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PKA-dependent activation of the vascular smooth muscle isoform of K_{ATP} channels by vasoactive intestinal polypeptide and its effect on relaxation of the mesenteric resistance artery

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Abstract

Vasoactive intestinal polypeptide (VIP) is a potent vasodilator and has been successfully used to alleviate hypertension. Consistently, disruption of VIP gene in mice leads to hypertension. However, its downstream targets in the vascular regulation are still not well demonstrated. To test the hypothesis that the vascular smooth muscle isoform of K_{ATP} channels is a downstream target of the VIP signaling, we performed the studies on the Kir6.1/SUR2B channel expressed in HEK293 cells. We found that the channel was strongly activated by VIP. Through endogenous VIP receptors, the channel activation was reversible and dependent on VIP concentrations with the midpoint-activation concentration ~10 nM. The channel activation was voltage-independent and could be blocked by K_{ATP} channel blocker glibenclamide. In cell-attached patches, VIP augmented the channel open-state probability with modest suppression of the single channel conductance. The VIP-induced Kir6.1/SUR2B channel activation was blocked by PKA inhibitor RP-cAMP. Forskolin, an adenylyl cyclase activator, activated the channel similarly as VIP. The effect of VIP was further evident in the native tissues. In acutely dissociated mesenteric vascular smooth myocytes, VIP activated the K_{ATP} currents in a similar manner as in HEK293 cells. In endothelium-free mesenteric artery rings, VIP produced concentration-dependent vasorelaxation that was attenuated by glibenclamide. These results therefore indicate that the vascular isoform (Kir6.1/SUR2B) of K_{ATP} channels is a target of VIP. The channel activation relies on the PKA pathway and produces mesenteric arterial relaxation.
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1. Introduction

Vasoactive intestinal polypeptide (VIP) is a 28-amnio-acid peptide hormone and neurotransmitter present in multiple organs and systems. VIP has broad effects on cellular functions including vasodilation, water reabsorption, neurotransmission, insulin secretion and immunomodulation [1–3]. These biological effects are mediated by specific VIP receptors (VPAC1 and VPAC2), both of which are coupled to G proteins, primarily the G_S proteins [4].

As a potent vasodilator, VIP containing nerve terminals innervate a variety of blood vessels in systemic and pulmonary circulations [5]. VIP released from the nerve terminals produces vascular smooth muscle relaxation. Such a vasodilation effect is 50–100 times more potent than acetylcholine [5]. Administration of VIP to patients with severe cardiovascular diseases such as primary pulmonary hypertension results in substantial improvement in their conditions without adverse side effects [6]. Moderate pulmonary arterial hypertension has been observed in mice lacking the VIP gene [7,8]. By taking advantage of the vasodilation effects, several species of animals have developed VIP-like peptides that are lethal vasodilatory toxins serving for defense and predatory purposes [9–11].

The downstream molecules of VIP in the vasodilation effect are still not fully understood [5]. In the vascular smooth muscle cells, the influx of Ca^{++} through voltage-dependent Ca^{++} channels contributes to vessel constriction, while the preclusion of

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this event leads to the vasodilation [12,13]. The opening and closure of these Ca^{++} channels are largely controlled by membrane potentials. Activation of the vascular K_{ATP} channels hyperpolarizes the vascular smooth muscle cells, prevents the Ca^{++} influx, and relaxes blood vessels. Therefore, the vascular K_{ATP} channels play an important role in vascular tone regulation [14].

Functional K_{ATP} channels are made of four pore-forming subunits Kir6.x (Kir6.1 or Kir6.2) and four regulatory subunits sulfonylurea receptor SURx (SUR1, SUR2A or SUR2B) [15]. The former belongs to the inwardly rectifying K^+ channels, and the latter is a member of the ATP-binding cassette (ABC) protein family [16–18]. A combination of different Kir6.x and SURx results in distinct K_{ATP} channels, such as the Kir6.2/SUR1 channel in pancreatic β-cells and the Kir6.2/SUR2A channel in cardiac muscles [19]. The Kir6.1/SUR2B channel is the major isoform of K_{ATP} channels in vascular smooth muscles, although there is evidence that the Kir6.2 also form functional channels with SUR2B in blood vessels [20–22]. The biophysical and pharmacological properties of the Kir6.1/SUR2B channel are comparable to those of K_{NDP} channels found in native coronary and mesenteric arteries [23,24]. Consistently, disruptions of the *Kcnj8* (Kir6.1) or *ABCC9* (SUR2) genes in mice cause abnormalities in coronary circulation, sudden cardiac death and fatal susceptibility to endotoxemia [25–27].

Previous studies have suggested that the vascular K_{ATP} channels are subjected to phosphorylation regulation by protein kinases A, C and G (PKA, PKC, PKG), allowing them to respond to several vasoactive hormones and neurotransmitters [28–32]. Since the PKA signaling system can be activated by VIP, it is possible that the vascular smooth muscle K_{ATP} channels play a role in vasodilation effect of VIP. To test the hypothesis, we performed these studies on the Kir6.1/SUR2B channel expressed in HEK293 cells, cell-endogenous K_{ATP} channels from dissociated smooth myocytes and isolated mesenteric arterial rings.

2. Methods and materials

2.1. Expression Kir6.1/SUR2B channel in HEK293 cells

Human embryonic kidney cells (HEK293) were used for the expression of Kir6.1/SUR2B channel. The HEK293 cells were cultured in DMEM/F12 medium with 10% fetal bovine serum and penicillin/streptomycin at 37 °C with 5% CO_2. A eukaryotic expression vector pcDNA3.1 containing Rat Kir6.1 (GenBank No. D42145) or SUR2B cDNAs (GenBank No. D86038, mRNA isoform NM_011511) was co-transfected to the cells. A 35-mm petri dish of cells was transfected with 1 μg Kir6.1 and 3 μg SUR2B using Lipofectamine^{2000} (Invitrogen Inc., Carlsbad, CA). To facilitate the identification of positively transfected cells, 0.5 μg green fluorescent protein (GFP) cDNA (pEGFP-N2, Clontech, Palo Alto, CA) was included in the cDNA mixture. 24 h after transfection, cells were disassociated with 0.25% trypsin, split and transferred to cover slips. Experiments were performed on the cells in the following 6–48 h.

2.2. Electrophysiology

Patch clamp experiments were carried out at room temperature as described previously [31–33]. In brief, fire-polished patch pipettes with 2–5 MΩ resistance were made from 1.2-mm borosilicate glass capillaries. Whole-cell currents were recorded in single-cell voltage clamp using the Axopatch 200B amplifier (Axon Instruments Inc., Foster City, CA), low-pass filtered (2 kHz, Bessel 4-pole filter, −3 dB), and digitized (10 kHz, 16-bit resolution) with Clampex 9 (Axon Instruments Inc.). Data were analyzed using Clampfit 9 (Axon Instruments Inc.). The bath solution contained (in mM): KCL 10, potassium gluconate 135, EGTA 5, glucose 5, and HEPES 10 (pH=7.4). The pipette was filled with a solution containing: KCl 10, potassium gluconate 133, EGTA 5, glucose 5, K_2ATP 1, NaADP 0.5, $MgCl_2$1, and HEPES 10 (pH=7.4). To avoid nucleotide degradation, all intracellular solutions were freshly made and used within 4 h.

All reagents and chemicals were purchased from Sigma unless otherwise stated. Pinacidil and glibenclamide were prepared as stock solution of 10 mM in DMSO. VIP was prepared in 1% acetic acid (v/v). All solvents were tested and showed no detectable effect on the K_{ATP} channels.

2.3. Mesenteric artery preparation and tension measurement

All animal experiments were performed in compliance with an approved protocol by the Institutional Animal Care and Use Committees (IACUC) at Georgia State University. Male Sprague–Dawley rats (200–250 g body weight) were deeply anesthetized followed by decapitation. Mesenteric arteries were dissected free and placed in PSS containing (in mM): NaCl 140, KCl 4.6, $CaCl_2$ 1.5 $MgCl_2$ 1, glucose 10, HEPES 5, pH 7.3. The arteries were cut into small rings (2 mm in length) and transferred to ice-cold Krebs solution containing: NaCl 118.0, $NaHCO_3$ 25.0, KCl 3.6, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.0, $CaCl_2$ 2.5. The endothelium-free rings were prepared by rubbing with a sanded polyethylene tubing, and confirmed with vasodilation response to acetylcholine (ACh) as described previously [31].The arterial ring was mounted on a force electricity transducer (Model FT-302, iWorx/CBSciences, Inc. Dover, NH) for measurements of isometric force contraction in a 5-ml tissue bath filled with the air bubbled Krebs solution. All rings were pre-tested with phenylephrine (PE) to ensure the tissue vitality. When endothelium needed to be removed, the rings were tested by PE for contraction followed by an exposure to ACh (1 μM). The rings were considered to be endothelium-free if more than 90% relaxation was eliminated. PE and ACh then were washed out, and the rings were allowed to equilibrate in the Krebs solution for another 30–60 min before experiments.

2.4. Acute dissociation of mesenteric vascular smooth cells

Single vascular smooth cells were prepared with two-step enzyme digestions. Main branch of mesenteric arteries was obtained as mentioned above. After clearance of connective tissue, 1–2 mm small segments were cut and placed in 5 ml solution containing (in mM): NaCl 140, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 0.1, HEPES 10 and D-glucose 10 for 10 min in room temperature. The tissues were then placed in 1 ml of the same solution with 20U of papain (Worthington, New Jersey), 1.25 mg dithiothreitol (DTT) and 1% fetal bovine serum. After 25-min digestion at 35 °C, the tissues were washed and incubated with 440U collagenase (CLS II, Worthington), 1.25 mg trypsin inhibitor (Sigma) and 1% fetal bovine serum for 10 min. After thorough washes, the tissues were triturated with a fire-polished Pasteur pipette to yield single cells. The dissociated smooth muscle cells were placed in a petri dish and allowed to attach to the dish surface before recordings. Patch clamp experiments were carried out in the cells that showed clear smooth muscle morphology and had no sign of swelling and shrinkage.

2.5. Data analysis

Data were presented as means±S.E. (standard error). Differences were evaluated using Student's *t*-tests or ANOVA, and statistical significance was accepted if $P<0.05$.

3. Result

3.1. Activation of the Kir6.1/SUR2B channel by VIP in HEK293 cells

The Kir6.1/SUR2B channel was transiently expressed in the HEK293 cells, and whole cell voltage-clamp was performed

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