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Puerarin blocks transient ourward K^+ current and delayed rectifier K^+ current in mice hippocampal CA_1 neurons[1]

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KEY WORDS hippocampus; puerarin; potassium channels; patch-clamp techniques

ABSTRACT

AIM: To study the effects of puerarin (Pue) on I_A and I_K in mouse hippocampal neurons. **METHODS**: The whole cell patch clamp techniques were used. **RESULTS**: Pue reduced the amplitude of I_A and I_K, in a concentration-dependent, but not rate- or use-dependent manner (IC_{50} were 461 μmol/L and 215 μmol/L, respectively). Pue (0.5 mmol/L) shifted the steady state activation curves of I_A and I_K to positive and negative potentials (V_h about 20.6 mV and 28.6 mV) respectively, but inactivation curves of I_A were not affected by Pue. **CONCLUSION**: Pue inhibited I_A and I_K in mouse hippocampal CA_1 neurons and its blocking effect on I_K was much stronger than on I_A.

INTRODUCTION

Puerarin (Pue), an active component extracted from the Chinese traditional medicine *Pueraria lobata* (wild) Ohwi, has been shown to be effective in treatment of hypertension, myocardial ischemia and arrhythmia[1-3]. Recently we have demonstrated that a puerariae isoflavone could antagonize cerebral ischemia and amnesia[4,5]. It was also reported that Pue could inhibit tetrodotoxin-resistant sodium current in rat dorsal root ganglion[6], but its mechanism of action is still unclear. Hypoxia and anoxia are known to induce membrane hyperpolarization and enhance extracellular K^+ by activation of K^+ channels, and K^+ channel blockers reduce the electrical reponse to hypoxia[7,8]. Therefore, the protective effects of Pue may be due to blocking of K^+ channels. In hippocampal pyramidal neurons, the two components of outward K^+ channels: a rapidly activating and inactivating transient outward K^+ current, I_A; and a delayed rectifier K^+ current, I_K, influence repolarization and frequency adaptation of action potentials. I_K affects the main spike repolarization; I_A is capable of controlling the approach of membrane potential to spike threshold at the early stage of repolarization and thus contribute to the excitability for repetitive firing[9]. Consequently, in the present study, we have investigated the effect of Pue on I_A and I_K, probe into its possibly mechanism of neuroprotective action in hippocampal neurons.

MATERIALS AND METHODS

Preparation of isolated cells Single hippocampal CA_1 pyramidal cells were acutely dissociated from 10 - 15 d old Kunming mice (♀♂, Grade Ⅱ, Certificate № 003, the Experimental Animal Center, Institute of Zoology, Chinese Academy of Sciences). The method has been described previously by Kay and Wong[10] and modified by Hu *et al*[11]. Briefly, hippocampal CA_1 region was cut into pieces about 400 - 500 μm thick and incubated at 32 ℃ for 2 h in artificial cerebrospinal solution (ACS) containing NaCl 124, KCl 5, K_2HPO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26, glucose 10 mmol/L, pH 7.4, and were transferred into ACS containing protease 1.5 g/L at 32 ℃ for 40 min, bubbled with 5 % CO_2 + 95 % O_2. Then the digested tissue was washed thrice with ACS, and dispersed by pipettes, the cells were transferred into a 35 mm culture dish, filled with extracellular solution 2 mL contained: NaCl 150, KCl 5, $CaCl_2$ 1.1, HEPES 10, glucose 10 mmol/L, pH 7.4. The cells were viable up to 4 - 5 h.

Whole cell patch-clamp recording Whole cell patch-clamp currents were recorded with an Axopatch

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200B patch clamp amplifier (Axon Instrument, USA), and stored in a PC 486 computer. The pCLAMP version 6.02 software (Axon Instrument, USA) was used to produce the signal, collect and process the data. The glass microelectrodes were pulled in two steps by a microelectrode puller (Narrishage, Japan) and had tip resistance of 2 – 5 MΩ when filled with intracellular solution containing: KCl 65, KOH 5, KF 80, HEPES 10, egtazic acid 10, Na_2-ATP 2 mmol/L, adjusted to pH 7.4. To record the potassium currents, TTX 1 μmol/L and $CdCl_2$ 0.2 mmol/L were added to the extracellular solution for blocking the sodium current and calcium current, respectively. Current traces were obtained after 15 min in the presence of the drug (after the activity of the drug reached a steady-state).

Drugs Pue, obtained from Department of Phytochemistry, China Pharmaceutical University, was dissolved in the extracellular solution. Tetrodotoxin (TTX) and Na_2-ATP were purchased from Sigma Co.

Data analysis All data were analyzed by the use of pCLAMP 6.02 CLAMPFIT (Axon Instrument) and Sigmaplot (Jandel Scientific) software, and were given as $\bar{x} \pm s$. Statistical significances were analyzed by paired or unpaired t test.

RESULTS

Effects of Pue on I_A and I_K To record the K^+ currents, the cell was held at a holding potential (HP) of – 100 mV, two components of outward currents were elicited by 160 ms depolarizing pulses from – 60 mV to + 90 mV in 10 mV steps, a rapidly activating and inactivating transient outward K^+ current, I_A, sensitive to 4-AP; and a slowly inactivating delayed rectifier K^+ current, I_K, sensitive to TEA ($n = 5$). I_A was estimated as the peak current, and I_K was determined as late current at 158 ms step depolarization.

Fig 1 shows current traces and current voltage relationship of I_A and I_K before and after the application of Pue 0.5 mmol/L. The results indicated that Pue reduced the amplitude of both I_A and I_K, and preferentially blocked I_K, its inhibition was partially reversible after washout for 5 min. The maximal suppression of Pue on I_A and I_K was 47 % ± 8 % and 56 % ± 11 % at + 90 mV of depolarization, respectively.

Effect of Pue on activation and inactivation kinetics of I_A and I_K On the basis of data obtained from current-voltage relationship, activation curves of peak I_A and I_K were determined before and after application of Pue 0.5 mmol/L, the activation curve was fitted by the Boltzmann equation $G/G_{max} = 1/[1 - \exp(V - V_h)/k]$, where G is the membrane conductance at po-

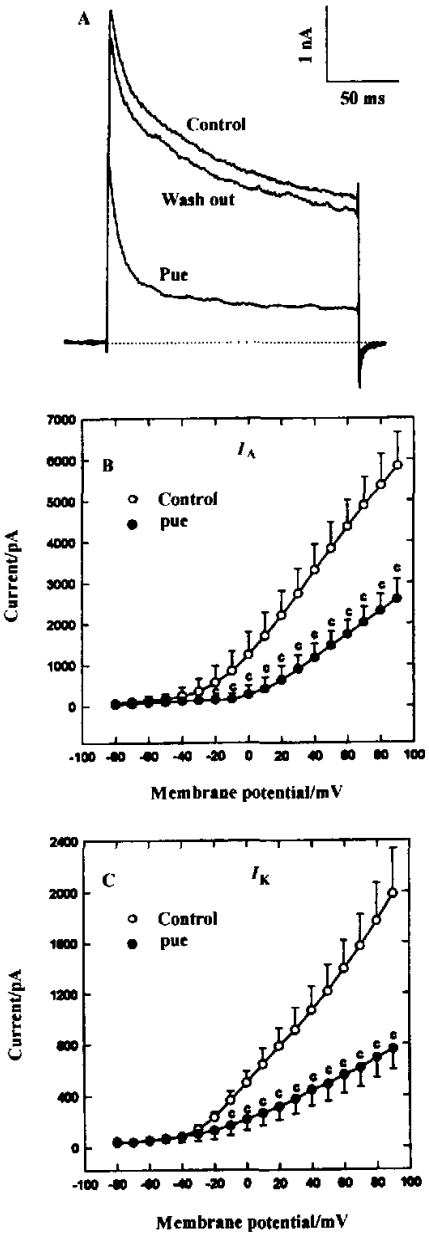

Fig 1. Effect of Pue 0.5 mmol/L on I_A and I_K. A) Current traces obtained in the absence and presence of the drug. B) and C) Current-voltage relations (I-V curve) of I_A and I_K. $n = 8$ cells. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

tential V, V_h is the half activation voltage, k is a slope factor. The V_h for action of I_A before and after the application of Pue were (7 ± 4) mV and (28 ± 6) mV ($P < 0.01$), with k of −25 ± 5 and −22 ± 5 ($P > 0.05$), respectively; V_h of I_K before and after the application of Pue were (18 ± 3) mV and (−11 ± 7) mV ($P < 0.01$), with k of −23 ± 6 and −21 ± 7 ($P > 0.05$), respectively. Pue shifted the steady-state activation curve of I_A towards more positive potential by 20.6 mV, that of I_K towards more negative potential by 28.6 mV, although the k remained unaltered (Fig 2,3).

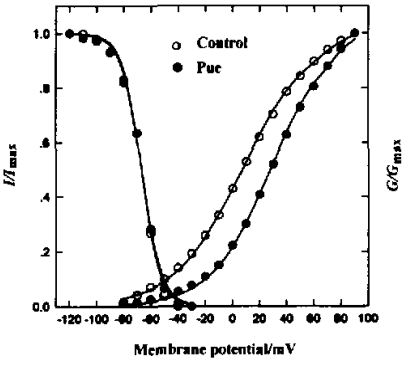

Fig 2. Effect of Pue 0.5 mmol/L on steady state activation and inactivation of I_A. $n = 8$ cells.

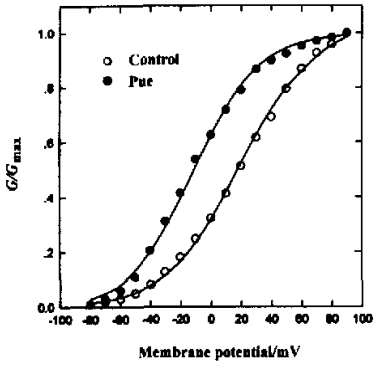

Fig 3. Effect of Pue 0.5 mmol/L on steady state activation of I_K. $n = 8$ cells.

The steady-state inactivation curves of I_A were obtained by use of a two-pulse protocol, a 80-ms conditioning prepulse to various potentials (from −120 mV to −20 mV) was followed by a 160 ms test pulse to +50 mV. The inactivation curve was also fitted to the Boltzmann equation $I/I_{max} = 1/[1 + \exp(V - V_h)/k]$, V_h is the half inactivation voltage, k is a slope factor. The V_h and k of I_A were (−67 ± 6) mV and (7.0 ± 0.8) mV in control; and −67 ± 5 and 7.4 ± 1.1 in the presence of Pue 0.5 mmol/L, respectively. Inactivation characteristics of I_A were not affected by Pue (Fig 2).

Concentration-dependent effect of Pue The currents were elicited by applying a single pulse to +50 mV from a HP of −100 mV before and after exposure to Pue. Fig 4 shows results from experiments demonstrating that Pue (10 − 1000 μmol/L) elicited a concentration-dependent inhibition of I_A and I_K, with IC_{50} 461 (402 − 514) μmol/L and 215 (176 − 257) μmol/L, respectively. The maximal suppression of Pue 1 mmol/L on I_A and I_K was up to 57 % ± 11 % and 78 % ± 34 %.

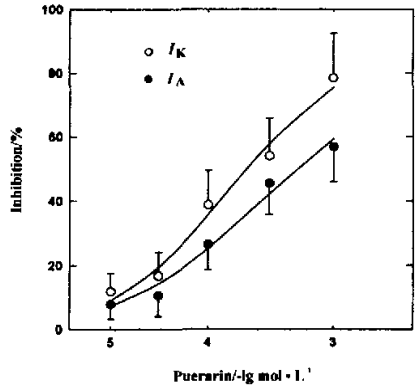

Fig 4. Concentration-response curves for blockade of I_A and I_K by Pue. $n = 8$ cells. $\bar{x} \pm s$.

Use-dependent effect of Pue Use-dependent effect of Pue 0.5 mmol/L was studied by a series of 30 depolarizing pulses from a HP −100 mV to +50 mV. When the frequencies were 0.5, 1, 2, and 4 Hz, the relation amplitude of I_A and I_K were not markedly changed in the absence and presence of the drug. So, we did not find the use-or rate-dependent effect of Pue.

DISCUSSION

The present results show that Pue inhibited voltage dependent K^+ currents in acutely dissociated mouse hippocampal CA_1 neurons, the inhibition was concentration-dependent and reversible. Pue's blocking potency on I_K is much stronger than I_A, which indicated that Pue had different sensitivity for I_A and I_K.

In addition, our experiment demonstrates that Pue obviously shifts the activation curves of I_A and I_K to positive and negative potentials, respectively, but inactivation

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curves of I_A are not affected, suggesting that Pue influence mainly activation state of I_A and I_K, and not the inactivation state of I_A.

The reduction of cerebral blood flow resulting in brain ischemia and oxygen deprivation may be one of the causes for induction of amnesia. Oxygen lack causes a dramatic electrophysiologic change in central neurons. The deprivation of oxygen induces an increase in extracellular K^+ and intracellular K^+ loss, which is possibly due to Na^+-K^+-ATPase inhibition secondary to depletion of intracellular ATP. A release of intracellular K^+ through K^+ channels were activated by a fall in cellular ATP. Anoxia also leads to a rise in intracellular free Ca^{2+} and thus activation of Ca^{2+}-sensitive K^+ channels. Thus blocking of K^+ channel may reduce the anoxia-induced K^+ leakage[7,8]. Pue's block on I_A and I_K retards the repolarization of the action potential, which is beneficial to neuron recovery from anoxic injury. In addition, Pue reduces oxygen deprivation, and inhibits depletion of intracellular ATP and accumulation of intracellular Ca^{2+} in cerebral ischemic tissue[4], and thus inhibits the activation of K^+ channel, resulting in attenuation of intracellular K^+ loss. Hence, the neuroprotective action of Pue on brain ischemia and amnesia may be attributed to inhibition of K^+ channel, and offers evidence for potential uses to treat cerebral ischemia and amnesia, clinically.

In conclusion, Pue inhibits I_A and I_K in mouse hippocampal CA_1 neurons, its therapeutic actions on brain ischemia and amnesia may be due to blocking of K^+ channels.

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葛根素抑制小鼠海马 CA_1 区神经元瞬间外向钾电流和延迟整流钾电流[1]

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关键词 海马；葛根素；钾通道；膜片箝技术

目的：研究葛根素对小鼠海马 CA_1 区神经元瞬间外向钾电流(I_A)和延迟整流钾电流(I_K)的作用. **方法**：膜片箝全细胞记录技术. **结果**：葛根素明显抑制 I_A 和 I_K，呈浓度依赖性，IC_{50}分别为 461 μmol/L 和 215 μmol/L，没有使用或频率依赖性. 葛根素 0.5 mmol/L 显著影响稳态激活曲线，分别使 I_A 的 V_h 右移，I_K 的 V_h 左移，但不影响 I_A 的失活曲线，表明其电压依赖性地影响激活过程. **结论**：葛根素抑制小鼠海马 CA_1 区锥体细胞 I_A 和 I_K，且对 I_K 的作用大于 I_A.

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