Effects of palmatine on potassium and calcium currents in isolated rat hepatocytes

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Abstract

AIM: To study the effects of palmatine on delayed rectifier potassium current and L-type calcium current (I_{CaL}) in guinea pig ventricular myocytes, on the potassium and calcium currents in isolated rat hepatocytes.

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

RESULTS: Palmatine 0.3-100 µM reduced I_{Kd} in a concentration-dependent manner with EC_{50} of 41.62±10.11 µM and n_{H}=0.48±0.07 (n=8). The effect of the drug was reversible after washout. When the bath solution was changed to tetraethylammonium (TEA) 8 mM, I_{Kd} was inhibited. Palmatine 10 µM and 100 µM shifted the I-V curves of I_{Kd} downward, and the block of I_{Kd} was voltage-independent. Palmatine 0.3-100 µM also inhibited I_{CRAC} in a concentration-dependent manner. The fitting parameters were as follows: EC_{50}=51.19±15.18 µM and n_{H}=0.46±0.07 (n=8). The peak value of I_{CRAC} in the I-V relationship was decreased by palmatine 10 µM and 100 µM. But the reverse potential of I_{CRAC} occurred at Voltage=0 mV in all cells. Palmatine 0.3-100 µM failed to have any significant effect on either inward or outward components of I_{K1} at any membrane potential examined.

CONCLUSION: The inhibitory effects on I_{Kd} and I_{CRAC} could be one of the mechanisms that palmatine exerts protective effect on hepatocytes.

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N\textsuperscript{\(\text{N}^-\)}, N\textsuperscript{\(\text{N}^-\)}-tetraacetatic acid (EGTA) 0.5 (pH 7.4) that yielded approximately 85 % to 95 % viable hepatocytes. A small aliquot of the medium containing single cell was transferred into a 1 mL chamber mounted on the stage of an inverted microscope (XD-101\textsubscript{B}, Nanjing, China). The spherical, smooth cells were used for the whole-cell voltage-clamp studies. All experiments were performed at room temperature (20 to 22 °C).

**Voltage-clamp recording**

A programmable vertical puller (pp-83, Narishige, Japan) was used to pull the electrodes. The resistance of the capillary glass electrodes (GG-17, Nanjing, China) was used to record 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 software (pClamp 6.0, Wuhu, China) and data were stored on computer for subsequent analysis. Drug actions were measured only after steady-state-conditions were reached, which were judged by the amplitudes and time courses of currents remaining constant with further perfusion of drug.

**Drugs and solutions**

Palmatine hydrochloride was obtained from Zhonglian Pharmaceutical Company of China as base powders, dissolved in distilled water and made a stock solution at 0.1 M. Palmatine was added to bath solution for extracellular application. All drugs were from Sigma Chemical Co unless otherwise indicated.

With studies of \(I_{\text{K}}\), the bath solution was a modified Tyrode's solution contained in mM: NaCl 144, KCl 4.0, CaCl\(_2\) 1.8, MgCl\(_2\) 0.53, Na\(_2\)HPO\(_4\) 0.33, HEPES 5 and Glucose 5.5 (pH 7.3). The patch pipette solution contained in mM: KCl 130, K\(_2\)ATP 5.0, creatine phosphate 5.0 and HEPES 5.0 (pH 7.4).

**Statistics**

All values were expressed as mean ± S.E.M. and error bars were plotted as S.E.M. Student’s \(t\) test was used to evaluate the statistical significance of differences between means. A value of \(P<0.05\) was considered to be statistically significant. Concentration-response curves were fitted by the Hill equation:

\[
\text{Inhibition of current} (\%) = 100 \times \frac{C}{C_0+1+E_{\text{EC50}}/C}\]

Where \(E_{\text{EC50}}\) is the concentration of palmatine for half-maximum block, \(C\) is the concentration of palmatine, and \(n_H\), the Hill coefficient.

**RESULTS**

**Effects of palmatine on \(I_{\text{K}}\)**

Palmatine 0.3-100 \(\mu\)M failed to have any significant effect on either inward or outward components of \(I_{\text{K}}\) at any membrane potential examined.

**Effects of palmatine on \(I_{\text{CRAC}}\)**

Hyperpolarizing and depolarizing potentials over a range from -200 mV to +175 mV were applied from a holding level of 0 mV\textsuperscript{[20]} the absolute value at the end of test pulse was measured as the amplitude of \(I_{\text{K}}\). Palmatine 0.3-100 \(\mu\)M failed to have any significant effect on either inward or outward components of \(I_{\text{K}}\) at any membrane potential examined.

**Discussion**

In this study we have, for the first time, characterized the effects of palmatine on the hepatocyte \(I_{\text{K}}\), \(I_{\text{KI}}\), and \(I_{\text{CRAC}}\) by patch-clamp techniques and demonstrated that palmatine effectively inhibited \(I_{\text{K}}\) and \(I_{\text{CRAC}}\) in isolated rat hepatocytes.

Membrane potential is important in regulating metabolic processes in the liver, including gluconeogenesis, amino acid transport, and the rate of uptake of bile salts\textsuperscript{[22,23]}. Changes in
**Figure 1** Effects of palmatine on $I_K$. (A) Family of $I_K$ recorded with changes in the absent or present of palmatine 100 µM. Dotted line indicates zero current level. (B) Dose-response curve for the effects of palmatine on $I_K$. The data are mean values from $n=8$ cells. (C) $I-V$ relationship of $I_K$ under control (●) and palmatine 10 µM (○), 100 µM (●). The voltage steps used to elicit $I_K$ are shown in the inset of panel (B). $^b P<0.05$, $^c P<0.01$ vs control ($n=8$). (D) Dependence of palmatine effects on test potential. The values for the mean percentage reductions in $I_K$ induced by palmatine 10 µM (square) and 100 µM (●) are plotted against the corresponding test potential. No significant voltage-dependence was observed for the blocks induced by palmatine.

**Figure 2** Effects of palmatine on $I_{CRAC}$. (A) Family of $I_{CRAC}$ recorded with changes in the absent or present of palmatine 100 µM. Dotted line indicates zero current level. (B) Dose-response curve for effects of palmatine on $I_{CRAC}$. The data are mean values from $n=8$ cells. (C) $I-V$ relationship of $I_{CRAC}$. Under control (●) and palmatine 10 µM (○), 100 µM (●). The voltage steps used to elicit $I_K$ are shown in the inset of panel (B). $^b P<0.05$, $^c P<0.01$ vs control ($n=8$).

$K^+$ permeability can affect the transmembrane potential. Transcellular bile acid transport is integrated in the regulation of intracellular pH, $K^+$ homeostasis and membrane potential. Hepatocellular $K^+$-depletion can result in inhibition of bile acid secretion despite rising intracellular concentration$^{[24-26]}$.

During ischemia and hypoxia, hepatocellular volume and $K^+$ conductance are increased. It was reported that the extracellular $K^+$ increase would result in hyperpolarization and hyperexcitability of cells. This would lead to cell death$^{[27-29]}$. Nietsch et al demonstrated membrane potential change by modulation of $K^+$ channel activity might be involved in the mechanism of apoptosis in human hepatoma cells$^{[30,31]}$. The inhibition of $K^+$ channels could delay hepatocyte apoptosis and death.

Calcium has been demonstrated to play an important role in hepatocyte damage. Elevation of intracellular Ca$^{2+}$...
concentration was associated with the development of cell damage and apoptosis.[32-35]

Recent developments suggest that an early disturbance in hepatocellular Ca\textsuperscript{2+} homeostasis might be involved in the hepatocellular damage induced by CCl\textsubscript{4}.[36-38]

Hepatocytes as the nonexcitable cells are short of the voltage-dependent Ca\textsuperscript{2+} channels but possess plasma membrane Ca\textsuperscript{2+} channels that have a high selectivity for Ca\textsuperscript{2+}, and are activated by a decrease in the concentration of Ca\textsuperscript{2+} in intracellular stores, which named I\textsubscript{CRAC}[39, 40]. The gating of I\textsubscript{CRAC} is independent of membrane voltage, there is, nevertheless, a strong dependence of Ca\textsuperscript{2+} influx on the driving force exerted by the membrane potential, ie, the influx rate increases with hyperpolarization and decreases with depolarization, which is different from cardiac myocytes that Ca\textsuperscript{2+} influx increases with depolarization and decreases with hyperpolarization[41].

Palmitate inhibits I\textsubscript{CRAC} with EC\textsubscript{50} of 51.19 μM, which is higher than the EC\textsubscript{50} of I\textsubscript{CL} in cardiac myocytes[42]. The differential drug sensitivity of the two currents also provides further support for the idea that I\textsubscript{CRAC} is different from voltage-gated Ca\textsuperscript{2+} channel.

In conclusion, palmitate blocks K\textsuperscript{+} channel and decreases the extracellular K\textsuperscript{+} to regulate the metabolic processes in the liver. Palmitate also inhibits I\textsubscript{CRAC} effectively and protects hepatocytes from calcium overload via the inhibition of I\textsubscript{CRAC}. The inhibitory effects on I\textsubscript{K} and I\textsubscript{CRAC} may partly contribute to the hepatoprotective action of palmitate.

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