Glibenclamide Specifically Blocks ATP-Sensitive K^+ Channel Current in Atrial Myocytes of Guinea Pig Heart

Eiji HAMADA, Reiko TAKIKAWA, Hiroyuki ITO, Mari IGUCHI,
Akira TERANO, Tsuneaki SUGIMOTO and Yoshihisa KURACHI*

The Second Department of Internal Medicine, Faculty of Medicine,
University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113, Japan

Accepted September 28, 1990

Abstract—Effects of glibenclamide on the control membrane ionic currents, acetylcholine or adenosine-induced K^+ current, and nicorandil-induced K^+ current were examined in single atrial myocytes of guinea pig heart. The nystatin-whole cell clamp technique was used. Nicorandil evoked the time-independent K^+ current which is probably the current through the ATP-sensitive K^+ channel. Glibenclamide inhibited this current in a concentration-dependent fashion, although it had no effect on the other currents. We concluded that glibenclamide specifically inhibits the ATP-sensitive K^+ channel current in cardiac myocytes.

A variety of cells, including cardiac myocytes, skeletal muscle cells, smooth muscle cells and pancreatic β cells, have the intracellular-ATP-sensitive K^+ channel (1–3). Sulfonylurea derivatives, such as tolbutamide and glibenclamide, are reported to inhibit the ATP-sensitive K^+ channel (4, 5) and are now used to examine the functional roles of the K^+ channel on the electrical activity of several tissues, including the heart, in a variety of physiological and pathophysiological conditions, on the assumption that the effects of glibenclamide are very specific to the ATP-sensitive K^+ channel (6–8). However, it was recently reported that glibenclamide inhibits the adenosine-induced sinus bradycardia in the canine heart (9), suggesting that the drug might not be specific to the ATP-sensitive K^+ channel.

In the present study, we examined the effects of glibenclamide on the control membrane currents: the Na^+ current, the L type Ca^{2+} current, the inward-rectifying i_{K1} current and the delayed outward K^+ current, the acetylcholine or adenosine-induced K^+ current and nicorandil-induced ATP-sensitive K^+ current (10) in isolated atrial myocytes of the guinea pig to evaluate the specificity of the

drug to cardiac membrane ionic currents.

Single cardiac atrial myocytes were isolated from the guinea pig heart by enzymatic dissociation (11). Briefly, the heart was perfused with the nominally Ca -free bathing solution containing 0.4 mg/ml collagenase (Sigma type I) for 15–20 min, and then it was rinsed with high K^+ /low Cl^- solution. The heart was then stored at 4°C in the solution until the experiments. The control bathing solution contained: 136.5 mM NaCl, 5.4 mM KCl, 0.53 mM $MgCl_2$, 0.3 mM NaH_2PO_4 , 5 mM HEPES-NaOH, and 10 mM glucose (pH 7.4). The high K^+ /low Cl^- solution had the following composition: 70 mM glutamic acid, 15 mM taurine, 30 mM KCl, 10 mM KH_2PO_4 , 0.5 mM $MgCl_2$, 11 mM glucose, 0.5 mM EGTA, and 10 mM HEPES-KOH buffer (pH 7.4). The pipette solution contained: 145 mM KCl and 5 mM HEPES-KOH buffer (pH 7.3). The solution also contained nystatin (Sigma, St. Louis, U.S.A.) at the concentration of 150 μ g/ml (12). Acetylcholine (ACh) and adenosine were purchased from Sigma. Glibenclamide and nicorandil were gifts from Hoechst (Frankfurt, F.R.G.) and Chugai Pharmaceutical Co. (Tokyo, Japan), respectively. All experiments were performed at 30°C.

Figure 1 shows the effects of glibenclamide

* To whom all correspondence should be addressed.

Fig. 1. Effects of glibenclamide on the acetylcholine- or adenosine-induced K^+ current and nicorandil-induced K^+ current. Whole cell membrane currents from an atrial myocyte. The myocyte was held at -40 mV under the whole cell voltage clamp condition using the nystatin method. Acetylcholine (ACh, $1 \mu\text{mol/l}$), adenosine (Ado, $10 \mu\text{mol/l}$), nicorandil ($500 \mu\text{mol/l}$) and glibenclamide (Gli, $1 \mu\text{mol/l}$) were added to the bath solution. Experimental protocols are indicated below the current trace. The arrow indicates the zero current level.

on the ACh and adenosine-induced K^+ currents and nicorandil-induced K^+ current in an atrial myocyte of the guinea pig. The cell was held at -40 mV. ACh ($1 \mu\text{mol/l}$) and adenosine ($10 \mu\text{mol/l}$) induced outward currents flowing through a specific population of inward-rectifying K^+ channels as reported previously (10). The currents desensitized to steady levels. Glibenclamide ($1 \mu\text{mol/l}$), applied to the bath solution, did not affect the ACh or adenosine-induced outward currents at all. In the same cell, nicorandil ($500 \mu\text{mol/l}$) induced a huge outward current. The nicorandil-induced outward current was completely suppressed by glibenclamide ($1 \mu\text{mol/l}$). Since it was reported that nicorandil activated the ATP-sensitive K^+ channel current in cardiac ventricular myocytes (10), glibenclamide probably inhibited the ATP-sensitive K^+ current in the atrial myocyte.

Figure 2 shows current traces at various membrane potentials (A) and the current-voltage relations of an atrial cell in the control, under the perfusion with $500 \mu\text{mol/l}$ nicorandil and under the perfusion with the drug plus $1 \mu\text{mol/l}$ glibenclamide (B). The cell was held at -40 mV to inactivate the Na^+ current. Thus, the conventional L-type Ca^{2+} current and the delayed outward current were elicited on depolarization and so was the inward-rectifying i_{K1} current on hyperpolarization. Nicorandil induced outward currents at potentials more positive than -90 mV and inward currents at potentials more negative than -90 mV, indicating the nicorandil-induced current is a K^+ current (10). Under

nicorandil perfusion, the conventional L-type Ca^{2+} current was apparently diminished, probably due to the overlapping outward K^+ current. When glibenclamide was further added to the bathing solution, the nicorandil-induced K^+ current was completely suppressed and the Ca^{2+} current re-appeared. The initial and steady-state current-voltage relations in the control bathing solution and those under the perfusion with nicorandil plus glibenclamide were superimposable. Thus, glibenclamide specifically inhibited the nicorandil-induced K^+ current, probably the ATP-sensitive K^+ current, and did not appreciably affect the conventional L type Ca^{2+} current, the delayed outward current and the inward-rectifying i_{K1} current in atrial myocytes. We examined the effect of glibenclamide on the Na^+ current using the tight-seal whole cell clamp technique. We held the cell at -70 mV and perfused with low Na^+ bathing solution. It was also observed that glibenclamide ($1 \mu\text{mol/l}$) did not affect the Na^+ current in atrial myocytes (not shown).

The present study shows that glibenclamide does not affect the control membrane ionic currents of atrial myocytes; i.e., Na^+ current, L type Ca^{2+} current, the delayed-outward current and the inward-rectifying i_{K1} current, but specifically inhibits the nicorandil-induced ATP-sensitive K^+ channel current in a voltage-independent fashion. This observation is consistent with the previous report that glibenclamide does not affect the control action potential of adult guinea pig ventricular cells but suppresses the dinitrophenol-induced

Fig. 2. The current traces at various membrane potentials (A) and the current-voltage relations of an atrial cell in the control, under the perfusion with nicorandil and under the perfusion with nicorandil plus glibenclamide (B). A: The membrane currents in the control (the left column), under the perfusion with 500 $\mu\text{mol/l}$ nicorandil (the middle) and under the perfusion with nicorandil plus 1 $\mu\text{mol/l}$ glibenclamide (the right). The holding potential (hp) was -40 mV. CP: the command potential. The voltage protocol is indicated at the top of the left column. The arrow heads are the zero current level. B: The initial current-voltage relations (a) and the relations at the end of the command pulses (300 msec) (b). The initial relations were measured at the peak of the inward Ca^{2+} current or at 10 msec after the onset of command pulses. ●—●: Control, ■---■: Nicorandil, 500 $\mu\text{mol/l}$, ○-----○: Nicorandil, 500 $\mu\text{mol/l}$, + Glibenclamide, 1 $\mu\text{mol/l}$.

ATP-sensitive K^+ channel current (4).

Since we used the nystatin-method of whole cell recording (12), it may be expected that the intracellular ATP concentration of the atrial myocytes was minimally affected in the present study. Thus, the present study suggests that a high dose of nicorandil can activate the ATP-sensitive K^+ channel in cardiac myocytes even under physiological conditions. Since it was reported that the ATP-sensitive K^+ channel is completely inhibited by 1 mmol/l of intracellular ATP in the cardiac cell membrane (1), and the intracellular ATP concentration in normal cardiac cells is 3–4 mmol/l (13), it may be indicated that nicorandil alters the sensitivity of the K^+ channels to intracellular ATP in cardiac myocytes.

Although it was reported that glibenclamide inhibited adenosine-induced sinus bradycardia in the isolated heart under Langendorff perfusion (9), a high dose (1 μ mol/l) of glibenclamide had no effects on the muscarinic K^+ channel current activated by acetylcholine or adenosine in the present study. We suggest that some difference of the experimental conditions might have influenced their results.

It was reported that another sulfonylurea derivative, tolbutamide, inhibits the ATP-sensitive K^+ channel with a K_i of 380 μ mol/l in cardiac myocytes (14). The value is 20–50 times higher than that (7–18 μ mol/l) in pancreatic β -cells. Glibenclamide blocked the nicorandil-induced ATP-sensitive K^+ channel with a K_i of around 4 nmol/l ($n=4$) in the present study (data not shown). The value is about 4–40 times larger than that in β -cells (0.1–1 nmol/l) (15). These observations suggest that the difference in the sensitivity of the ATP-sensitive K^+ channel to sulfonylurea derivatives between the cardiac myocytes and pancreatic β -cells might represent an intrinsic difference in the properties of the K^+ channels in these cells.

The present study showed that glibenclamide specifically inhibits the ATP-sensitive K^+ channel current and does not affect the control ionic currents, including the ACh- or adenosine-induced K^+ current in cardiac myocytes with relatively small K_i (4 nmol/l). Thus, those results may indicate that glibenclamide can be used as an excellent specific

blocker of the ATP-sensitive K^+ channel under various physiological and pathophysiological conditions in cardiac preparations.

Acknowledgement: This work was supported by grants from the Ministry of Education, Culture and Science of Japan and the Research Program on Cell Calcium Signalling in the Cardiovascular System and by a grant for the study of cardiovascular disease (1A-1) from the Ministry of Health and Welfares of Japan.

References

- 1 Noma, A.: ATP-regulated K^+ channel in cardiac muscle. *Nature* **305**, 147–148 (1983)
- 2 Cook, D.L. and Hales, C.N.: Intracellular ATP directly blocks K^+ channel in pancreatic B-cells. *Nature* **311**, 271–273 (1984)
- 3 Trube, G. and Hescheler, J.: Inward-rectifying channels in isolated patches of the heart cell membrane: ATP-dependence and comparison with cell-attached patches. *Pflugers Arch.* **401**, 178–184 (1984)
- 4 Fosset, M., De Weille, J.R., Green, R.D., Schmid-Antomarchi, H. and Lazdunski, M.: Antidiabetic sulfonylureas control action potential properties in heart cells via high affinity receptors that are linked to ATP-dependent K^+ channels. *J. Biol. Chem.* **263**, 7933–7936 (1988)
- 5 De Weille, J.R., Fosset, M., Mourre, C., Schmid-Antomarchi, H., Bernardi, H. and Lazdunski, M.: Pharmacology and regulation of ATP-sensitive K^+ channels. *Pflugers Arch.* **414**, Supp. 1, S80–S87 (1989)
- 6 Grigg, J.J. and Anderson, G.: Glucose and sulfonylureas modify different phases of the membrane potential change during hypoxia in rat hippocampal slices. *Brain Res.* **489**, 302–310 (1989)
- 7 Standen, N.B., Quayle, J.M., Davies, N.W., Brayden, J.E., Huang, Y. and Nelson, M.T.: Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science* **245**, 177–180 (1989)
- 8 Wilde, A.A.M., Escande, D., Schumacher, C.A., Thuringer, D., Mestre, M. and Fiolet, J.W.T.: Glibenclamide inhibition of ATP-sensitive K^+ channels and ischemia-induced K^+ accumulation in the mammalian heart. *Pflugers Arch.* **414** Supp. 1, S176 (1989)
- 9 Belloni, F.L., Chiruzzo, F.Y. and Hintze, T.H.: Inhibition of adenosine-induced sinus bradycardia by glibenclamide. *Circulation* **80**, Supp. II, II-132 (1989)
- 10 Hiraoka, M. and Fan, Z.: Activation of ATP-sensitive outward K^+ current by nicorandil (2-

- nicotinamidoethyl nitrate) in isolated ventricular myocytes. *J. Pharmacol. Exp. Ther.* **250**, 278–285 (1989)
- 11 Kurachi, Y., Nakajima, T. and Sugimoto, T.:** On the mechanisms of activation of muscarinic K⁺ channels by adenosine in isolated atrial cells: involvement of GTP-binding proteins. *Pflugers Arch.* **407**, 264–274 (1986)
 - 12 Kurachi, Y., Asano, Y., Takikawa, R. and Sugimoto, T.:** Cardiac Ca²⁺ current does not run down and is very sensitive to isoprenaline in the nystatin-method of whole cell recording. *Naunyn Schmiedeberg's Arch. Pharmacol.* **340**, 219–222 (1989)
 - 13 Khairallah, P.A. and Mommaerts, W.F.H.M.:** Nucleotide metabolism in cardiac activity; I. Methods and initial observations. *Circ. Res.* **1**, 8–11 (1953)
 - 14 Belles, B., Hescheler, J. and Trube, G.:** Changes of membrane currents in cardiac cells induced by long whole-cell recordings and tolbutamide. *Pflugers Arch.* **409**, 582–588 (1987)
 - 15 Schmidt-Antomarchi, H., De Weille, J., Fosset, M. and Lazdunski, M.:** The receptor from anti-diabetic sulfonylureas controls the activity of the ATP-modulated K⁺ channel in insulin-secreting cells. *J. Biol. Chem.* **262**, 15840–15844 (1987)