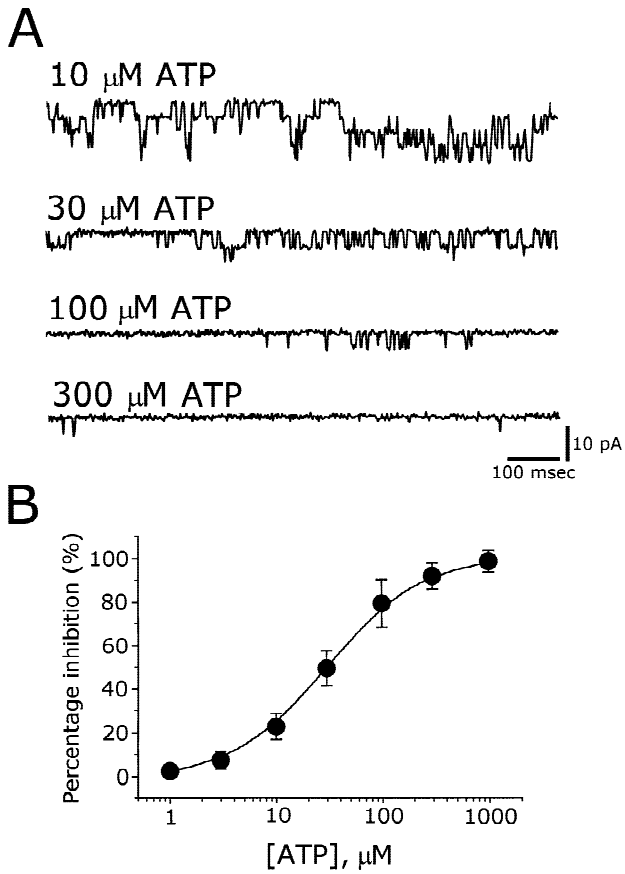

Fig. 5. Concentration-dependent effect of Mg-ATP on the activity of K_{ATP} in GH_3 cells. The experiments were conducted with symmetrical K^+ concentration. Under the inside-out configuration, the holding potential was −60 mV. (*Panel A*) Original current traces obtained in the presence of various concentrations (1 μM − 1 mM) of Mg-ATP. Channel openings are shown as a downward deflection. (*Panel B*) Concentration-response curve for the Mg-ATP-induced inhibition of K_{ATP} channels. The curve was fitted with the Hill equation as described under Materials and Methods. The IC_{50} value and maximally inhibited percentage was 30 μM and 99%, respectively. The Hill coefficient was 1.1. Each point represents mean ± SEM (n = 5–8).

Effect of Mellitin on K_{ATP} Channel Activity in GH_3 Cells

Whether the activity of phospholipase A_2 affects the opening probability of K_{ATP} channels present in GH_3

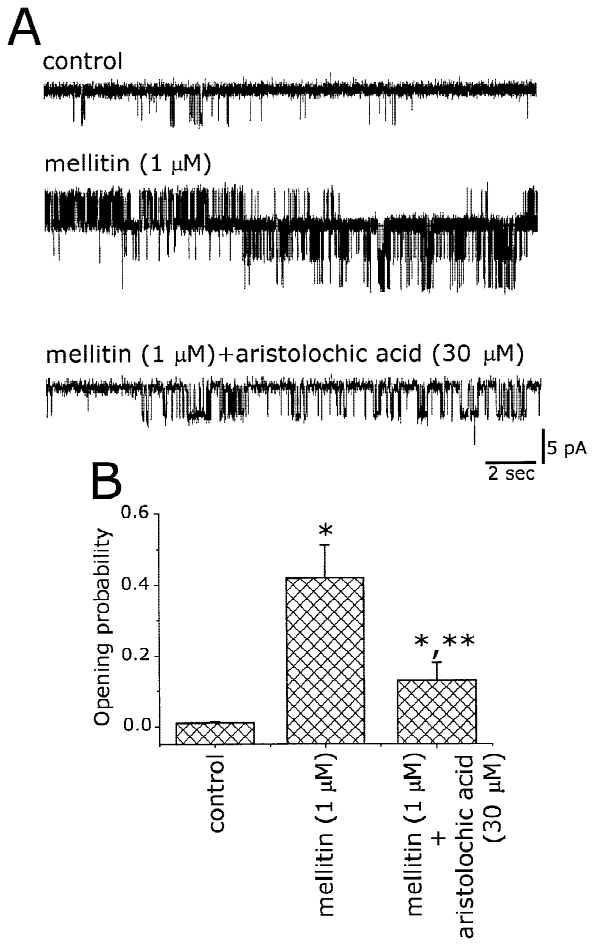

Fig. 6. Current traces (*Panel A*) and bar graph (*Panel B*) showing the effect of mellitin and aristolochic acid on the activity of K_{ATP} channels. In *Panel A,* upper part is control, middle part was recorded in the presence of mellitin (1 μM) and lower part was after the addition of aristolochic acid (30 μM), but still in the presence of 1 μM mellitin. In *Panel B,* *indicates significant difference from control group; **denotes significant difference between mellitin alone group and mellitin plus aristolochic acid group. Each point represents mean ± SEM (n = 5–9).

cells was also examined. Mellitin is a bee venom peptide and known to be an activator of phospholipase A_2 (Clark et al., 1991). It is of interest that when mellitin (1 μM) was applied to the bath, channel activity was greatly increased (Fig. 6). The presence of mellitin significantly increased the channel activity from 0.010 ± 0.002 to 0.421 ± 0.088 (n = 5). However, there was no significant difference in the amplitude of the unitary inward current between the absence and presence of mellitin (2.39 ± 0.12 pA [n = 8] *vs.* 2.40 ± 0.13 pA [n = 7]). In addition, the effect of mellitin on channel activity was attenuated by aristolochic acid. Aristolochic acid was reported to be an inhibitor of phospholipase A_2 (Vishwanath, Kini & Gowda, 1987). After the further addition of aristolochic acid (30 μM), channel activity was significantly reduced to 0.128 ± 0.045 (n = 7). The mellitin-induced increase in channel activity was also decreased by the presence of glibenclamide (10 μM). Thus, these results can be interpreted to mean that the activity of phospholipase A_2 present in GH_3 cells can regulate the opening of K_{ATP} channels.

Effect of 4,4′-Dithiodipyridine on K_{ATP} Channel Activity in GH_3 Cells

In this study, we also investigated whether the exposure of cells to sulfhydryl-oxidizing agent can cause any change in the activity of K_{ATP} channels. 4,4′-Dithiodipyridine is known to be a lipophilic sulfhydryl-oxidizing agent. As shown in Fig. 7, when cells were exposed to 4,4′-dithiodipyridine, the opening probability of K_{ATP} channels was profoundly increased. For example, at the level of −60 mV, 4,4′-dithiodipyridine (100 μM) caused a significant increase in channel activity from 0.122 ± 0.041 to 0.662 ± 0.142 (n = 7). However, no significant difference in unitary current amplitude was demonstrated between the absence and presence of 4,4′-dithiodipyridine (4.28 ± 0.06 pA *vs.* 4.30 ± 0.05 pA [n = 7]). Moreover, the further addition of reduced glutathione (10 mM) significantly decreased channel activity to 0.196 ± 0.041 (n = 6). These results suggest that the increased production of reactive oxygen species caused by 4,4′-dithiodipyridine may cause a stimulatory effect on K_{ATP} channels in GH_3 cells.

Diazoxide, Mellitin and 4,4′-Dithiodipyridine Activate the Same Population of K^+ Channels

To compare the kinetic properties of K^+ channels induced by the presence of diazoxide, mellitin and 4,4′-dithiodipyridine, we also analyzed the mean lifetimes of the channel openings. The open-lifetime histogram was obtained from 30-sec recordings in the presence of each agent (Fig. 8). The distribution of open times in the presence of diazoxide, mellitin or 4,4′-dithiodipyridine was fitted by a single exponential. However, there was no significant difference in the mean open lifetime among the presence of 100 μM diazoxide, 1 μM mellitin and 100 μM 4,4′-dithiodipyridine (1.70 ± 0.04 msec [n = 5], 1.72 ± 0.04 msec [n = 6], and 1.73 ± 0.03 msec [n = 5], P > 0.05). The results suggested that diazoxide, mellitin and 4,4′-dithiodipyridine appeared to interact with the same type of K^+ channels with similar opening kinetics, although these compounds were different in potency and caused no significant change in single channel conductance. The target K^+ channels for these compounds in GH_3 cells are thus likely to be K_{ATP} channels.

Effect of Diazoxide on Spontaneous Action Potentials in GH_3 Cells

The effect of diazoxide on the change in membrane potentials was also examined. Under the current-clamp

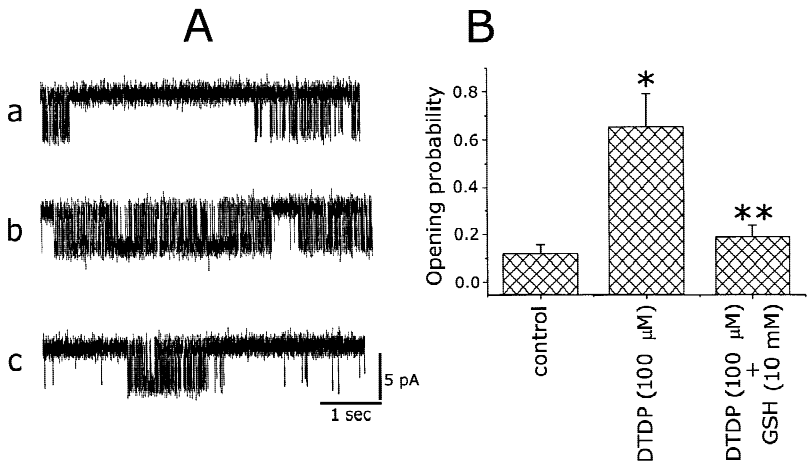

Fig. 7. Current traces (*Panel A*) and bar graph (*Panel B*) showing the effect of 4,4′-dithiodipyridine (DTDP) and reduced glutathione (GSH) on the activity of K_{ATP} channels. In *Panel A*, upper part is control, middle part was recorded in the presence of 4,4′-dithiopyridine (100 μM) and lower part was after reduced glutathione (10 mM), but still in the presence of 100 μM 4,4′-dithiodipyridine. In *Panel B*, *indicates significant difference from control group; **denotes significant difference between 4,4′-dithiodipyridine alone group and 4,4′-dithiodipyridine plus reduced glutathione group. Each point represents mean ± SEM (n = 5–9).

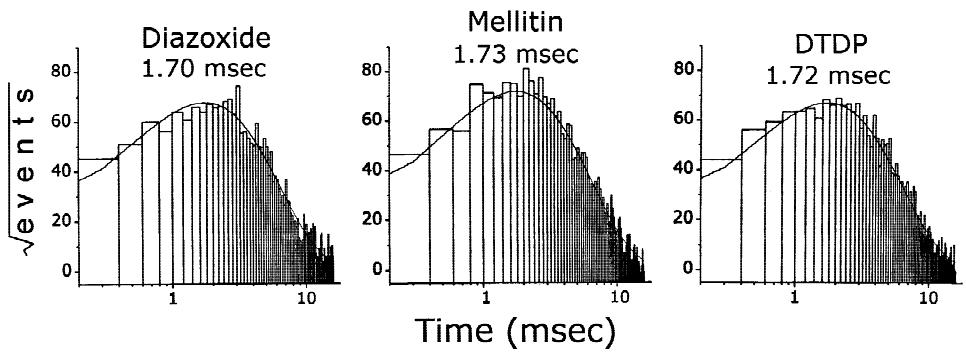

Fig. 8. Open-time histograms in the presence of diazoxide (100 μM), mellitin (1 μM) and dithiodipyridine (DTDP; 100 μM). The experiments were conducted in cell-attached membrane patches, holding potential was −60 mV and each agent was applied to the bath. Open-time histograms were fitted by a one-exponential function. The abscissa shows the logarithm of the open time (msec) and the ordinate shows the square root of the number of events ($n^{1/2}$).

conditions, GH_3 cells, bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$, had a resting membrane potential of −45 ± 6 mV (n = 21). About 70% of GH_3 cells have been known to exhibit the repetitive firing of action potentials (Wu et al., 2000). The typical effects of diazoxide on spontaneous action potentials in these cells are illustrated in Fig. 9. When cells were exposed to 10 and 100 μM diazoxide, spontaneous spiking discharge was significantly decreased to 0.5 ± 0.1 Hz (n = 5) and 0.3 ± 0.1 Hz (n = 6), respectively, from a control value of 1.1 ± 0.2 Hz (n = 8). After addition of diazoxide (100 μM), cells were also significantly hyperpolarized to −53 ± 8 mV from a control value of −46 ± 6 mV (n = 6). However, no significant change in action potential duration between the absence and presence of diazoxide (100 μM) was found (78 ± 8 msec [n = 5] *vs.* 79 ± 8 msec [n = 6]). In addition, nicorandil (100 μM) caused no significant effect on the repetitive firing of action potentials. Thus, the decrease in the firing of action potentials caused by diazoxide may be explained by its stimulatory effect on the activity of K_{ATP} channels expressed in GH_3 cells.

Discussion

The important findings of the present study are that pituitary GH_3 cells express a functionally K_{ATP} channel. Recent studies have shown that these channels are formed by the molecular interaction between an inwardly rectifying K^+ channel subunit (Kir6.1 or Kir6.2) and a high affinity receptor for the sulphonylureas (SUR1 or SUR2) (Inagaki et al., 1995; Babenko et al., 1998). When expressed in isolation, these proteins did not result in forming molecules that recapitulated the properties of native K_{ATP} channels. However, either channel subunit was able to couple with each sulfonylurea receptor to produce functional K_{ATP} channels (Inagaki et al., 1996; Gopalakrishnan et al., 2000).

The single channel conductance of K_{ATP} channels in GH_3 cells measured with the use of 145 mM K^+ on both sides of the membrane was 74 ± 2 pS (n = 8). This value is similar to those of typical K_{ATP} channels reported in pancreatic β cells (Ashcroft & Gribble, 1998), but greater than that of K_{ATP} channels (26 pS) seen in adenohypophysis cells (Bernardi et al., 1993). One of

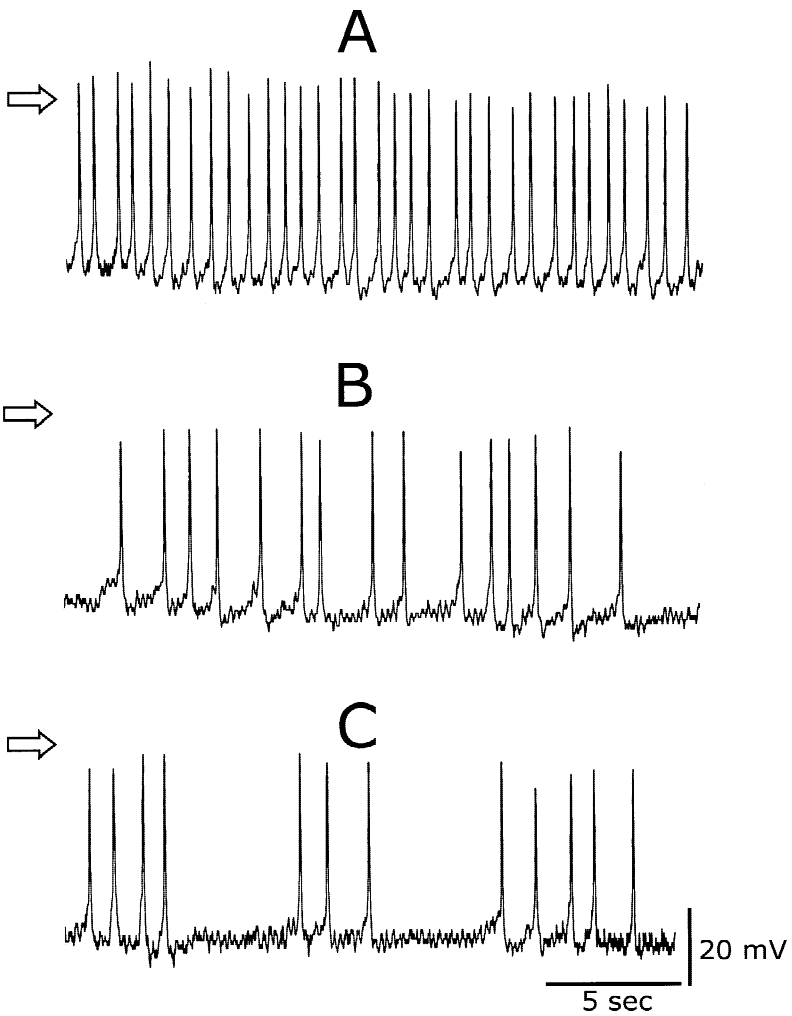

Fig. 9. Effect of diazoxide on the firing of action potentials in GH_3 cells. Cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$. The change in membrane potential was measured under current-clamp condition. Potential trace shown in *Panel A* is control, and those in *Panel B* and *C* were obtained 1 min after application of 10 and 100 μM diazoxide, respectively. Open arrows shown in each potential trace indicate the 0 mV potential.

the reasons for this discrepancy could be due to the different K^+ concentrations used. However, the open-time (1.7 msec), channel-bursting, ATP-sensitivity, voltage-insensitivity and glibenclamide-sensitivity were all nearly identical to those described in native pancreatic β cells (Mukai et al., 1998). The concentration of Mg-ATP required for the inhibition of K_{ATP} channels in our study was 30 μM. This value is also similar to that observed in an inside-out patch containing many hundreds of wild-type (SUR1/Kir6.2) K_{ATP} channels (Gribble et al., 1997). In addition, diazoxide that significantly induced K_{ATP} channel activity in GH_3 cells was not found to activate SUR2/Kir6.2 channels (Inagaki et al., 1996; D'hahan et al., 1999). Therefore, on the basis of pharmacological and biophysical properties, the K_{ATP} channel in GH_3 cells appears to be similar to those described in pancreatic β cells (Ashcroft & Gribble, 1998; Mukai et al., 1998). However, it remains to be clarified whether these channels are heteromers composed of SUR1 and Kir6.2 subunits (Babenko et al., 1998; Gopalakrishnan et al., 2000), before the molecular nature of the K_{ATP} channel in GH_3 cells is fully characterized. Indeed, it has been reported that mRNA for SUR2A was expressed at low levels in pituitary gland (Inagaki et al., 1995). Pharmacological properties of K_{ATP} channels were also believed to depend on a family of structurally related but functionally distinct sulfonylurea receptors (Inagaki et al., 1996; Babenko et al., 1998; Gopalakrishnan et al., 2000).

It is of interest to mention that mellitin, an activator of phospholipase A_2, caused a stimulatory effect on the activity of K_{ATP} channels in GH_3 cells and that the further addition of aristolochic acid suppressed the mellitin-induced increase in channel activity. These findings suggest that the activity of phospholipase A_2 can play a role in the regulation of K_{ATP} channel activity in these cells. However, the stimulatory action of mellitin presented here could be interpreted to conflict with a previous report showing that mellitin can interact with K_{ATP} channels to reduce their opening probability in a insulin-secreting cell line (Eddlestone, 1995). The reason for this discrepancy is currently unclear. It is possible that diffusible cytosolic messengers inside the cells (e.g., ara-

chidonic acid) may contribute to the mellitin-induced effect on the opening of K_{ATP} channels present in GH_3 cells. Nonetheless, the stimulation of K_{ATP} channels caused by the activation of phospholipase A_2 with mellitin may serve as a negative feedback mechanism to limit Ca^{2+} influx once secretion has begun. Our study appears to be consistent with previous reports showing that the stimulation of phospholipase A_2 activated the K^+ currents in the light green cells of the mollusc *Lymnaea stagnalis* (van Tol-Steye et al., 1999) and in neurons from hypothalamus and brain stem (Zhu et al., 1998*a*). However, further research is still needed to find out whether the increased activity of phospholipase A_2 with mellitin can stimulate K_{ATP} channel activity in a variety of cells.

The present study demonstrating the stimulatory effect of 4,4′-dithiodipyridine on K_{ATP} channel activity in GH_3 cells is in agreement with previous reports in cardiac myocytes and pancreatic β cells (Nakazaki et al., 1995; Ichinari et al., 1996; Krippeit-Drews et al., 1999). In addition, the further addition of reduced glutathione can reverse 4,4′-dithiodipyridine-induced increase in channel activity. However, aristolochic acid had no effect on 4,4′-dithiodipyridine-stimulated channel activity. Thus, it is likely that the oxidation of critical SH groups by 4,4′-dithiodipyridine increases the opening of K_{ATP} channels expressed in GH_3 cells, even though its effect does not seem to be associated with the increased activity of phospholipase A_2. These SH groups involved in channel gating appear to be localized at the inner surface of the cell membrane. Moreover, the present finding showed that neither open-time kinetics nor unitary conductance of the channel was affected by diazoxide, mellitin, or 4,4′-dithiodipyridine, suggesting that the increased responsiveness of the channel to these compounds was secondary to the alterations remote from the pore region of the channel. However, it remains to be determined whether the stimulatory effect of the lipid soluble oxidative agent 4,4′-dithiodipyridine on channel activity results from its reduction of intracellular ATP content and/or inhibition of glucose-dependent pathway of ATP synthesis in these cells (Nakazaki et al., 1995).

In our study, the repetitive firing of action potentials in GH_3 cells can be suppressed by the presence of diazoxide. These results allow us to suggest that the change in membrane potential could be influenced by the activity of K_{ATP} channels. Indeed, because single GH_3 cells exhibit a very high input resistance (1–5 GΩ), a small amount of current is sufficient to alter the level of membrane polarization. Further investigation is required to clarify the physiological role of K_{ATP} in GH_3 cells or other neuroendocrine cells. Previous reports have indeed shown that the release of growth hormone derived from anterior pituitary cells of diabetic rats was reduced (Olchovsky et al., 1990), and the level of serum prolactin in diabetic rats was also lower (Lau et al., 1993). Because pituitary cells are vulnerable to ischemic conditions, it is also tempting to speculate that this current could play a protective role under conditions of metabolic deprivation as has been postulated for K_{ATP} channel currents elsewhere in neurons (Ashcroft & Gribble, 1998; Liu et al., 1999). In any case, it appears to be important, perhaps required, for the regulation of hormonal secretion that the change in the activity of K_{ATP} channels alters electrical activity of pituitary lactotrophs.

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