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Unique action of sodium tanshinone II-A sulfonate (DS-201) on the Ca^{2+} dependent BK_{Ca} activation in mouse cerebral arterial smooth muscle cells

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ABSTRACT

Sodium tanshinone II-A sulfonate (DS-201) is a water-soluble derivative of tanshinone IIA, a main active constituent of *Salvia miltiorrhiza* which has been used for treatments of cardio- and cerebro-vascular diseases. DS-201 activates large conductance Ca^{2+}-sensitive K^+ channels (BK_{Ca}) in arterial smooth muscle cells, and reduces the vascular tone. Here we investigated the effect of DS-201 on the BK_{Ca} channel kinetics by analyzing single channel currents. Smooth muscle cells were freshly isolated from mouse cerebral arteries. Single channel currents of BK_{Ca} were recorded by patch clamp. DS-201 increased the total open probability (*NPo*) of BK_{Ca} in a concentration-dependent manner. But this action required intracellular Ca^{2+}, and the effect depended on the Ca^{2+} concentration ($[Ca^{2+}]_{free}$). DS-201 activated BK_{Ca} with the half maximal effective concentration (EC_{50}) of 111.5 μM at 0.01 μM $[Ca^{2+}]_{free}$, and 68.5 μM at 0.1 μM $[Ca^{2+}]_{free}$. The effect of DS-201 on *NPo* was particularly strong in the range of $[Ca^{2+}]_{free}$ between 0.1 and 1 μM. Analysis of the channel kinetics revealed that DS-201 had only the effect on the channel closing without affecting the channel opening, which was a striking contrast to the effect of $[Ca^{2+}]_{free}$, that is characterized by changing the channel opening without changing the channel closing. DS-201 may be bound to the open state of BK_{Ca}, and have an inhibitory effect on the transition from the open to closed state. By this way DS-201 may enhance the activity of BK_{Ca}, and exhibit a strong vasodilating effect against vasoconstriction in the range of $[Ca^{2+}]_{free}$ between 0.1 and 1 μM.

1. Introduction

Large conductance calcium-activated potassium channels (BK_{Ca}) are broadly expressed in the vascular smooth muscle cells (SMCs) and play a critical role in regulating the vascular tone (Lingle et al., 1996; Ramanathan et al., 1999; Wanner et al., 1999). The channels are activated by both the membrane depolarisation and the increase of intracellular calcium concentration (Ko et al., 2008). Activation of BK_{Ca} leads to cell membrane hyperpolarisation which deactivates the voltage-dependent calcium channels and then causes vasodilatation (Jaggar et al., 2000; Ledoux et al., 2006; Perez et al., 2001). In physiological condition, activation of BK_{Ca} in SMCs counteracts the membrane depolarisation and reduces the vascular tone. Therefore, activation of the BK_{Ca} may become a new strategy for treatment of hypertension and other cardiovascular diseases.

Danshen (*Salvia miltiorrhiza*), is a traditional Chinese medical herb. This has been widely used in China and many other countries for treating patients with cardiovascular and cerebro-vascular diseases with minimal side effects. It has been reported that Danshen is effective in prevention of angina pectoris, hyperlipidemia and acute ischemic stroke (Chan et al., 2004; Cheng, 2006; Valli and Giardina, 2002; Zhou et al., 2005). Tanshinone II-A is the main active diterpene quinine of Danshen. Sodium tanshinone II-A sulfonate (DS-201) is a water-soluble derivative of tanshinone II-A after sulfonation. The underlying ionic mechanism for tanshitone II-A is still not well understood, whereas some reports have shown its effects, such as inhibition of L-type calcium channel in bovine adrenal medullary cells (Mao et al., 2009), activation of tetraethylammonium-sensitive K^+ channels in the smooth muscle cells (Lam et al., 2005), and activation of adenosine triphosphate-sensitive K^+ channels (K_{ATP}) in aortic smooth muscle cells (Chan et al., 2009). We have shown in a previous report that DS-201 relaxed the coronary artery strips and increased the BK_{Ca} macroscopic currents and the spontaneous transient outward K^+ currents (STOCs), and those effects are reversible (Yang et al., 2008). In the present study, we addressed our attention to the effects of DS-201 on the kinetic properties and the Ca^{2+} dependence of BK_{Ca} by analyzing single channel currents recorded from membrane patches of the mouse cerebral arterial smooth muscle cells.

2. Materials and methods

2.1. Single cell isolation

This study was approved by the Ethics Committee of Luzhou medical college. Mice were obtained from the animal care centre of Luzhou Medical College. Animals were deeply anesthetized with pentobarbital sodium (60 mg/kg i.p.). The brain was dissected out,

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and placed in ice-cold normal physiological saline solution (PSS). Cerebral arteries were carefully dissected out from the brain, and then exposed to low Ca^{2+} PSS (0.1 mM $CaCl_2$). The arteries were enzymatically digested in low Ca^{2+} PSS containing (in mg/ml) 0.3 papain and 0.2 dithioerythritol for 9.5 min, and further digested for 9.5 min in low Ca^{2+} PSS containing (in mg/ml) 0.8 collagenase F, 1.0 collagenase II and 1.0 dithiothreitol at 37 °C. Single cerebral arterial smooth muscle cells were obtained by gently triturating the digested tissues in culture dish filled with PSS, then stored at 4 °C for later electrophysiological experiments.

All the measurements were conducted at room temperature (23 ± 2 °C).

2.2. Electrophysiological methods

Electrophysiological recordings were performed practically the same way as those described previously (Yang et al., 2008). Single channel currents were recorded 20–30 min after a membrane patch formation and more than 5 min after each drug application. The total open probability (*N*Po), the amplitude, and the kinetic characteristics of the channels were analyzed with the pCLAMP software 10.0 and QUB software (http://www.qub.buffalo.edu). In inside-out patch experiments, the pipette solution consisted of (in mM): K-aspartate (K-Asp) 40, KCl 100, hydroxyethyl piperazine ethanesulfonic potassium (HEPES-K) 10 (pH 7.2) and ethylene glycol-bis(2-aminoethylether)-N, N, N', N'-tetraacetic acid (EGTA) 2, and the bath solution (in mM): K-Asp 100, KCl 40, HEPES-K 10 (pH 7.4) and EGTA 1. The free Ca^{2+} concentration in the bath solution ($[Ca^{2+}]_{free}$) was calculated according to the previous report (Yang et al., 2008). To make $[Ca^{2+}]_{free}$ of 0, 10, 100, 500, 1000 or 10,000 nM, the $CaCl_2$ concentration was changed to 0, 0.11, 0.55, 0.86, 0.92 or 1 mM, respectively. We refer to the free Ca^{2+} concentration at 0 mM $CaCl_2$ and 1 mM EGTA as $[Ca^{2+}]_{free} = 0$ in this paper. The membrane potential (Vm) was expressed as that of the intracellular side.

2.3. Drug and chemicals

DS-201 was purchased from Bioproduct Research Institute of Chengdu, China (purity ≥ 98%). The drug was dissolved in de-ionized water to obtain 2 mM stock solution, and was added to the bath solution to obtain the desired concentration. K-aspartate, HEPES, EGTA, iberiotoxin (IbTX) and the enzymes were obtained from Sigma (USA).

2.4. Statistical analysis

Numerical values were expressed as mean ± S.E.M. Student's *t* test for paired data and independent test were used for statistical analysis. A value of $P<0.05$ was considered to be statistically significant and "NS" ($P>0.05$), "*" ($P<0.05$) and "**" ($P<0.01$) were indicated in the figures. The relationship between drug concentration and normalized *N*Po was fitted to the Hill equation,

$$y = x^b / \left(c^b + x^b\right) \quad (1)$$

where *x* is the concentration of DS-201 or calcium, *c* is the half maximal effective concentration (EC_{50}) of DS-201 or dissociation constant (K_d) of calcium and *b* is the slope factor (Hill coefficient, n_H).

3. Results

3.1. Properties of single BK_{Ca} currents in cerebral arterial smooth muscle cells

We first confirmed that BK_{Ca} in the mouse cerebral arterial smooth muscle cells possesses its peculiar properties found in other tissues. Fig. 1A shows representative records of single BK_{Ca} currents under inside-out configuration at different voltage with the symmetrical

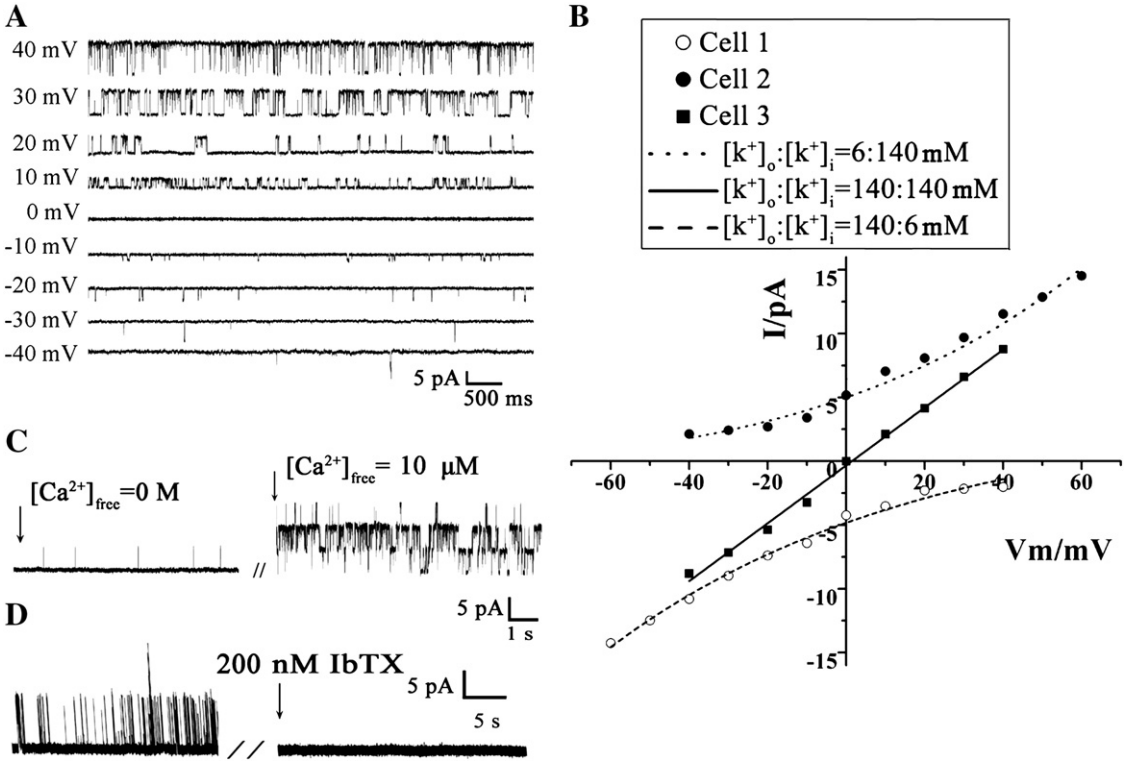

Fig. 1. Properties of BK_{Ca} in mouse cerebral artery smooth muscle cells. (A) A typical recording of single channel currents in an inside-out membrane patch configuration. The upward deflection indicates outward currents. (B) I–V relations obtained from three inside-out patches at different K^+ gradients (substituting Na^+ for K^+). Ratios indicated $[K^+]_o$:$[K^+]_i$ (both in mM). Curves are drawn according to the Goldman–Hodgkin–Katz current equation for K^+. (C) An example of testing Ca^{2+} dependence of BK_{Ca} (inside-out patch, at +40 mV membrane potential). (D) An example of testing IbTX blockage of BK_{Ca} (outside-out patch, at +40 mV membrane potential).