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Inhibitory effects of berberine on ATP-sensitive K^+ channels in cardiac myocytes

Yong-Xiao Wang [a,*], Yun-Min Zheng [b], Xiao-Bao Zhou [b]

[a] *Department of Pharmacology, Fourth Military Medical University, Xian 710032, People's Republic of China*
[b] *Department of Aviation Medicine, Fourth Military Medical University, Xian 710032, People's Republic of China*

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Abstract

The effects of berberine on cardiac action potentials were measured in isolated guinea-pig papillary muscles exposed to hypoxia and cromakalim using the standard microelectrode technique. In addition, the patch clamp technique was used to determine the effects of berberine on cromakalim-induced outward currents in isolated ventricular myocytes and on ATP-sensitive K^+ (K_{ATP}) channels in inside-out membrane patches. Berberine, at 3 μM significantly inhibited, while at 100 μM completely blocked the shortening of action potential duration and effective refractory period induced by hypoxia or cromakalim (100 μM). Under the whole-cell voltage clamp conditions, berberine (3–100 μM) attenuated or even abolished the cromakalim-elicited outward K^+ currents. Berberine (3–100 μM) inhibited K_{ATP} channel activity in a concentration-dependent fashion in inside-out membrane patches exposed to 0.1 mM ATP. This inhibition appeared to be mainly due to a decrease in the open channel probability without affecting unitary conductance or the time constants for open and closed channel times. Glibenclamide (10 μM) partially blocked the hypoxia-evoked but fully reversed the cromakalim-evoked abbreviation of action potential duration and effective refractory period. Both the whole-cell outward K^+ currents induced by cromakalim and the opening of single K_{ATP} channels induced by the low intracellular ATP concentration were also completely abolished by 10 μM glibenclamide. We conclude that berberine is a blocker of the cardiac K_{ATP} channel. The reported beneficial effect of berberine on ischemia-induced arrhythmias is likely attributed to its inhibition of K_{ATP} channel activation and subsequent shortening of action potential duration and effective refractory period during ischemia.

Keywords: Berberine; K^+ channel, ATP-sensitive; Patch clamp; Microelectrode recording; Cardiac cell

1. Introduction

Berberine, a benzodioxoloquinozine alkaloid isolated from the plants of genera *Berberis* and *Coptis*, has been shown to produce a significant protection against cardiac arrhythmias induced by coronary artery occlusion and other factors (Ribeiro et al., 1982; Wang et al., 1986, 1992a, 1993a,b, 1994). Moreover, this alkaloid exerts a beneficial effect on cardiac arrhythmias in patients (Huang, 1990). Using the standard microelectrode technique, berberine has been demonstrated to decrease the maximal velocity of depolarization (V_{max}), to prolong the action potential duration and effective refractory period, and to suppress the development of delayed afterdepolarizations and triggered activity in cardiac myocytes in vitro and in vivo (Wang et al., 1987, 1991, 1993b, 1994; Wang and Tan, 1987; Neto, 1993). However, so far the ionic mechanisms responsible for its effects are not fully clear.

The ATP-sensitive K^+ (K_{ATP}) channel is activated by a decrease in intracellular ATP concentration in cardiac cells, which can be induced by hypoxia, ischemia or metabolic blockade (Noma, 1983; Trube and Hescheler, 1983, 1984; Noma and Shibasaki, 1984; Sanguinetti et al., 1988; Nichols and Lederer, 1991; Weiss et al., 1992; Edwards and Weston, 1993; Deutsch and Weiss, 1993). The activation of K_{ATP} channels brings about a marked shortening of action potential duration and effective refractory period in cardiomyocytes. K_{ATP} channel openers can mimic the effects of hypoxia on action potential duration and effec-

* Corresponding author. Present address: Department of Animal Biology, University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104, USA. Tel.: (1-215) 898-2840; Fax: (1-215) 898-9923; e-mail: yongxiao@pobox.upenn.edu

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tive refractory period, whereas K_{ATP} channel blockers prevent the effects of hypoxia and K_{ATP} channel openers (Kojima and Ban, 1988; Mestre et al., 1988; Smallwood and Steinberg, 1988; Sanguinetti et al., 1988; Nichols and Lederer, 1991; Edwards and Weston, 1993).

The present study was designed to investigate the effects of berberine on cardiac action potentials in isolated guinea-pig papillary muscles exposed to hypoxia or cromakalim using the standard microelectrode technique, and on cromakalim-induced outward currents in isolated ventricular myocytes and K_{ATP} channels in inside-out membrane patches using the patch clamp technique. This could lead to a further insight into the ionic mechanisms for berberine's effects.

2. Materials and methods

2.1. Action potential recording

Experimental procedures have been described previously (Wang et al., 1992b, 1994). Briefly, right ventricular papillary muscles (diameter, 0.6–0.9 mm) were rapidly obtained from male or female guinea pigs weighing 300–450 g and placed in a 1-ml bath chamber. Preparations were continuously superfused with Tyrode's solution at a rate of 5 ml/min. The solution was bubbled with 95% O_2 and 5% CO_2 and warmed to 35°C where it was maintained within 0.5°C. The solution composition was (mM): NaCl 137.6, $NaHCO_3$ 12.0, NaH_2PO_4 1.8, $MgCl_2$ 0.5, KCl 4.0, $CaCl_2$ 2.0, glucose 5.5 (pH 7.4). Electrical stimuli of 3 ms duration and 1.5 times the threshold voltage at 1 Hz frequency were delivered by a stimulator (Nihon Kohden, SEN-710032) through a bipolar platinum electrode. After a 1-h equilibration period, transmembrane action potentials were measured by the standard glass microelectrode technique using a microelectrode amplifier (Nihon Kohden, MEZ-7101). The V_{max} was obtained by an electronic differentiator (Dan Yang, BME-1) with a linear differentiation in the range from 0 to 1000 mV. The effective refractory period was measured by introducing a premature stimulation (3 ms duration, 5 times the threshold voltage) after 7 basic pacing stimulations (3 ms duration and 1.5 times the threshold voltage at 1 Hz frequency). The delayed interval of the premature stimulation was increased progressively up to the longest interval at which the premature stimulation failed to induce an action potential. Action potential variables measured were as follows: action potential amplitude, resting potential, V_{max}, action potential duration at 50% and 90% repolarization and effective refractory period.

To determine the effect of berberine during hypoxia, preparations were superfused with the hypoxic solution, which had the same composition as that mentioned above, except it was glucose-free and gassed by 95% N_2 and 5% CO_2. This hypoxic solution yielded pH of 6.8–7.0 and PO_2 of 6–9 mmHg.

2.2. Current recording

The isolation procedure of single myocytes is similar to that of our previous paper (Wang and Korth, 1995). Male or female guinea pigs weighing 300–400 g were killed by cervical dislocation. The heart was rapidly taken out, and mounted on a Langendorff apparatus. A retrograde coronary perfusion was performed at a constant rate of 10 ml/min with the following solutions (37°C): the Krebs-Henseleit solution (2 mM Ca^{2+}) for 4 min, and then the nominally Ca^{2+}-free Joklik solution (Joklik-MEM, Biochrom) for 4 min, and finally the enzyme Joklik solution containing 0.42 mg/ml collagenase (Worthington), 0.5 mg/ml protease (Sigma), 0.2 mg/ml trypsin (Serva), 1 mg/ml albumin (Sigma) and 50 μM Ca^{2+} for 8–10 min. After that, the ventricles were triturated and incubated for 5 min in the enzyme Joklik solution. The enzyme Joklik solution containing single cells was filtered through a mesh with a diameter of 200 μm and centrifuged at 800 r.p.m. for 3 min. Cells were resuspended and stored in the Joklik solution containing 1% albumin and 50 μM Ca^{2+} at room temperature.

Membrane currents were measured by whole-cell and inside-out configurations of the patch clamp technique (Hamill et al., 1981) using a patch clamp amplifier (L/M EPC7, List Medical Electronic, Germany). In whole cell clamp conditions, the electrode resistance ranged between 2 and 4 MΩ. Pipettes having resistance of 5–8 MΩ were used for recording single channel currents. Following the formation of a gigaohm seal, membrane patches were disrupted by a slight suction to get whole cell patch configurations, and then capacitance and series resistance were compensated. The average membrane capacitance was 87.3 ± 12.4 pF. The degree of series resistance compensation was up to 60%. Inside-out configurations were obtained through pulling a small membrane vesicle and exposing the vesicle to the air for about 1 s. Drug application was performed with a microperfusion system at a rate of 3 μl/min. All experiments were carried out at 30 ± 0.5°C. Current and voltage signals were stored on a videocassette recorder. Then data were reproduced, filtered at 1 kHz and analyzed off-line on a computer.

In the whole-cell patch clamp experiments, pipettes were filled with (mM): K-aspartate 110, KCl 27, $MgCl_2$ 2, HEPES 11, EGTA 10, K_2-ATP 3, (pH adjusted to 7.4 with KOH); the composition of extracellular solution was (mM): NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, HEPES 5.5, glucose 5.5 (pH 7.4 with NaOH). In the single channel recordings, the pipette solution contained (mM): K-aspartate 110, KCl 27, $MgCl_2$ 2, HEPES 11, EGTA 10, K_2-ATP 0.1, (pH 7.4 with KOH). The bath solution consisted of the same composition as the pipette solution.

2.3. Drugs

Berberine was purchased from Sigma (St. Louis, MO, USA) and dissolved in distilled water to make a 10 mM stock solution. Appropriate portions of this stock solution were added to the bath solution just before use to achieve the final concentrations needed. Cromakalim and glibenclamide from Sigma were dissolved in dimethylsulfoxide. A maximal solvent concentration was 0.1% in the final bath solution.

2.4. Statistics

Results are expressed as means ± S.D. Student's paired *t*-test was used to determine the significance of differences between observations within groups. One-way ANOVA (analysis of variance) for repeated measurements was used to asses the statistical differences of the values between groups. A P value of less than 0.05 was considered significant.

3. Results

3.1. Effect on action potentials under hypoxic conditions

Superfusion with the hypoxic solution produced a marked shortening of action potential duration at 50% and 90% repolarization and effective refractory period, accompanied by a slight reduction in action potential amplitude, resting potential and V_{max} in isolated guinea-pig right ventricular papillary muscles. These changes became detectable within 5 min, reached a steady-state at about 30 min, and were stably sustained up to 60 min (Fig. 1A and Table 1).

To test the effect of berberine, muscles were simultaneously exposed to this alkaloid for 30 min during continued hypoxia after 30 min of hypoxia. Berberine at low concentration (3 μM) significantly inhibited the hypoxia-induced shortening of repolarization, restoring action potential duration at 50% and 90% repolarization and effective refractory period from 49.1%, 56.4% and 55.8% of the control to 59.7%, 67.5% and 65.6% ($n = 6$, $P < 0.05$), whereas at high concentration (100 μM) the alkaloid abolished the hypoxia effects. Action potential duration at 50% and 90% repolarization and effective refractory period were restored to 96.7%, 97.3% and 96.6% ($n = 6$). Moreover, berberine (100 μM) caused a further decrease in V_{max}, action potential amplitude and resting potential (Fig. 1B and Table 1).

As seen from Fig. 1C and Table 1, a K_{ATP} channel blocking agent, glibenclamide (10 μM) partially antagonized the hypoxia-induced decrease in action potential duration and effective refractory period. Thirty minutes after superfusion of the agent, the reduction of action potential duration at 50% and 90% repolarization and effective refractory period were respectively returned to 74.8%, 82.5% and 79.6% of the control ($n = 6$, $P < 0.05$ compared with before the addition of glibenclamide). Unlike berberine, glibenclamide (10 μM) had no statistically significant effects on V_{max}, action potential amplitude and resting potential during the period of hypoxia.

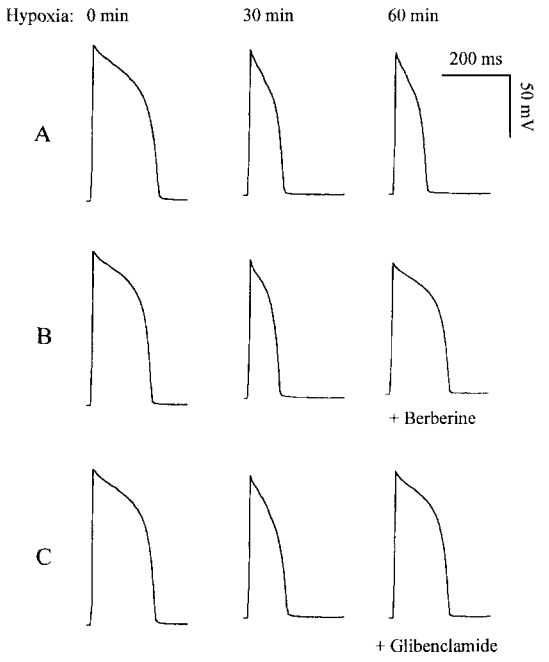

Fig. 1. Effect of berberine and glibenclamide on the hypoxia-induced change of action potential in the isolated guinea-pig papillary muscle. Panel (A) (control recording) shows that superfusion of the hypoxic solution for 30 min produced a marked shortening in action potential duration, with a slight reduction in action potential amplitude and resting potential. They were stable up to 60 min. In panel (B), exposure of 100 μM berberine to another muscle for 30 min during the continued superfusion of the hypoxic solution after 30 min of hypoxia abolished the hypoxia-induced shortening of action potential duration. In panel (C), 10 μM glibenclamide also had an inhibitory effect on the hypoxia-induced response.

3.2. Effect on action potentials in the presence of cromakalim

In good agreement with published papers (Mestre et al., 1988; Sanguinetti et al., 1988), the application of 100 μM cromakalim (an opener of K_{ATP} channels) greatly and time-dependently shortened action potential duration and effective refractory period. Thirty minutes after addition of the drug, its action was up to a maximum. Prolonged exposure to 60 min did not cause a further shortening. V_{max}, action potential amplitude and resting potential were not significantly modified (Table 2).

Berberine (100 μM) fully antagonized the cromakalim-induced abbreviation of action potential duration and effective refractory period. The reduction in action potential duration at 50% and 90% repolarization and effective refractory period after this alkaloid were 96.6%, 96.8% and 96.1% ($P < 0.05$, $n = 6$), compared with 42.8%, 46.3% and 44.5% before this alkaloid. As in papillary muscles subsequently exposed to hypoxia, berberine also reduced V_{max}. However, both action potential amplitude and resting potential were not altered (Table 2).

Table 1
Effect of berberine and glibenclamide on the hypoxia-induced changes in action potentials of isolated guinea-pig ventricular papillary muscles

	Drug	(μM)	Hypoxia		Hypoxia + drug tested
			Before	After	
APA (mV)	Control		126 ± 3	120 ± 4 [a]	118 ± 4
	Berberine	3	127 ± 4	117 ± 3 [a]	116 ± 3
	Berberine	100	124 ± 4	118 ± 5 [a]	112 ± 3 [b]
	Glibenclamide	10	127 ± 3	119 ± 5 [a]	117 ± 4
RP (mV)	Control		−89 ± 4	−84 ± 3 [a]	−82 ± 3
	Berberine	3	−88 ± 4	−83 ± 4 [a]	−83 ± 3
	Berberine	100	−86 ± 3	−82 ± 4 [a]	−76 ± 4 [b]
	Glibenclamide	10	−88 ± 4	−84 ± 2 [a]	−82 ± 2
V_{max} (V/s)	Control		261 ± 48	236 ± 54 [a]	233 ± 52
	Berberine	3	277 ± 29	232 ± 26 [a]	221 ± 23 [b]
	Berberine	100	264 ± 33	227 ± 28 [a]	209 ± 25 [b]
	Glibenclamide	10	253 ± 38	230 ± 32 [a]	233 ± 31
APD_{50} (ms)	Control		118 ± 17	61 ± 10 [a]	58 ± 9
	Berberine	3	114 ± 11	56 ± 5 [a]	68 ± 6 [b]
	Berberine	100	121 ± 14	65 ± 8 [a]	117 ± 11 [b]
	Glibenclamide	10	115 ± 12	58 ± 7 [a]	86 ± 8 [b]
APD_{90} (ms)	Control		182 ± 24	106 ± 16 [a]	104 ± 15
	Berberine	3	179 ± 17	101 ± 9 [a]	121 ± 9 [b]
	Berberine	100	187 ± 19	111 ± 12 [a]	182 ± 18 [b]
	Glibenclamide	10	178 ± 20	103 ± 11 [a]	147 ± 12 [b]
ERP (ms)	Control		157 ± 21	88 ± 18 [a]	87 ± 17
	Berberine	3	154 ± 16	86 ± 9 [a]	101 ± 10 [b]
	Berberine	100	149 ± 15	85 ± 13 [a]	144 ± 16 [b]
	Glibenclamide	10	152 ± 18	83 ± 12 [a]	121 ± 13 [b]

Data are means ± S.D. from 6 different tissues. [a] $P < 0.05$ compared with prehypoxia; [b] $P < 0.05$ compared with hypoxia. APA, action potential amplitude; RP, resting potential; V_{max}, maximal velocity of depolarization; APD_{50} and APD_{90}, action potential duration at 50 and 90% depolarization; ERP, effective refractory period.

Table 2
Effect of berberine 100 μM and glibenclamide 10 μM on cromakalim (100 μM)-induced changes in action potentials in isolated guinea-pig ventricular papillary muscles

		Cromakalim		Cromakalim + drug tested
		Before	After	
APA (mV)	Control	124 ± 5	125 ± 4	126 ± 4
	Berberine	125 ± 4	127 ± 5	126 ± 3
	Glibenclamide	124 ± 4	126 ± 3	124 ± 4
RP (mV)	Control	−87 ± 3	−89 ± 4	−90 ± 4
	Berberine	−88 ± 4	−91 ± 4	−89 ± 2
	Glibenclamide	−86 ± 3	−88 ± 2	−86 ± 3
V_{max} (V/s)	Control	259 ± 41	263 ± 35	261 ± 38
	Berberine	248 ± 33	247 ± 32	224 ± 30 [b]
	Glibenclamide	251 ± 39	257 ± 25	256 ± 26
APD_{50} (ms)	Control	124 ± 16	56 ± 7 [a]	54 ± 7
	Berberine	119 ± 19	51 ± 5 [a]	115 ± 18 [b]
	Glibenclamide	121 ± 14	59 ± 8 [a]	120 ± 15 [b]
APD_{90} (ms)	Control	193 ± 21	92 ± 15 [a]	88 ± 16
	Berberine	188 ± 24	87 ± 20 [a]	182 ± 21 [b]
	Glibenclamide	185 ± 19	85 ± 13 [a]	186 ± 17 [b]
ERP (ms)	Control	160 ± 17	75 ± 8 [a]	72 ± 9
	Berberine	155 ± 20	69 ± 11 [a]	149 ± 22 [b]
	Glibenclamide	152 ± 16	70 ± 9 [a]	154 ± 15 [b]

Data are means ± S.D. from 6 preparations. [a] $P < 0.05$ compared with pre-cromakalim; [b] $P < 0.05$ compared with cromakalim. Abbreviations are the same as in Table 1.

Different from that under the hypoxic condition, 10 μM glibenclamide completely abolished the shortening of action potential duration and effective refractory period induced by cromakalim. Action potential duration at 50% and 90% repolarization and effective refractory period were, respectively, restored to 96.6%, 100.0% and 101.3% of the control ($n = 6$). This compound had no effects on V_{max}, action potential amplitude and resting potential (Table 2).

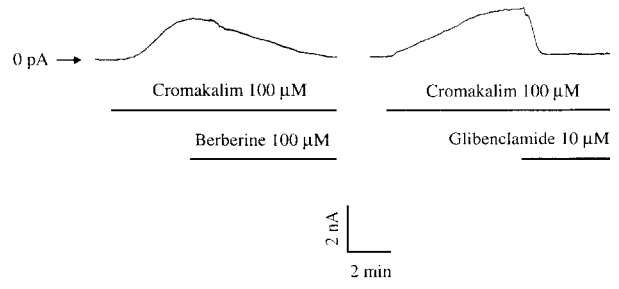

Fig. 2. Berberine (100 μM) or glibenclamide (10 μM) completely blocked the holding membrane outward current induced by 100 μM cromakalim in single guinea-pig ventricular myocyte. Cells were held at −40 mV under whole-cell voltage clamp conditions. Bars under the current traces show the protocol of application of cromakalim, berberine and glibenclamide.

3.3. Effects on whole-cell membrane currents

As shown in Fig. 2, application of 100 μM cromakalim to ventricular myocytes clamped at −40 mV induced holding outward currents. In the 9 cells, its mean amplitude was 2.15 ± 0.32 nA. Glibenclamide (10 μM) completely inhibited the cromakalim-induced outward currents with a mean time at half-block of 34.3 ± 2.8 s ($n = 4$).

Similar to glibenclamide, 100 μM berberine exerted a complete block of the cromakalim-induced outward currents. The time at half-block of the induced currents was 138.5 ± 11.4 s ($n = 5$), which was slower than that with glibenclamide. This result suggests that berberine might be a K_{ATP} channel blocker.

To investigate the effects of berberine and glibenclamide on the current-voltage relationship of the outward currents induced by cromakalim (100 μM), command voltage pulses of 500 ms in duration, from a holding potential of −40 mV to various membrane potentials

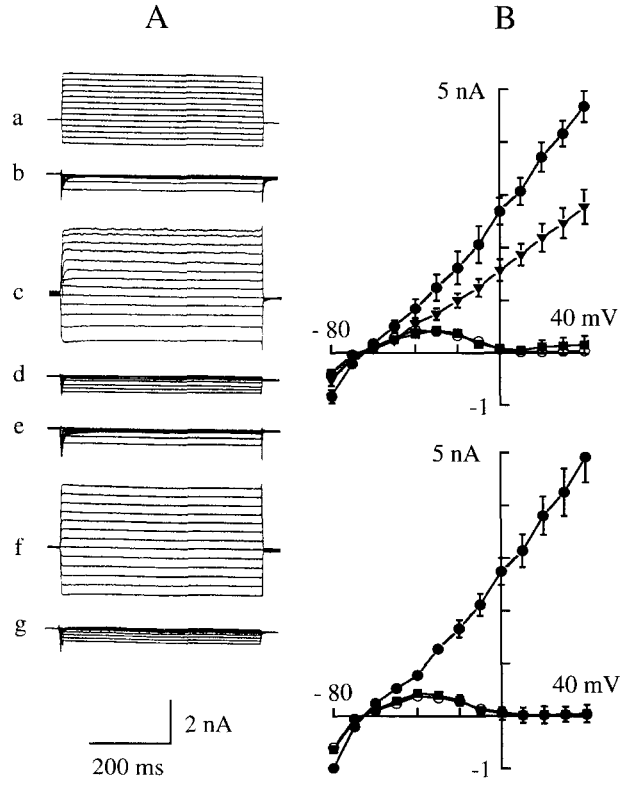

Fig. 3. Inhibitory effect of berberine and glibenclamide on current-voltage relationships of outward membrane currents induced by 100 μM cromakalim in single guinea-pig ventricular myocytes. The holding potential was held at −40 mV. Command voltage pulses of 500 ms in duration to various potentials from −80 to 40 mV were applied to the cells at 0.2 Hz. Panel (A) shows voltage protocol and sample current traces. (a) Voltage protocol; (b) and (e) control; (c) and (f) cromakalim; (d) cromakalim and 100 μM berberine; (g) cromakalim and 10 μM glibenclamide. Panel (B) summarizes effects of berberine (at the top) and glibenclamide (at the bottom). (○) Control; (●) 100 μM cromakalim; (▼) 3 μM berberine and 100 μM cromakalim; (■) 100 μM berberine or 10 μM glibenclamide in the presence of 100 μM cromakalim. Each point represents means ± S.D. from 6 different cells.

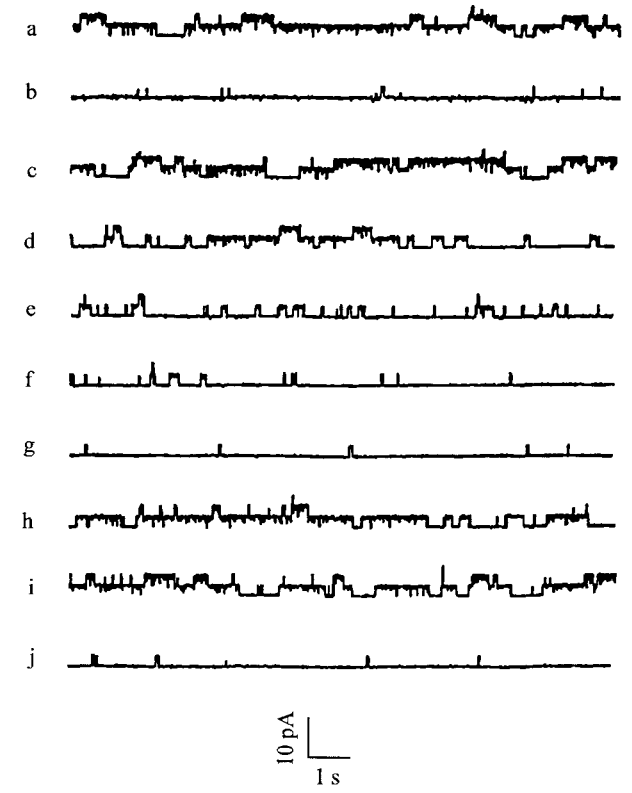

Fig. 4. Inhibitory effects of berberine and glibenclamide on K_{ATP} channels in inside-out membrane patches from guinea-pig ventricular myocytes. Membrane potential was held at +40 mV. Panels (a) and (b), respectively, depict the activity of K_{ATP} channels in the presence of 0.1 and 3 mM ATP concentration. The activity of K_{ATP} channels was dramatically decreased, as ATP concentration was increased to 3 mM (b) from 0.1 mM ((a), control). In panels (c)–(h), berberine inhibited the K_{ATP} channel activity in a concentration-dependent manner. Its action was reversible after the washout of the alkaloid. (c) Control; (d) 3 μM berberine; (e) 10 μM berberine; (f) 30 μM berberine; (g) 100 μM berberine; (h) washout of berberine. Panels (i) and (j) show, respectively, the activity of K_{ATP} channels before and after 10 μM glibenclamide. The agent fully blocked the activity of K_{ATP} channels.

(from −80 to 40 mV) were applied to ventricular cells at 0.2 Hz. When 100 μM cromakalim was given, prominent outward currents were brought about (Fig. 3). This result was similar to that of Shen et al. (1992).

Berberine (3 μM) significantly inhibited the outward currents elicited by cromakalim. At a higher concentration (100 μM), this drug exerted a more potent inhibiting action, producing a nearly complete blockade (top panel of Fig. 3). Glibenclamide (10 μM) also fully abolished the cromakalim-evoked outward currents (bottom panel of Fig. 3).

3.4. Effects on inside-out single channel currents

When inside-out membrane patches were bathed in 0.1 mM ATP, the activation of at least 3 K_{ATP} channels was detected (Fig. 4a, c, h and i). These channels exhibited a large elementary conductance of 76 ± 4 pS and high activity ($P_o = 0.944 \pm 0.081$, $n = 14$). P_o was expressed as a

cumulative open probability, which was calculated as a fraction of the total length of time that any channel was in an open state over the total recording duration. The channels were sensitive to intracellular ATP. As the ATP concentration increased to 3 mM from 0.1 mM, the activity of K_{ATP} channels was dramatically decreased (Fig. 4). In the total of 4 patches, P_o was reduced from 0.951 ± 0.035 to 0.034 ± 0.007 ($P < 0.01$).

Fig. 4 also illustrates sample current traces from an inside-out membrane patch exposed to berberine (3–100 μM). This compound produced a concentration-dependent suppressive action on the activation of K_{ATP} channels without altering their unitary conductance. At the concentration of 3 μM it had a detectable effect (Fig. 4d). P_o was decreased from 0.974 to 0.908. 100 μM of this alkaloid fully inhibited the channel openings (Fig. 4g). Its action was reversible after the washout of the drug (Fig. 4h). The concentration-response curve of berberine on the K_{ATP} channel activity in the presence of 0.1 mM ATP is summarized in Fig. 5. The steady-state channel activity was determined after the application of each concentration of berberine and normalized to the channel activity in the control. The relation could be fit by the Hill equation using the least-squares method:

$$y = 1/\left\{1 + ([D]/K_i)^H\right\}$$

where y is the relative P_o, [D] is the concentration of berberine, K_i is the berberine concentration at the half-maximal inhibition of the channel, and H is the Hill coefficient. The K_i and H were 18.25 μM and 2.78 respectively ($n = 6$).

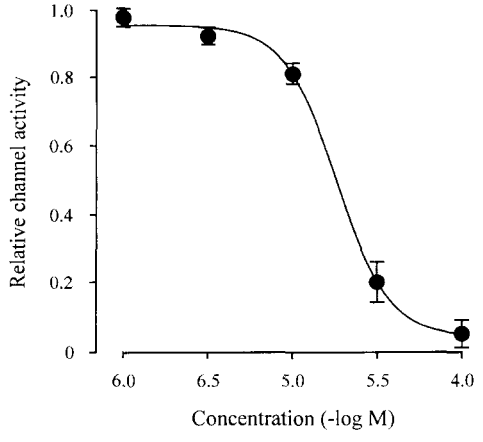

Fig. 5. Relation between the concentration of berberine and the cumulative open probability of the K_{ATP} channels in inside-out membrane patches from guinea-pig ventricular myocytes. Open probability (P_o) was calculated as a fraction of the total length of time that each channel was in an open state over the total recording duration. The relation could be fitted by the Hill equation: $y = 1/\{1 + ([D]/K_i)^H\}$, where y is the relative P_o, [D] is the concentration of berberine, K_i is the berberine concentration at the half-maximal inhibition of the channel, and H is the Hill coefficient. The K_i and H were 18.25 μM and 2.78 respectively. The continuous line in the graph was a curve fit to the Hill equation using the least-squares method. Data points indicate the means ± S.D. from 6 different cells.

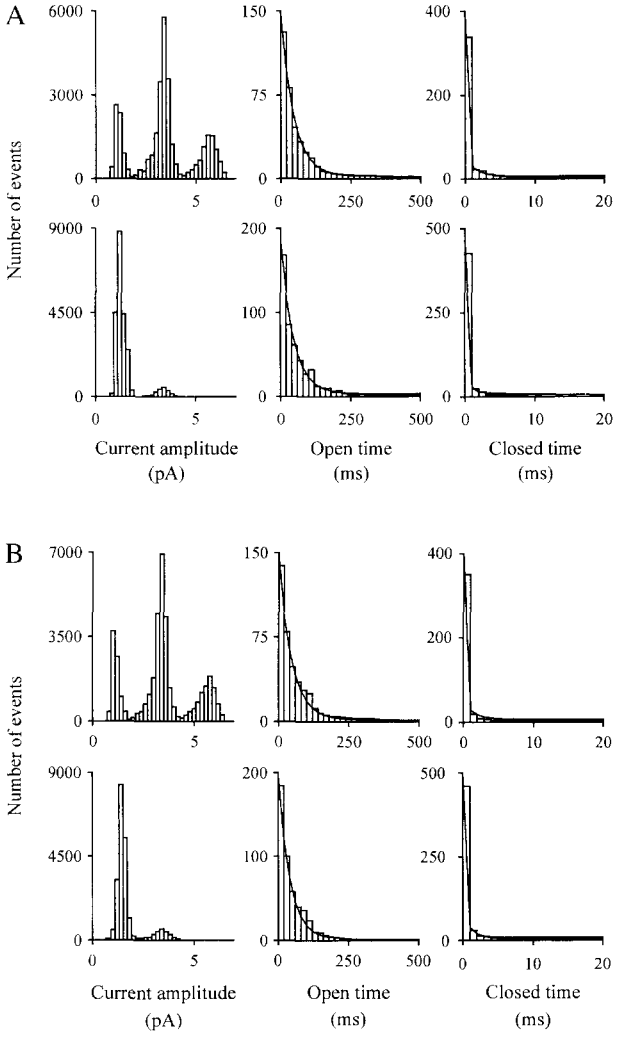

Fig. 6. Distribution of amplitude, open time and closed time of K_{ATP} channel currents recorded from inside-out membrane patches of guinea-pig ventricular cells. Panel (A) shows that 100 μM berberine inhibited the channel activity (left side), but did not alter the time constant for open time (middle) and closed time (right side). In panel (B), 10 μM glibenclamide had the same action as that of berberine.

Glibenclamide (10 μM) completely blocked the activity of K_{ATP} channels in the presence of 0.1 mM ATP (Fig. 4j). After application of glibenclamide, P_o was decreased from 0.945 ± 0.098 to 0.029 ± 0.006 ($P < 0.01$, $n = 4$), but the unitary conductance was not significantly changed (77 ± 3 vs. 76 ± 3 pS).

The measurement of the current amplitude histograms, as depicted in Fig. 6, further indicated that in the presence of 0.1 mM ATP, there existed robust multiple channel openings (top panels of Fig. 6A and B). Berberine (bottom panel of Fig. 6A), like glibenclamide (bottom panel of Fig. 6B), possessed an inhibitory effect on K_{ATP} channels.

As we can see in Fig. 6, the open-time histograms of the K_{ATP} channel were well fitted with single-exponential functions, whereas the closed-time histograms were well fitted with two-exponential functions. In the control, the

time constant for the open time was 42 ± 7 ms (n = 14); the fast and slow components of the time constant for the closed time were 0.39 ± 0.08 and 0.41 ± 0.03 ms (n = 14). Both berberine and glibenclamide did not alter the time constants for the open time and closed time of the K_{ATP} channels. This result suggests that both berberine and glibenclamide may not directly interact with the channel gating kinetics.

4. Discussion

In this study, we showed that berberine at 3 μM significantly inhibited, and at 100 μM completely abolished the shortening of action potential duration and effective refractory period induced by hypoxia and by cromakalim in isolated guinea-pig ventricular papillary muscles. It has been assumed that the mechanism responsible for the shortening in action potential duration and effective refractory period produced by hypoxia is a decrease in the slow inward Ca^{2+} current and the activation of one or more outward K^+ currents. These K^+ currents include K_{ATP} currents, inward rectifier K^+ and arachidonate-activated K^+ currents, etc. However, the activation of the K_{ATP} channel plays the major role in this effect of hypoxia. This consideration, taken along with the fact that cromakalim is thought to shorten action potential duration and effective refractory period by opening K_{ATP} channels, leads to the suggestion that berberine may block cardiac K_{ATP} channels.

To test the aforementioned suggestion, whole-cell and inside-out configurations of the patch clamp technique were used to study the effects of berberine on cardiac K_{ATP} channels. Under whole-cell conditions, this alkaloid could inhibit or even abolish the cromakalim-induced outward currents. In inside-out membrane patches, application of berberine (3 μM) significantly reduced the activity of K_{ATP} channels. At a higher concentration (100 μM), this agent fully inhibited the opening of K_{ATP} channels. These results provide a further support for the conclusion that berberine is a blocker of the cardiac K_{ATP} channels. However, since berberine has been shown to reduce the maximal velocity of depolarization, to prolong the action potential duration and effective refractory period and to increase contractile force and slow inward Ca^{2+} current in normoxic myocardial cells (Sabir et al., 1978; Shaffer, 1985; Wang et al., 1987, 1992a, 1993a,b; Wang and Tan, 1987; Sun et al., 1989; Neto, 1993), this agent most likely is not a specific K_{ATP} channel blocker.

Tung and Kurachi (1991) have hypothesized that a functional model of the K_{ATP} channels consists of the ATP-binding site, transducer unit and channel gate. Berberine did not alter the unitary conductance as well as the time constant for the open time and the closed time of K_{ATP} channels. These results suggest that this drug may not inhibit the K_{ATP} channels by directly affecting the channel gate. Future studies are required to reveal the exact site of its interaction.

Glibenclamide (10 μM) completely abolished the abbreviation of action potential duration and effective refractory period as well as the outward current induced by cromakalim. Under the single channel recording, this compound also completely inhibited the activity of single K_{ATP} channels in the presence of 0.1 mM ATP. In contrast to glibenclamide, berberine only at high concentration (100 μM) completely prevented the activation of the K_{ATP} channels. It indicates that berberine produced less potent inhibiting effects on the K_{ATP} channels than that of glibenclamide. However, since berberine at low concentration (3 μM) not only inhibited the hypoxia-induced shortening of action potential duration and effective refractory period, but also the activity of the K_{ATP} channels, the prevention of the hypoxic effects by this alkaloid may be due in part to a decreased activity in K_{ATP} channels.

In contrast to the effects on either the cromakalim-induced changes in action potentials and whole-cell currents or the activity of single K_{ATP} channels, glibenclamide at the same concentration only partially prevented the hypoxia-induced shortening of action potential duration and effective refractory period, which is similar to the published papers (Nakaya et al., 1991; Tweedie et al., 1993). Such results support the argument that the activation of K_{ATP} channels may not be solely responsible for the shortening of action potential duration and effective refractory period caused by hypoxia or ischemia in cardiac muscles.

Berberine showed a nearly similar inhibitory effect on the shortening of action potential duration and effective refractory period induced by hypoxia to that by cromakalim. This result, with the findings that berberine produces a positive inotropic action (Sabir et al., 1978; Maroko and Ruzyllo, 1983; Vik-Mo et al., 1983; Shaffer, 1985; Wang et al., 1987; Wang and Tan, 1987; Marin-Neto et al., 1988) and an increase in slow inward Ca^{2+} current (Sun et al., 1989) as well as a prolongation of action potential duration and effective refractory period in normoxic cardiac cells (Wang et al., 1987, 1993b, 1994; Wang and Tan, 1987; Neto, 1993), suggests that its effect on the hypoxic action potentials might well involve not only the inhibition of K_{ATP} currents, but also an increase of the slow inward Ca^{2+} current and/or the inhibition of other outward K^+ currents such as arachidonic acid or phosphatidylcholine-activated K^+ currents.

Whether K_{ATP} channel blockers exert antiarrhythmic or proarrhythmic effects in the ischemic hearts remains controversial. From a theoretical standpoint, the effects of agents of this kind on the heart subject to ischemia are twofold. On one hand, they would inhibit the shortening of action potential duration and effective refractory period in the ischemic zone and therefore limit the dispersion in refractoriness between injured and normally oxygenated tissues, producing an antiarrhythmic action. On the other

hand, it might be expected that K_{ATP} channel blockers possess proarrhythmic properties, because shortening action potential duration reduces the amount of Ca^{2+} entering the cells and thus prevents the Ca^{2+} overload-related arrhythmogenic activity. Besides, it may ultimately suppress both electrical and mechanical activity in the ischemic cells. The local akinesia saves the energy for cell survival, whereas arrest of the electrical activity is highly desirable to prevent the appearance of foci of abnormal automaticity and impaired conduction. However, in conditions such as regional ischemia of the heart which is more similar to the clinical situation than global ischemia, where re-entrant mechanisms may be the main contributors to arrhythmogenesis, the K_{ATP} channel opener pinacidil has been shown to be proarrhythmic (Chi et al., 1990). Presumably this is because this agent decreases the refractoriness of the ischemic tissue, whereas glibenclamide has been shown to be markedly antiarrhythmic under these conditions (Wolleben et al., 1989; Kantor et al., 1990). It has been reported that berberine reduces the incidences of ventricular arrhythmia and mortality induced by regional ischemia, elevates the ventricular fibrillation threshold elicited by electrical stimulation in the ischemic zone in rats, and prevents the development of ventricular fibrillation evoked by programming electrical stimulation after the left anterior descending coronary artery occlusion in dogs (Ribeiro et al., 1982; Wang et al., 1986, 1993a; Xu et al., 1989). Therefore, it is possible that the effectiveness of this alkaloid against cardiac arrhythmias induced by the heart regional ischemia might be associated with the prevention of the shortening in refractoriness by inhibiting the activity of K_{ATP} channels.

It should be noted that berberine also caused a further reduction in the maximal velocity of depolarization in hypoxic cardiac cells (Table 1). This effect might lead to the inhibition of excitability and conduction in ischemic tissues, thereby converting the unidirectional block of conduction into the bidirectional block and subsequently canceling the re-entrant activity. This mechanism might also contribute to its effect on ischemic cardiac arrhythmias.

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