Korean J Physiol Pharmacol
Vol 5: 157–163, April, 2001

Block of ATP-Sensitive K^+ Channels Expressed in *Xenopus* Oocytes by Dimethyl Sulfoxide

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The effects of dimethyl sulfoxide (DMSO) were studied in two groups of *Xenopus* oocytes, one expressing ATP sensitive K^+ (K_{ATP}) channel comprised of sulfonylurea receptor SUR1 and inwardly rectifying K^+ channel subunit Kir6.2, and the other expressing renal K_{ATP} channel ROMK2. At concentrations of 0.3~10% (vol/vol) DMSO inhibited whole cell Kir6.2/SUR1 currents elicited by bath application of sodium azide (3 mM) in a concentration-dependent manner. The inhibition constant and Hill coefficient were 2.93% and 1.62, respectively. ROMK2 currents, however, was not affected significantly by DMSO. The results support the idea that DMSO inhibits K_{ATP} channel expressed in *Xenopus* oocyte through a protein-specific mechanism(s) that remains to be further elucidated.

Key Words: Dimethyl sulfoxide, ATP-sensitive K^+ channel, ROMK2, *Xenopus* oocyte

INTRODUCTION

ATP-sensitive potassium (K_{ATP}) channels inhibited by cytoplasmic ATP have been described in a number of cell types; excitable cells such as cardiac cells (Noma, 1983) and neurons (Jonas et al, 1991), and non-excitable cells such as pancreatic β-cell (Cook & Dale, 1984) and kidney epithelium (Quast, 1996) where they provide not only a link between the metabolic status of the cell and membrane potential but also a mechanism for K^+ secretion in epithelia. K_{ATP} channels are members of a rapidly expanding family of inwardly rectifying K^+ channels containing two transmembrane domains (Ho et al, 1993; Inagaki et al, 1995). In pancreatic β-cell, the K_{ATP} channel comprises a complex of two proteins: an inwardly rectifying K^+ channel subunit Kir6.2 and the sulfonylurea receptor SUR1 (Inagaki et al, 1995; Sakura et al; 1995). SUR1 that belongs to the ATP-binding cassette (ABC) transporter family acts as a receptor for sulfonylurea drugs (Auguilar-Bryan et al, 1995) and may be required for high sensitivities of K_{ATP} channel to K^+ channel openers or ATP (Inagaki et al, 1996). The K_{ATP} channel in the principal cells of the cortical collecting duct has a composite structure similar to that of β-cell. The α-subunits are those making up the ROMK2 channel (Kir1.1b) and the function of the SUR appears to be served by the cystic fibrosis transmembrane conductance regulator (CFTR) (McNicholas et al, 1996).

DMSO is an ampiphatic compound that possesses both a hydrophilic sulfoxide moiety and two hydrophobic methyl groups. As a result, DMSO is able to solubilize a wide range of compounds including biological agents that are poorly soluble in water. However, DMSO is not inert and many effects on biological systems have been suspected. For example, DMSO up-regulates intracellular calcium level ($[Ca^{2+}]_i$) in invertebrate photoreceptors (Brown & Rydqvist, 1990) as well as in cells from primary cultures and a variety of established cell lines (Morley & Whitfield, 1993). DMSO inhibits Na^+ current thereby blocking propagation of action potential in nerve (Sams, 1967; Larsen et al, 1996). In heart, DMSO prolongs the duration of action potential in ventricular

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cells through inhibition of delayed rectifier K^+ current (Ogura et al, 1995). To our knowledge, however, there has been no study on the effects of DMSO on cloned proteins expressed in *Xenopus* oocyte. In the present study, we demonstrate that DMSO blocks K_{ATP} channel expressed in *Xenopus* oocytes, which is one of the most frequently used expression system.

METHODS

In vitro transcription

Kir6.2 and SUR1 cloned into the vectors pBK-CMV and pcDNA3, respectively, were used for the experiment. Kir6.2 refers to BIR1 (Genebank accession number D50581) cloned from a mouse insulinoma cDNA library (Sakura et al, 1995) and SUR1 was cloned from hamster insulinoma cells (HIT-T15; Genebank accession number L40623) (Aguilar-Bryan et al, 1995). ROMK2 clone (Genebank accession number L29403) was obtained from cDNA library made from rat kidney poly(A)+ RNA in the plasmid vector pSPORT (Zhou et al, 1994). Capped mRNA was synthesized by *in vitro* transcription from linearized cDNA and stored in 10 mM Tris-HCl (pH 7.4) at −80°C.

Oocyte preparation

Immature *Xenopus* oocytes were prepared as described previously (Cho et al, 1993) with some modifications. Sections of ovaries from wild-type *Xenopus lavies* females were removed through a minilaparatomy under anesthesia. In order to partially dissociate the follicle cells, the sections were incubated for 1 hr in Ca^{2+}- free buffer solution containing collagenase (2 mg/ml) followed by repeated washes. Stage V-VI oocytes were then isolated and defolliculated manually.

Using a microinjection pipette mounted on a micropositioner, the oocytes were injected with 50 nl (1 ng/nl) of mixture of Kir6.2 and SUR1 mRNA while being observed at 25x magnification with a stereoscope. Equal amounts (25 ng) of each mRNA were mixed prior to injection. ROMK2 mRNA (1 ng/nl) was also injected using the same protocol. For controls, oocytes were either injected with 50 nl distilled water or left uninjected. All oocytes were then stored in incubation medium at 18~19°C to allow for translation of the mRNA. The incubation medium consists of 5 mM pyruvate and 5 g/mg gentamycin in ND96 containing (in mM) 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES (pH 7.55 with NaOH). Experiments were undertaken 3 days after injection.

Electrophysiology

Whole-cell K_{ATP} currents were measured using a two-electrode voltage-clamp technique. Test pulses were delivered, and membrane responses were recorded by means of a voltage-clamp amplifier (OC-725B, WPI, New Haven, CT) interfaced to an analog-to-digital converter (Digidata1200A, Axon Instrument) and a personal computer running pClamp software (Axon Instrument). The currents were filtered and digitized at 2 kHz. The electrodes were fabricated from glass capillaries containing an inner filament (OD 1.5 mm, ID 1.12 mm; WPI, New Haven, CT), which were back filled with 3 M KCl. Electrode resistances for current and voltage electrodes were 1~2 and 4~6 $M\Omega$ in ND96 solution, respectively.

Experiments were performed in a 2 ml acryl chamber that was continuously perfused at a rate of 3 ml/min. In order to stabilize the membrane potential, the oocytes were bathed in ND96 solution. Once membrane potential reaches a steady state, the bathing solution was replaced by a high K^+ -solution containing (in mM) 2 NaCl, 90 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES. The pH was adjusted to 7.55 with NaOH.

Measurement of $[Ca^{2+}]_i$

For measuring $[Ca^{2+}]_i$, 70 nl of 1 mM cell-impermeant Fura-2 was injected into each oocyte. The oocytes were then incubated in the standard extracellular medium for 1~2 hrs to allow the injected Fura-2 dispersed. Standard extracellular medium contains (in mM) 115 NaCl, 2 KCl, 0.1 $CaCl_2$, 1 $MgCl_2$ and 5 HEPES. The pH was adjusted to 7.20 with KOH. Fura-2 was excited alternately by UV light at 340 nm and 380 nm using a rotating filter wheel. The intensity of fluorescence was measured through a 510 nm filter with a photomultiplier (PTI). The signal at 340 nm was divided by that at 380 nm and from this ratio, $[Ca^{2+}]_i$ was determined.

Drugs

DMSO, glibenclamide and sodium azide were purchased from Sigma Co. (St.Louis, MO). Glibenclamide and sodium azide were dissolved in DMSO and distilled water, respectively and stored at 4°C until use. Immediately before use, sodium azide (3×10^{-1} M) and glibenclamide (10^{-1} M) were diluted in the perfusion solution.

RESULTS

Induction of K_{ATP} current

Voltage steps over the range of −70 to +40 mV were applied from holding potential of 0 mV, which produced no resolvable current in both controls and the oocytes coinjected with Kir6.2 and SUR1 mRNA (Fig. 1). However, when metabolic inhibitor azide (3 mM) was added to the perfusion solution, whole cell currents (1.5 ± 0.5 μA, n=12) were developed in the oocytes coinjected with Kir6.2 and SUR1 but not in controls. The currents increased gradually and reached the peak in 9.2 ± 2.4 min (n=8), which persisted throughout the experiments. The sodium azide-induced current was almost completely blocked by a subsequent application of glibenclamide (0.1 mM, n=3), suggesting that a reduction in intracellular ATP by sodium azide unblocked K_{ATP} channels (Gribble et al, 1997). In some oocytes, the azide-induced K_{ATP} current declined slowly after the peak was attained, which were excluded from the use.

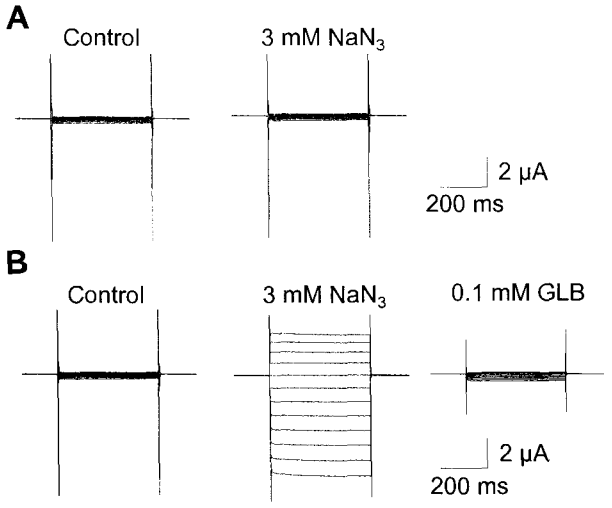

Fig. 1. Whole cell K_{ATP} current induced by a metabolic inhibitor azide in *Xenopus* oocyte coinjected with Kir6.2 and SUR1 mRNA. Current was elicited in response to voltage step from −70 to +40 mV in 10 mV increment from holding potential of 0 mV in control (A) and the oocyte coinjected with Kir6.2 and SUR1 mRNA (B). Each pulse was applied every 5 seconds. Glibenclamide (GLB, 0.1 mM) completely blocked the sodium azide-induced Kir 6.2/SUR1 currents.

Effects of DMSO on K_{ATP} current

To examine whether DMSO affects K_{ATP} current, oocytes expressing Kir6.2/SUR1 were exposed to increasing concentrations of DMSO from 0.3 to 10% added to the bath perfusion for 5 min. Before and during application of DMSO, a 400 ms hyperpolarization step down to −60 mV was applied from holding potential of 0 mV and the currents elicited were measured. DMSO inhibited the holding current (at 0 mV) and the azide-induced K_{ATP} current at −60 mV in a concentration-dependent manner (Fig. 2A). The current measured at each concentration of DMSO was normalized against steady-state peak current, averaged from 5∼8 cells and fit to Hill equation (Fig. 2B). Half-maximal inhibition occurred at 2.9% with the Hill coefficient of 1.6.

Voltage dependence of DMSO effects on K_{ATP} current was also investigated in the oocytes expressing Kir6.2/SUR1 (Fig. 3). In the presence of azide (3 mM), the inward and outward currents were elicited in response to 300 ms voltage steps ranging from −70 to +40 mV, which presumably are K_{ATP} current (Fig. 3A). DMSO (3%) markedly suppressed both inward and outward currents. The effect of DMSO was reversible on washout of the drug. The currents remained after DMSO application was further blocked by subsequent addition of 0.1 mM glibenclamide (data not shown). At the end of 300 ms test pulse the amplitude of the currents were measured then plotted for current-voltage (I-V) relationships (Fig. 3B). DMSO block of whole cell Kir6.2/SUR1 current was found to be voltage independent.

To examine whether the inhibition of K_{ATP} current by DMSO is protein specific, the effects of DMSO on another K^+ current was also tested. In the oocytes expressing ROMK2, similar inward and outward currents were resolved in response to the voltage steps in the absence of azide (Fig. 4A). Oocytes were held at 0 mV and voltage steps were applied over the range of −70 to +40 mV for 300 ms. DMSO (3%)

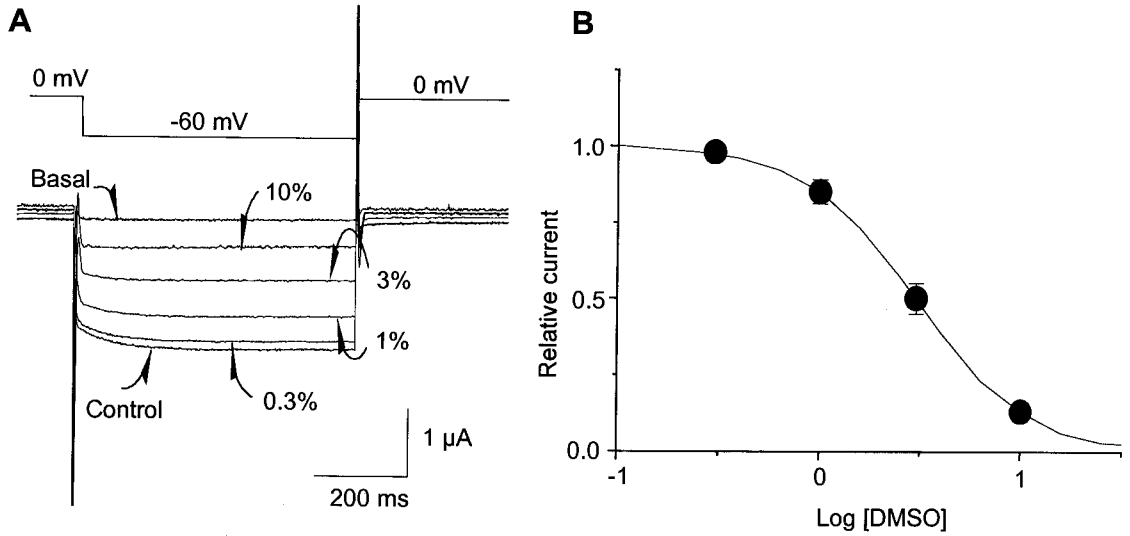

Fig. 2. Concentration-dependent block of whole cell K_{ATP} currents by DMSO. Whole cell Kir6.2/SUR1 currents in the presence of 3 mM azide were rapidly activated by 500 ms hyperpolarization step to −60 mV from holding potential of 0 mV. A: Basal current recorded immediately before addition of azide, control current, a maximum current induced by metabolic inhibition and the currents stabilized after addition of 0.3, 1, 3 and 10% DMSO are shown. B: Inhibition of K_{ATP} current, normalized against control current, is plotted as a function of DMSO concentration. Data were fit to Hill equation, giving a half-maximal inhibitory concentration of 2.9% and a Hill coefficient of 1.6. Each data point represents the mean±SEM of 5 to 6 experiments.

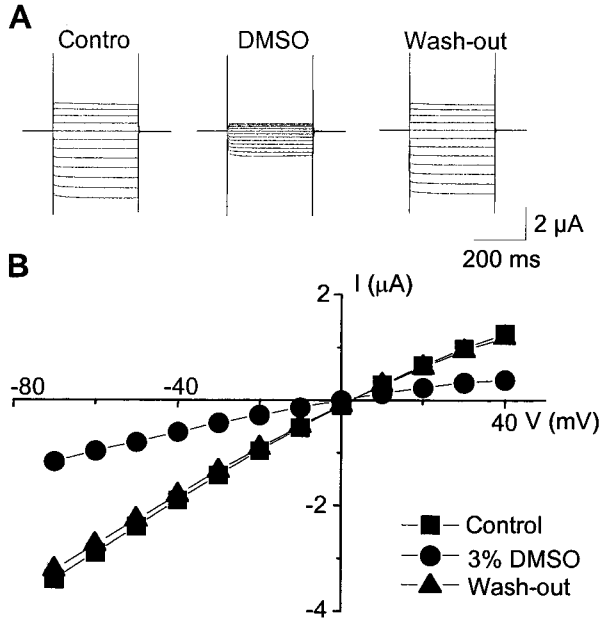

Fig. 3. Effects of DMSO on whole cell K_{ATP} currents expressed in *Xenopus* oocytes. Whole cell currents were recorded in response to voltage steps from −70 to +40 mV in 10 mV increments from holding potential of 0 mV in the presence of 3 mM azide. Whole cell current records (A) and current-voltage relationships (B) of Kir6.2/SUR1 currents before, during and after application of 3% DMSO.

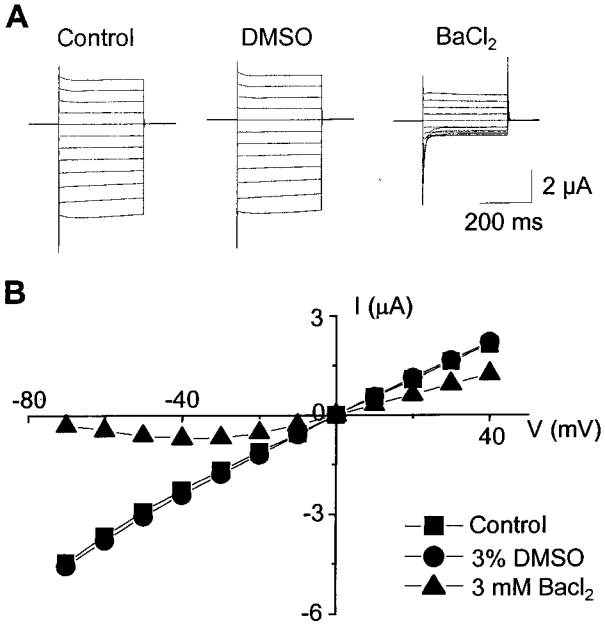

Fig. 4. Effects of DMSO on whole cell ROMK2 currents expressed in *Xenopus* oocytes. Whole cell current traces were recorded in response to voltage steps from −70 to +40 mV in 10 mV increments from holding potential of 0 mV. Whole cell currents traces (A) and current-voltage relationships (B) of the currents obtained from an oocytes injected with ROMK2 mRNA in control, with 3% DMSO or with 3 mM $BaCl_2$.

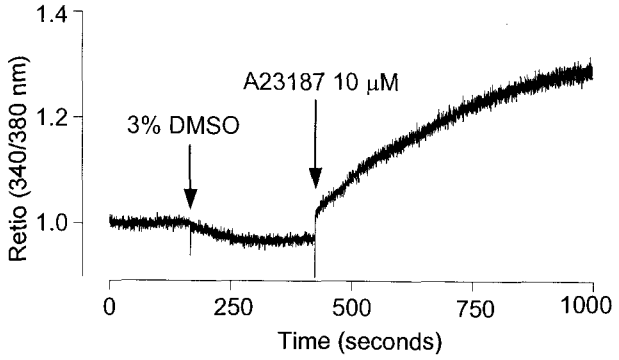

Fig. 5. DMSO effects on intracellular free calcium ion concentration ($[Ca^{2+}]_i$) of *Xenopus* oocytes. 3% DMSO applied extracellularly caused no change in $[Ca^{2+}]_i$. Subsequent application of calcium ionophore A23187 (10 μM) produced a rapid increase in $[Ca^{2+}]_i$. Trace shows the Ca^{2+}-dependent fluorescence ratio recorded from an oocyte loaded with Fura-2.

had almost no effect on the ROMK2 currents. ROMK has been known to share many basic characteristics with renal K_{ATP} currents (Zhou et al, 1994; Quast, 1996). Addition of $BaCl_2$ (3 mM) inhibited the current substantially. The block by Ba^{2+} was voltage-dependent, as it has been shown previously (Zhou et al, 1994). The outward currents were only slightly inhibited by 0.3 mM Ba^{2+}, while this concentration reduced inward currents by >75% (Fig. 4B).

Finally, the effects of DMSO on $[Ca^{2+}]_i$ of *Xenopus* oocytes was examined (Fig. 5). Fura-2 had been injected into the cells with microinjection pipette. A final concentration of Fura-2 microinjected into each oocyte was 100 μM. Application of 3% DMSO in 90 mM external K^+ solution caused no significant change in $[Ca^{2+}]_i$ of *Xenopus* oocytes (n=5). As a positive control, A23187 (10 μM) was applied.

DISCUSSION

The data in the present study showed that DMSO inhibited K_{ATP} current composed of Kir 6.2 and SUR1 but did not inhibit renal K_{ATP}, ROMK2 currents. Although the mechanisms for the action of DMSO on biological systems are not clear, it has been suggested that the primary mechanism(s) might involve an increase in $[Ca^{2+}]_i$ (Morley & Whitfield, 1993), an alteration of cell structure (Lyman et al, 1976) or direct interaction with subunit proteins (Henderson et al, 1975).

A possible mechanism for the DMSO inhibition of K_{ATP} currents could be the disturbance of the lipid-bilayer by DMSO, which might indirectly affect gating properties of Kir6.2/SUR1 channels. In guinea pig papillary muscle, the action potential prolongation induced by DMSO was partly related to cell shrinkage (Ogura et al, 1995). Gating of the K_{ATP} channel is mechanosensitive and mechanosensitive modulation may be significant under physiological and pathophysiological conditions (Van Wagner, 1993). Therefore, it is worthwhile considering whether the cell-shrinking effects of DMSO might have contributed to the modified electrical activity, for relatively high concentrations of DMSO were also used in our study. Another possible explanation for the K_{ATP} channel inhibition by DMSO might be a direct effect of osmolarity on K_{ATP} channel. Zimmerberg et al (1990) found that approximately 3.5 times hyperosmotic sucrose solutions applied to the inside and outside of perfused squid giant axons depressed axon delayed rectifier K^+ current by about 80%. However, 3% mannitol or 3% sucrose did not affect the amplitude of whole cell Kir6.2/SUR1 currents in *Xenopus* oocyte (Park et al, unpublished data), suggesting that osmotic stress does not explain the inhibition of whole cell Kir6.2/SUR1 currents.

An alternative explanation for K_{ATP} channel inhibition by DMSO may be a direct modification of channel proteins. Several lines of reports have suggested that DMSO inhibits various ion channel activity through direct interaction with these membrane proteins rather than the lipid bilayer and this interaction is reversible after removal of DMSO (Nakahiro et al, 1992). In the present study we cannot completely exclude the possibility that DMSO inhibits K^+ channel activity through the disturbance of cell membrane. However, selective block of Kir6.2/SUR1 current by DMSO over ROMK2 suggested that DMSO block of K_{ATP} currents in *Xenopus* oocyte could be mediated by protein specific mechanism(s).

Intracellular calcium spike or protein kinase activation by DMSO has been proved as a key step to stimulate cell differentiation in various cell lines. In the present study, however, DMSO did not affect the $[Ca^{2+}]_i$ in *Xenopus* oocytes (Fig. 5), suggesting that the DMSO inhibition of K_{ATP} current is not related to the change of $[Ca^{2+}]_i$. The suspicion was also supported by the result that Kir6.2/SUR1 current but not ROMK2 current was inhibited by DMSO, because increase in $[Ca^{2+}]_i$ has been shown to inhibit both of

pancreatic (Larsson et al, 1996) and renal K_{ATP} currents (Mauerer, 1998).

Interference with ion diffusion into the channel could be another possible effect by DMSO. However, ionic diffusion in a K^+ channel mouth does not appear to be rate limiting (Wagoner & Oxford, 1987).

In conclusion, DMSO blocked K_{ATP} currents expressed in *Xenopus* oocyte and it could be mediated by protein specific mechanism(s), which is independent on $[Ca^{2+}]_i$.

ACKNOWLEDGEMENTS

We thank Dr. S. Seino and Dr. L.G. Palmer for kind donation of Kir6.2 and SUR1 cDNA, and ROMK2 cDNA, respectively. The present study was supported by the Basic Science Research Institute program, Ministry of Education (BSRI-96-4435).

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