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Cerebrosides of baifuzi, a novel potential blocker of calcium-activated chloride channels in rat pulmonary artery smooth muscle cells

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Abstract

Calcium-activated chloride channels (CaCCs) are crucial regulators of vascular tone by promoting a depolarizing influence on the resting membrane potential of vascular smooth muscle cells. However, the lack of a special blocker of CaCCs has limited the investigation of its functions for long time. Here, we report that CB is a novel potential blocker of $I_{Cl(Ca)}$ in rat pulmonary artery smooth muscle cells (PASMC). Cerebrosides (CB) were isolated from *Baifuzi* which is dried root tuber of the herb *Typhonium giganteum* Engl used for treatment of stroke in traditional medicine. Using the voltage-clamp technique, sustained Ca^{2+}-activated Cl^- current ($I_{Cl(Ca)}$) was evoked by a K^+-free pipette solution containing 500 nM Ca^{2+} which exhibited typical outwardly rectifying and voltage-/time-dependence characterization. Data showed that CB played a distinct inhibitory role in modulating the CaCCs. Moreover, we investigated the kinetic effect of CB on $I_{Cl(Ca)}$ and found that it could slow the activation dynamics of the outward current, accelerate the decay of the inward tail current and change the time-dependence characterization. We conclude that CB is a novel potent blocker of CaCCs. The interaction between CB and CaCCs is discussed.
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Keywords: Calcium-activated chloride channels; Calcium channels; Pulmonary artery smooth muscle; Cerebrosides; Patch clamp

1. Introduction

Calcium-activated chloride channels (CaCCs) play important roles in cellular physiology, including epithelial secretion of electrolytes and water, sensory transduction, regulation of neuronal and cardiac excitability, and regulation of vascular tone. In vascular smooth muscle cells, a variety of cationic channels (Ca^{2+}, K^+, Na^+) in the plasma membrane have been characterized, and their functional roles in controlling vascular tone have been extensively studied. For example, K^+ channel dysfunction plays an important role in the development of pulmonary hypertension (Yuan et al., 1998). Activity of K^+ channels regulates the membrane potential (E_m) of pulmonary artery smooth muscle cells (PASMC) and in turn elevates $[Ca^{2+}]_i$ by opening voltage-dependent Ca^{2+} channels which are implicated in stimulating vascular SMC proliferation and inducing vasomotor tone (Somlyo and Somlyo, 1994; Platoshyn et al., 2000).

Analogous to K^+ as the predominant intracellular cation, Cl^- is the most abundant intracellular and extracellular anion under physiological conditions. CaCCs have been extensively studied in smooth muscle cells derived from a variety of tissues and appear to be involved in both regulation of myogenic tone and contraction stimulated by agonists (Large and Wang, 1996; Davis and Hill, 1999). Activation of CaCCs in smooth muscle

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can occur by Ca^{2+} entry through voltage-gated calcium channels (VGCCs) or by Ca^{2+} released from intracellular stores by inositol 1,4,5-trisphosphate (IP_3) generated through the phospholipase C (PLC) pathway (Large and Wang, 1996; Davis and Hill, 1999). Because E_{Cl} is positive to the resting potential in smooth muscle, opening CaCCs will produce a depolarization (Chipperfield and Harper, 2000).

Norepinephrine, which contracts smooth muscle by activation of Gq-coupled α-adrenergic receptors, increases membrane Cl^- efflux (Wahlstrom, 1973), which leads to membrane depolarization (Bolton, 1979) by activation of $I_{Cl(Ca)}$ (Byrne and Large, 1985; Byrne and Large, 1988). The depolarization is almost abolished by removing external Cl^- in pregnant guinea pig myometrium, guinea pig mesenteric vein, and the anococcygeus muscle (Van Helden, 1988; Large, 1984). This depolarization could increase the open probability of VGCCs, thereby enhancing Ca^{2+} entry and further increasing muscle contraction. Thus smooth muscle contraction is under the control of the release Ca^{2+} from intracellular stores in response to muscle activators and Ca^{2+} entry through VGCCs activated by the depolarization induced by CaCC activation.

Further support for the role of CaCCs in smooth muscle contraction was gathered using the Cl^- channel blocker NFA (Criddle et al., 1996; Criddle et al., 2002; Greenwood and Large, 1995; Lamb and Barna, 1998; Yuan, 1997). NFA blocks both rabbit portal vein $I_{Cl(Ca)}$ and rat aorta contraction induced by norepinephrine by about 50%. Smooth muscle cells also express calcium-dependent potassium current (I_{KCa}). Thus an increase in $[Ca^{2+}]_i$ can open both CaCC and I_{KCa}, which will induce depolarization and hyperpolarization. Spontaneous depolarizations, which may result from the activation of $I_{Cl(Ca)}$ by Ca^{2+} sparks, have been observed in smooth muscle in the absence of agonists. Although these depolarizations could alter the smooth muscle tone, the precise physiological significance of these depolarizations is unknown (Large and Wang, 1996).

Despite the fact that CaCCs are so broadly expressed in cells and play such important functions, understanding these channels has been limited by the absence of specific blockers and the fact that the molecular identities of CaCCs remains in question. Here, we report the CB is a novel potential blocker of $I_{Cl(Ca)}$ in rat PASMC. Cerebrosides (CB) were isolated from *Baifuzi*, which is dried root tuber of the herb *Typhonium giganteum* Engl which has been used for treatment of cerebral apoplexy for a long time in China (Chen et al., 2002). Cerebrosides are a kind of glycosphingolipid built a long-chain amino-alcohol known as a sphingoid base or long-chain base (LCB), a fatty acid residue and a saccharine head (Tringal, 2001).

It was reported that cerebrosides may act by signal transduction through cell membranes and exhibit significant activities such as inhibitting the entry of HIV-1 in neural cell lines in 1991 by Harouse et al. (1991) and show ionophoric activity for Ca^{2+} ions in 1990 by Kitagawa (Shibuya et al., 1990). However, the activity of CB on ion channels is completely unknown. We tested the effect of CB on CaCCs because CB was believed as the major pharmacological component for treatment of cerebral apoplexy. And activation of CaCCs induces artery contract is considered a very important process for cerebral apoplexy in arterial smooth muscle. We expected to investigate the relationship between cerebrosides and CaCCs and illustrate possible medicinal mechanism why CB could cure cerebral apoplexy. The present study focuses on the kinetic effect of CB on CaCCs' electrophysiological properties such as the activation, deactivation, voltage-/time-dependence of $I_{Cl(Ca)}$ and the potential role of CB in modulating the $I_{Cl(Ca)}$. We conclude that CB is a novel potent blocker of CaCCs and our finding may open the functional gate of CaCCs.

2. Materials and methods

2.1. Preparation of PASMC

Cells were prepared from isolated rat main pulmonary artery. After dissection and removal of connective tissue the artery was rubbed with incurvate scissors softly to remove endothelial cells in D-Hanks' balanced salt solution containing (in mg/ml) 0.4 KCl, 0.06 KH_2PO_4, 8.0 NaCl, 0.06 Na_2HPO_4, 0.35 $NaHCO_3$, pH7.2. The tissue was then cut into small strips and incubated in 2 mL D-Hanks' balanced salt solution containing 2 mg/mL collagenase for 1 h and then added 1 mL 0.15% trypsinase for 5 min at 37 °C to create a single cell suspension. The digestion was stopped by 2 mL DMEM supplemented with 10% fetal bovine serum and the cells were released by gentle agitation with a wide bore Pasteur pipette. Single pulmonary artery smooth muscle cells were resuspended and plated onto a glass coverslip and incubated in a humidified atmosphere of 5% CO_2-95% air at 37 °C in 20% fetal bovine serum culture medium for 2–3 days before use.

2.2. Preparation of cerebrosides

CB was isolated from *T. giganteum* Engl. The detailed process was described in our previous paper (Chen et al., 2002). Because CB cannot be dissolved in water, we packed it with liposome. CB contained the main cerebroside typhoniside A and some analogue of it. Their difference was the length of the fatty acid, such as 16, 18, 22 carbon atoms.

2.3. Electrophysiology

Conventional whole-cell patch-clamp measurements were performed using an EPC-9 patch-clamp amplifier and PULSE software (HEKA, Lambrecht, Germany). In experiments, $I_{Cl(Ca)}$ were evoked by pipette solutions containing 500 nM Ca^{2+} as this concentration of Ca^{2+} generated large and robust Cl^- currents in pulmonary artery smooth muscle cells (Piper and Greenwood, 2003; Greenwood et al., 2001). The pipette solution contained (mM): TEA-Cl 20; CsCl 106; HEPES 5; BAPTA 10; MgATP 3; $GTPNa_2$ 0.2; $MgCl_2$ 0.42, and the pH was set to 7.2 by adding CsOH. Free $[Ca^{2+}]$ was set at 500 nM by the addition of 7.8 mM $CaCl_2$ determined by the EQCAL buffer program. The external solution contained (mM): NaCl 126; HEPES 10; pH 7.4, glucose 11; $CaCl_2$ 1.8; $MgCl_2$ 1.2; TEA-Cl 10 and 4-aminopyridine 5. All reagents were purchased from Sigma unless otherwise stated.

2.4. Experimental protocols: patch-clamp recording from single cell

Patch pipettes pulled from borosilicate glass capillaries had resistances of 2–6 megohms by excising with internal solution. Currents were obtained by excising the patch from cells in the whole-cell configuration and typically digitized at 20 kHz. Macroscopic records were filter at 2 or 2.9 kHz during digitization. During recording, drugs and control/wash solutions were puffed locally onto the cell via a puffer pipette containing eight solution channels. The tip (300 μm diameter) of the puffer pipette was located about 120 μm from the cell. As determined by the conductance tests, the solution around

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