

• BASIC RESEARCH •

# Inhibitory effects of berberine on ion channels of rat hepatocytes

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## Abstract

**AIM:** To examine the effects of berberine, an isoquinoline alkaloid with a long history used as a tonic remedy for liver and heart, on ion channels of isolated rat hepatocytes.

**METHODS:** Tight-seal whole-cell patch-clamp techniques were performed to investigate the effects of berberine on the delayed outward potassium currents ( $I_K$ ), inward rectifier potassium currents ( $I_{K1}$ ) and  $\text{Ca}^{2+}$  release-activated  $\text{Ca}^{2+}$  currents ( $I_{\text{CRAC}}$ ) in enzymatically isolated rat hepatocytes.

**RESULTS:** Berberine 1-300  $\mu\text{mol/L}$  reduced  $I_K$  in a concentration-dependent manner with  $\text{EC}_{50}$  of  $38.86 \pm 5.37 \mu\text{mol/L}$  and  $n_H$  of  $0.82 \pm 0.05$  ( $n = 8$ ). When the bath solution was changed to tetraethylammonium (TEA) 8 mmol/L,  $I_K$  was inhibited. Berberine 30  $\mu\text{mol/L}$  reduced  $I_K$  at all examined membrane potentials, especially at potentials positive to +60 mV ( $n = 8$ ,  $P < 0.05$  or  $P < 0.01$  vs control). Berberine had mild inhibitory effects on  $I_{K1}$  in rat hepatocytes. Berberine 1-300  $\mu\text{mol/L}$  also inhibited  $I_{\text{CRAC}}$  in a concentration-dependent fashion. The fitting parameters were  $\text{EC}_{50} = 47.20 \pm 10.86 \mu\text{mol/L}$ ,  $n_H = 0.71 \pm 0.09$  ( $n = 8$ ). The peak value of  $I_{\text{CRAC}}$  in the I-V relationship was decreased by berberine 30  $\mu\text{mol/L}$  at potential negative to -80 mV ( $n = 8$ ,  $P < 0.05$  vs control). But the reverse potential of  $I_{\text{CRAC}}$  occurred at voltage 0 mV in all cells.

**CONCLUSION:** Berberine has inhibitory effects on potassium and calcium currents in isolated rat hepatocytes, which may be involved in hepatoprotection.

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## INTRODUCTION

Many natural drugs for liver diseases are currently used in popular medicine. For example, quaternary protoberberine alkaloids from *Flissitigma* and *Goniothalamus* have been used in popular medicine for hepatomegaly and hepatosplenomegaly<sup>[1,2]</sup>. The use of alkaloids from *Berberis aristata* for liver injury induced by chemical carcinogenesis and alkaloids from *Enantica* for disorders of bilirubin has also been reported<sup>[3,4]</sup>.

Berberine is an isoquinoline alkaloid with a long history in

both Ayurvedic and Chinese medicine. It exists in *Hydrastis canadensis* (golden seal), *Coptis chinensis* (Coptis or golden thread), *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), and *Berberis aristata* (tree turmeric). Berberine alkaloid can be found in roots, rhizomes, and stem bark of plants<sup>[5]</sup>. Berberine has been extensively studied and is known to exhibit multiple pharmacological activities such as antiprotozoal, antihypertensive<sup>[6]</sup>, antibacterial<sup>[7]</sup>, anti-inflammatory, anticholinergic<sup>[8]</sup> and antiarrhythmic activities<sup>[9]</sup>. Hwang *et al.* reported that berberine inhibited hepatotoxicity induced by *tert*-butyl hydroperoxide (*t*-BHP) via its antioxidant potential and could function as a chemopreventive agent in living systems<sup>[10]</sup>. Previous studies have shown that berberine could block delayed rectifier potassium currents, inward rectifier potassium currents ( $I_{K1}$ ) and L-type calcium currents ( $I_{\text{Ca,L}}$ ) in guinea pig ventricular myocytes<sup>[11,12]</sup>. So far, however, its hepatoprotective mechanism still remains unknown. No data are available on the relationship between ion currents in hepatocytes and the hepatoprotective effect of berberine.

Therefore, this study used patch-clamp techniques to record the whole-cell currents in isolated rat hepatocytes in order to investigate the hepatoprotective mechanism of berberine.

## MATERIALS AND METHODS

### Cell preparation

Rat hepatocytes were enzymatically isolated from Sprague-Dawley (SD) rats of either sex (150-200 g) by using slightly modified procedures described previously<sup>[13]</sup>. Briefly, adult animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 mg/kg) in strict accordance to the guidelines established by the Institutional Animal Care and Use Committee, which follow all applicable state and federal laws. Portal vein and inferior vena cava were cannulated. The liver was initially perfused at a flow rate of 25 mL/min via a constant-flow system with modified oxygenated  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -free Hanks' solution for several minutes, followed by perfusion with a  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -free Hanks' solution containing collagenase (0.3 g/L, type I) for 10 min. The solutions were gassed with 950 mL/L  $\text{O}_2$ +50 mL/L  $\text{CO}_2$  and heated to 37 °C. After these perfusions, the liver was excised and then minced in  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -free Hanks' solution at 0 °C. The cells were filtered through a 200  $\mu\text{m}$  nylon mesh, and washed 3 times by centrifugation at 50 g for 2 min. The cell pellets were resuspended in KB medium that yielded approximately 85% to 95% viable hepatocytes. A small aliquot of the medium containing single cells was transferred into a 1-mL chamber mounted on the stage of an inverted microscope (IX-70, Olympus, Japan). Spherical and smooth cells were used for the whole-cell patch-clamp studies. All experiments were performed at room temperature (20 °C to 22 °C).

### Voltage-clamp recording

A programmable vertical puller (pp-83, Narishige, Japan) was used to pull the electrodes. The resistance of capillary glass electrodes (GC150TF-10, Clark Electromedical Instruments, UK) used was 2 to 4 M $\Omega$  when filled with internal solution. A patch-clamp amplifier (EPC-9, Germany) was used to record the whole-cell currents with four-pole Bessel filter set at 1 kHz, digitized at 5 kHz. The protocols for voltage-clamp and data analysis were established with routines using pClamp 6.0 software (Axon Instrument, USA), and data

were stored in computer for subsequent analysis. Drug actions were measured only after steady-state conditions reached, which were judged by the amplitudes and time courses of currents remaining constant with further perfusion of drugs.

### Drugs and solutions

Berberine hydrochloride was obtained from Yichang Pharmaceutical Company of China as base powders, dissolved in distilled water and made into a stock solution at 0.1 mol/L. Berberine was added to bath solutions for extracellular application. All drugs were purchased from Sigma (USA) unless otherwise indicated.

Ca<sup>2+</sup>, Mg<sup>2+</sup>-free Hanks' solution for cell-isolation (mmol/L) contained NaCl 137, KCl 5.4, NaH<sub>2</sub>PO<sub>4</sub> 0.5, Na<sub>2</sub>HPO<sub>4</sub> 0.58, NaHCO<sub>3</sub> 4.16 and glucose 5.5 (pH 7.3). Kraft-bruhe (KB) medium for cell-preservation (mmol/L) contained L-glutamic acid 70, KCl 130, taurine 15, KH<sub>2</sub>PO<sub>4</sub> 10, MgCl<sub>2</sub> 0.5, glucose 11, N-(hydroxyethyl) piperazine-N'-2-ethanesulphonic acid (HEPES) 10 and ethylene glycol-O-O'-bis (2-aminoethyl) -N,N,N',N'-tetraacetic acid (EGTA) 0.5 (pH 7.4).

In studies of  $I_K$ , the bath solution was a modified Tyrode's solution (mmol/L) containing NaCl 144, KCl 4.0, CaCl<sub>2</sub> 1.8, MgCl<sub>2</sub> 0.53, Na<sub>2</sub>HPO<sub>4</sub> 0.33, HEPES 5 and glucose 5.5 (pH 7.3). The patch pipette solution contained (mmol/L) KCl 130, K<sub>2</sub>ATP 5.0, creatine phosphate 5.0 and HEPES 5.0 (pH 7.4).

For experiments on  $I_{K1}$ , both the bath solution and the pipette solution contained (mmol/L) KCl 7, MgCl<sub>2</sub> 2, EGTA 1, K-glutamate 130 and HEPES 10 (pH 7.4).

For  $I_{CRAC}$  recording, the bath solution (mmol/L) contained NaCl 140, KCl 2.8, CaCl<sub>2</sub> 10, MgCl<sub>2</sub> 0.5, glucose 11 and HEPES 10 (pH 7.4). The pipette solution used (mmol/L) contained K-glutamate 145, NaCl 8, MgCl<sub>2</sub> 1, MgATP 0.5, EGTA 10 and HEPES 10 (pH 7.2).

### Data analysis

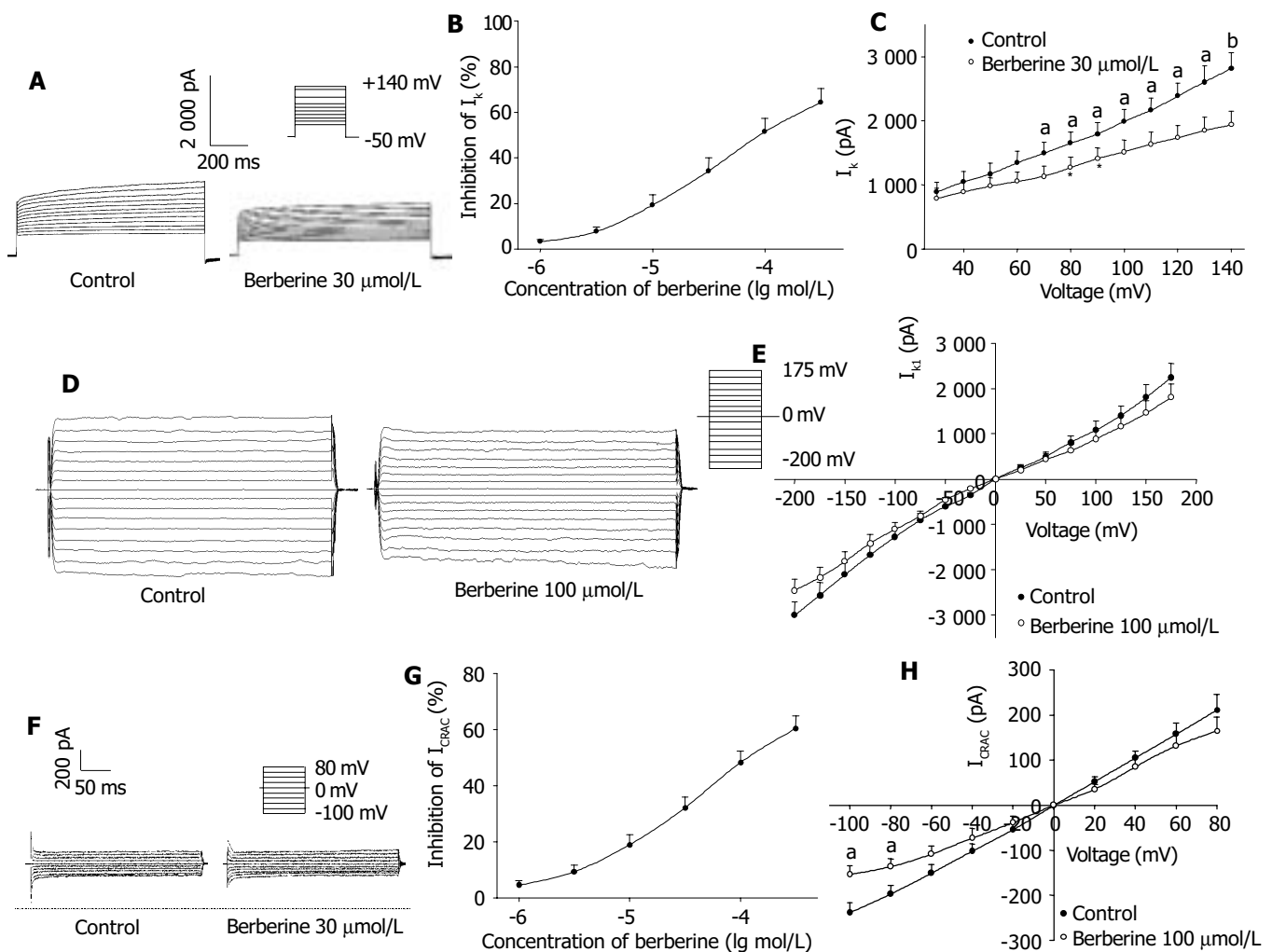
All the data were expressed as mean  $\pm$  SD and error bars were plotted as SD. Statistical significance was evaluated by a *t* test. Statistical differences were considered to be significant when *P* value was less than 0.05.

## RESULTS

### Effects of berberine on $I_K$

Under conventional whole-cell patch-clamp mode, the membrane potential was clamped at -50 mV, and  $I_K$  was elicited in isolated rat hepatocytes by depolarizing pulse to +140 mV for 900 ms. The current at the end point of the test pulse was measured as the amplitude of  $I_K$ .

The percentage block of  $I_K$  was defined as  $(I_{\text{Control}} - I_{\text{berberine}}) / I_{\text{Control}}$  and plotted as a function of logarithm [berberine] in



**Figure 1** Effects of berberine on  $I_K$ . A: Families of  $I_K$  recorded with changes in the absence or presence of berberine 30  $\mu\text{mol/L}$  and 100  $\mu\text{mol/L}$ . Dotted line indicates zero current level; B: Dose-response curve for effects of berberine on  $I_K$ ; C: I-V relationship of  $I_K$  under control (●) and berberine 30  $\mu\text{mol/L}$  (○). D: Families of  $I_{K1}$  recorded with changes in the absence or presence of berberine 100  $\mu\text{mol/L}$ . Dotted line indicates zero current level; E: I-V relationship of  $I_{K1}$  under control (●) and berberine 100  $\mu\text{mol/L}$  (○). F: Families of  $I_{CRAC}$  recorded with changes in the absence or presence of berberine 30  $\mu\text{mol/L}$ . Dotted line indicates zero current level; G: Dose-response curve for effects of berberine on  $I_{CRAC}$ ; H: I-V relationship of  $I_{CRAC}$  under control (●) and berberine 30  $\mu\text{mol/L}$  (○). The voltage steps used to elicit  $I_K$  are shown in the inset. *n* = 8, mean  $\pm$  SD, <sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01 vs control.

Figure 1B. At +140 mV, berberine 1-300  $\mu\text{mol/L}$  decreased the  $I_K$  amplitude concentration-dependently in all cells tested, which was poorly reversible after washout. The data points were fitted according to the Hill equation: Inhibition of current (%) =  $100/[1 + (\text{EC}_{50}/C)^{n_H}]$ , and an  $\text{EC}_{50}$  value of  $38.86 \pm 5.37 \mu\text{mol/L}$  and  $n_H$  of  $0.82 \pm 0.05$  were obtained ( $n = 8$ ). When the bath solution was changed to tetraethylammonium (TEA) 8 mmol/L,  $I_K$  was inhibited.

Figure 1A shows the effects of berberine 30  $\mu\text{mol/L}$  on the steady-state I-V relationship for  $I_K$  generated by applying 12 steps voltage command pulse from +30 mV to +140 mV for 900 ms with a 10 mV increment from a holding potential of -50 mV. By comparing these two I-V curves shown in Figure 1C,  $I_K$  was reduced by berberine 30  $\mu\text{mol/L}$  at all membrane potentials examined, especially at potentials positive to +60 mV ( $n = 8$ ,  $P < 0.05$  or  $P < 0.01$  vs control). The results clearly indicated that berberine had a depressant action on  $I_K$  in rat hepatocytes.

### Effects of berberine on $I_{K1}$

Hyperpolarizing and depolarizing potentials over a range from -200 mV to +175 mV with a 25 mV increment for 40 ms were applied from a holding level of 0 mV. The absolute value at the end of test pulse was measured as the amplitude of  $I_{K1}$ . After administration of berberine 100, 300  $\mu\text{mol/L}$ , the  $I_{K1}$  amplitude at -200 mV was decreased from  $-3044.75 \pm 262.06 \text{ pA}$  to  $-2451.33 \pm 226.45 \text{ pA}$  and  $-2310.66 \pm 182.65 \text{ pA}$ , respectively. The inhibition rates were  $17.49 \pm 2.51\%$  and  $24.11 \pm 3.60\%$ , respectively. Figures 1D and 1E shows the I-V relations of berberine 100  $\mu\text{mol/L}$  on  $I_{K1}$ . As shown in Figures 1D and 1E, berberine only had mild inhibitory effects on  $I_{K1}$  in isolated rat hepatocytes.

### Effects of berberine on $I_{CRAC}$

When the holding potential was 0 mV, and the cells were depolarized to -100 mV for 200 ms at a frequency of 0.2 Hz,  $I_{CRAC}$  was evoked. As shown in Figure 1G,  $I_{CRAC}$  also was blocked by berberine in a concentration-dependent fashion, with an  $\text{EC}_{50}$  value of  $47.20 \pm 10.86 \mu\text{mol/L}$  and  $n_H$  of  $0.71 \pm 0.09$  ( $n = 8$ ). Figure 1H shows the effect of berberine 30  $\mu\text{mol/L}$  on the steady-state I-V relationships generated by applying a series depolarizing pulses from a holding potential of 0 mV to different membrane potentials (-100 mV to +80 mV) with a 20 mV increment. The peak value of  $I_{CRAC}$  was reduced on either inward or outward components, especially from -100 mV through -80 mV ( $n = 8$ ,  $P < 0.05$  vs control). But the reverse potential of  $I_{CRAC}$  occurred at voltage 0 mV in all cells.

## DISCUSSION

In this study, for the first time, we characterized the effects of berberine on  $I_K$ ,  $I_{K1}$  and  $I_{CRAC}$  by patch-clamp techniques and demonstrated that berberine mainly inhibited  $I_K$ ,  $I_{CRAC}$  and  $I_{K1}$  in isolated rat hepatocytes.

Potassium channels are ubiquitous in eukaryotic cells and could play roles in resting membrane potential, frequency of action potential, membrane potential repolarization rates and cell functions. It has been found that membrane potential is important in regulating metabolic processes in the liver, including gluconeogenesis, amino acid transport, and the rate of uptake of bile salts<sup>[14,15]</sup>. Changes in  $\text{K}^+$  permeability could affect the transmembrane potential. Bile formation was involved in anion accumulation within the apical lumen of hepatocytes. Potassium flux through hepatocellular basolateral membrane channels might provide the counterion for apical anion efflux<sup>[16]</sup>. Transcellular bile acid transport could be integrated in the regulation of intracellular pH,  $\text{K}^+$  homeostasis and membrane potential. Hepatocellular  $\text{K}^+$ -depletion could result in the inhibition of bile acid secretion despite increasing the intracellular concentration<sup>[17-19]</sup>.

During ischemia and hypoxia, hepatocellular volume and  $\text{K}^+$  conductance were increased and would lead to cell death<sup>[20,21]</sup>.

Nietsch *et al.* demonstrated TNF (25 ng/mL) elicited a 2- and 5-fold increase in  $\text{K}^+$  current in hepatoma tissue culture cells.  $\text{K}^+$  channel activation might participate in pathways that leading to TNF-mediated cell death, thus representing potential therapeutic targets to attenuate liver injury from TNF<sup>[22]</sup>. The inhibition of  $\text{K}^+$  channels could delay hepatocyte apoptosis and death. Churchill *et al.* reported that potassium channel antagonists had protective effects on rat liver via regulating the energy metabolism<sup>[23]</sup>.

Previous studies showed that berberine (0.5, 5 mg/kg, i.p.) protected rat liver from hepatotoxicity induced by *t*-BHP<sup>[10]</sup>. As shown previously, *t*-BHP could shrink hepatocytes by release of cellular  $\text{K}^+$ . Single-channel patch-clamp studies demonstrated *t*-BHP activated a 35-pS  $\text{K}^+$  channel and contributed to the  $\text{K}^+$  release of hepatocytes following exposure to *t*-BHP<sup>[24]</sup>. In this study, we observed that the concentration of berberine on  $I_K$  was consistent with the dose reported by Hwang<sup>[10]</sup>. The hepatoprotective effect of berberine on *t*-BHP may have some relations with the inhibitory effect on potassium channel.

However, as we reported previously, AP-Q had a protective effect on  $\text{CCl}_4$ -induced liver injury, probably by selectively increasing  $I_K$ . In this experiment, we observed that berberine had a protective effect on *t*-BHP induced liver injury by decreasing  $I_K$ . The contradiction may be due to the different mechanism of  $\text{CCl}_4$ -induced and *t*-BHP induced liver injury.  $\text{CCl}_4$ -induced hepatocyte injury paralleled with membrane depolarization by blocking the potassium channel in damaged hepatocytes<sup>[25-27]</sup>. However, *t*-BHP-induced liver injury is concerned with the release of cellular  $\text{K}^+$  by activating potassium channel. A previous study showed that berberine had not any protective effects on  $\text{CCl}_4$ -induced liver injury<sup>[28]</sup>.

Calcium has been demonstrated to play an important role in hepatocyte damage. An increase in calcium ion concentration in cytoplasm due to the influence of various toxic agents caused disturbances in the structure and function of hepatocytes, leading to their damage and even death<sup>[29]</sup>. Previous studies showed that hepatocellular  $\text{Ca}^{2+}$  overload and impaired  $\text{Ca}^{2+}$  signaling were related to hemorrhage/resuscitation liver injury. Increased  $\text{Ca}^{2+}$  uptake could result from a receptor-gated  $\text{Ca}^{2+}$  influx and/or oxygen-free radical induced membrane  $\text{Ca}^{2+}$  leaks. A protective effect of calcium channel blockers on hepatotoxins has been reported<sup>[30]</sup>.

Calcium ions could enter the cells mostly through calcium channels. However, hepatocytes as the nonexcitable cells were short of the voltage-dependent  $\text{Ca}^{2+}$  channels but possessed plasma membrane  $\text{Ca}^{2+}$  channels that had a high selectivity for  $\text{Ca}^{2+}$ , and were activated by a decrease in the concentration of  $\text{Ca}^{2+}$  in intracellular stores, which was named  $I_{CRAC}$ <sup>[31,32]</sup>. Berberine inhibited  $I_{CRAC}$  with  $\text{EC}_{50}$  of 47.20  $\mu\text{mol/L}$ , which was different from the  $\text{EC}_{50}$  of  $I_{Ca,L}$  in cardiac myocytes<sup>[33]</sup>. The differential drug sensitivity of the two currents also provided further support for the idea that  $I_{CRAC}$  is different from voltage-gated  $\text{Ca}^{2+}$  channel.

Berberine inhibited  $I_K$ ,  $I_{K1}$  and  $I_{CRAC}$  concentration-dependently in isolated rat hepatocytes and the inhibitory extent was  $I_K > I_{CRAC} > I_{K1}$ . Therefore, berberine could block  $\text{K}^+$  channel and decrease the extracellular  $\text{K}^+$  to regulate the metabolic processes in the liver. Berberine could also inhibit  $I_{CRAC}$  effectively and protect hepatocytes from calcium overload via the inhibition of  $I_{CRAC}$ . The inhibitory effects on potassium and calcium current may partly contribute to the hepatoprotective action of berberine. As berberine has already been used for liver damage in human beings<sup>[34]</sup>, it may be a good candidate for further study and used in treatment of liver damage.

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