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Study in the Ca^{2+}-Dependent K^+-Current and Ca^{2+} Entry Induced by Activation of α_{1B}-Adrenoceptor Subtype in HEK-293 Cell①

Guan Yongyuan[1] Tao Liang[1] He Hua[1] Han Qide[2] Zhang Youyi[2] Sun Jiajun[1]

(1 Department of Pharmacology, Sun Yat-sen University of Medical Sciences, Guangzhou, 510089
2 Institute of Vascular Medicine, Beijing Medical University, Beijing, 100083)

Abstract **Objective**: To study the characterization of the outward K^+-current and Ca^{2+} entry induced by activation of α_{1B}-adrenoceptor subtype at the HEK-293 cells. **Methods**: The single K^+-current was recorded from the cell attached configuration. The cytoplasmic Ca^{2+} was measured by fura-2 probe. **Results**: adrenaline evoked an outward K^+-current with a conductance of 160 pS. The current was markedly inhibited by 50 $\mu mol \cdot L^{-1}$ chloroethylclonidine, 5 $mmol \cdot L^{-1}$ EGTA or 2 $mmol \cdot L^{-1}$ TEA. Nifedipine did not change this current and adrenaline-induced Ca^{2+} entry wihich was inhibited by 1 $mmol \cdot L^{-1}$ $LaCl_3$. **Conclusion**: Activation of α_{1B}-subtype receptor at HEK-293 cells evokes Ca^{2+} entry through the nifedipine resistant Ca^{2+} channel, followed by an outward K^+-current.

Subject headings calcium channels; potassium channels; receptors, adrenergic, alpha-1; HEK-293 cell; electrophysiology

HEK-293 细胞 α_{1B}-肾上腺素受体引起的 Ca^{2+} 依赖性 K^+ 电流和 Ca^{2+} 内流

关永源[1] 陶 亮[1] 贺 华[1] 韩启德[2] 张幼怡[2] 孙家钧[1]

(1 中山医科大学药理教研室;广州,510089 2 北京医科大学血管医学研究所;北京,100083)

摘 要 目的:研究 HEK-293 细胞上 α_{1B}-肾上腺素受体亚型引起向外 K^+ 电流和 Ca^{2+} 内流的特性。方法:细胞贴附式单通道记录 K^+ 通道和 Fura-2 荧光测定胞浆游离 Ca^{2+} 浓度。结果:肾上腺素或苯肾上腺素可引发一电导为 160 pS 的外向 K^+ 电流。该电流可被 50 $\mu mol \cdot L^{-1}$ 氯乙醛可乐定(CEC),5 $mmol \cdot L^{-1}$ 依他酸(EGTA)或 2 $mmol \cdot L^{-1}$ 四乙铵(TEA)抑制。硝苯吡啶(nifedipine)不改变该电流及 α_{1B} 亚型引起的 Ca^{2+} 内流;后者可被 1 $mmol \cdot L^{-1}$ $LaCl_3$ 抑制。结论:激活转染在 HEK-293 细胞上的 α_{1B}-受体亚型可引起通过硝苯吡啶不敏感 Ca^{2+} 通道的 Ca^{2+} 内流,并跟随产生一外向 K^+ 电流。

主题词 钙通道;钾通道;受体,肾上腺素能 α_1;HEK-293 细胞;电生理学
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It has been suggested that α_1-adrenoceptor (α_1-AR) can be divided into α_{1A}, α_{1B} and α_{1D} three subtypes. Because these α_1-AR subtypes coexist in many tissues[1], it is difficult to identify the functions of individual α_1-AR subtype. Recently, a permanently transformed cell line derived from the human embryo renal cortical cells (HEK-293 cell line) has usually been used to express the cloned cDNA of various receptors[2~4]. HEK-293 cells were respectively transfected with α_{1A}-, α_{1B}- and α_{1D}-AR subtypes. However, there is no report about the K^+-currents induced by activation of α_{1B}-AR in HEK-293 cells. In this study, we determined the Ca^{2+}-dependent K^+-currents and the change of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), induced by activation of α_{1B}-adrenoceptor subtype at the transfected HEK-293 cells.

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1 Material and methods

1.1 Cell culture

Subclone of HEK-293 transfected with hamster α_{1B} was kindly provided by Dr. Minneman. Subclones were maintained in DMEM containing 100 ml·L^{-1} calf serum, and propagated in the continued presence of selective antibiotic of 50 mg·L^{-1} hygromycin-pREP4.

1.2 Single channel recording

Standard patch clamp techniques[5] were used for recording the membrane currents in the α_{1B} transfected HEK-293 cells. Single K^+-current was made from the cell attached configuration at 30 ℃. The pipette solution contained: KCl 139 mmol·L^{-1}, $MgCl_2$ 0.5 mmol·L^{-1}, $CaCl_2$ 0.1 mmol·L^{-1}, HEPES 10, egtazic acid (EGTA) 0.09 mmol·L^{-1}, glucose 10 mmol·L^{-1}, pH 7.4 with KOH. The extracellular solution contained: NaCl 130 mmol·L^{-1}, $MgCl_2$ 1.2 mmol·L^{-1}, $CaCl_2$ 1.8 mmol·L^{-1}, KCl 5.4 mmol·L^{-1}, HEPES 10 mmol·L^{-1}, glucose 5.2 mmol·L^{-1}, pH 7.4 with NaOH. The pipettes had outside tip diameters between 1~2 μm and had resistance values in the range 4~8 mΩ. A patch clamp amplifier (Axopatch-1D; Axon Instruments, Inc.) was used to record currents with a lowpass filter set at 3 kHz.

Data were recorded from patches with apparent activity from only one channel as evident from single open levels over the 15 s recording periods. A measure of the channel open probability was found by summing the individual open times and dividing this value by the total recording time at a specified potential.

1.3 Intracellular Ca^{2+} concentration measuring

Cells (10^3 cells·L^{-1}) were incubated with 1 μmol·L^{-1} fura-2/AM for 40 min at 37 ℃. The extracellular fura-2/AM was washed out with HEPES solution containing NaCL 110 mmol·L^{-1}, KCl 5.4 mmol·L^{-1}, $MgCl_2$ 1.0 mmol·L^{-1}, $CaCl_2$ 1.0 mmol·L^{-1}, HEPES 20 mmol·L^{-1}, pH 7.4. The $[Ca^{2+}]_i$ was monitored by a RF-5000 fluorescence spectrophotometer with dual excitation at 340 nm/380 nm and emission at 500 nm. $[Ca^{2+}]_i$ was calculated from the formula as following: $[Ca^{2+}]_i = Kd\ S_{f380/b380}(R-R_{min})/(R_{max}-R)$. Where, Kd is 225 nmol·$L^{-1}$ in the cytoplasmic environment; $S_{f380/b380}$ is the ratio of the intensities of the free and bound dye forms at 380 nm; R is the fluorescence ratio (340 nm/380 nm) of the intracellular fura-2; R_{max} and R_{min} are the maximal and minimal fluorescence ratios which are obtained by additions of Triton X-100 (final concentration is 0.9 ml·L^{-1}) and EGTA (final concentration is 3 mmol·L^{-1}) respectively.

Nifedipine (Sigma) and fura-2/AM (Sigma) were dissolved in ethanol at 10 mmol·L^{-1} and in DMSO at 1 μmol·L^{-1} stock solutions respectively, and stored in dark. These stock solutions were freshly diluted to desired concentrations with demineralized water. Other reagents were obtained from Sigma Company.

2 Results

2.1 Signal channel recording

4.5 μmol·L^{-1} adrenaline or 20 μmol·L^{-1} phenylephrine evoked an outward K^+ current which was significantly inhibited by 50 μmol·L^{-1} chloroethylclonidine (CEC), a selective α_{1B}-adrenoceptor subtype blocker (fig.1).

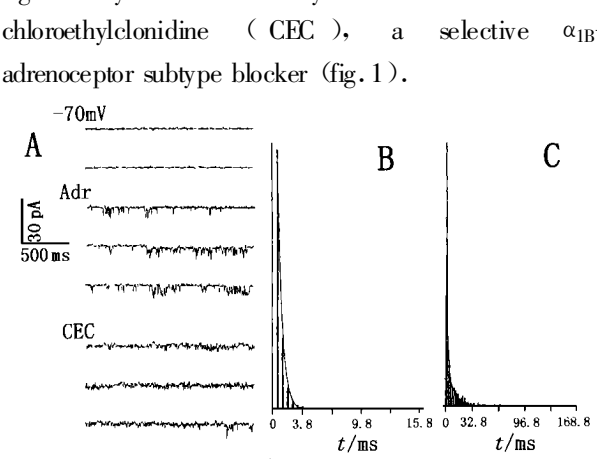

Fig.1 The outward K^+-current was recorded by cell attached configuration with a pipette potential of −70 mV

A. 4.5 μmol·L^{-1} Adrenaline (Adr) increased the channel open probability that was decreased by 50 μmol·L^{-1} chloroethylclonidine (CEC). B. The histogram of the channel open time distribution with time constant of 0.45 ms. C. The histogram of the channel close time distribution with the time constants of 0.96 and 7.98 ms

The opening time distribution of this K^+ channel required an one component fit with time constant of 0.45 ms, and the channel close time distribution required a two components fit with time constants of 0.96 and 7.98 ms. This current was voltage-dependent and had a

conductance of 160 pS (fig. 2).

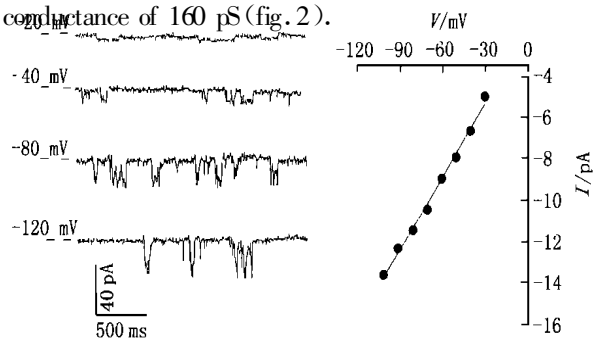

Fig. 2 The current was voltage-dependent

On the left: the current was recorded in the different pipette potentials. On the right: I-V relationship curve

The current was inhibited by 2 mmol·L^{-1} TEA (a Ca^{2+}-dependent K^+ channel blocker) and completely blocked by 5 mmol·L^{-1} EGTA. However, 0.2 μmol·L^{-1} nifedipine by which Ca^{2+} entry induced by depolarization was blocked[6] did not significantly inhibit this current (fig. 3 and tab. 1). Before and after addition of nifedipine, the channel opening probabilities were 0.057±0.002 and 0.058±0.002 respectively (n = 4; P > 0.05).

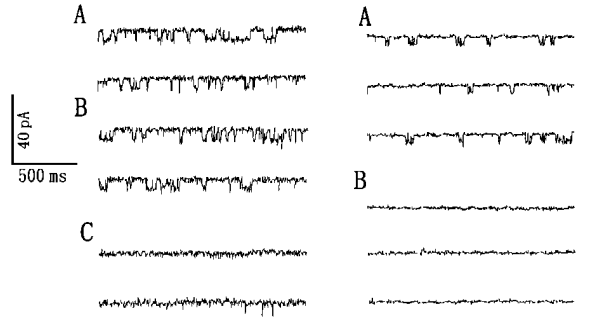

Fig. 3 The current was recorded with a pipette potential of −60 mV

On the left, A, B and C express the current recorded pretreatment, 1 μmol·L^{-1} nifedipine and 2 mmol·L^{-1} TEA respectively. Nifedipine did not significantly change the current which was markedly inhibited by TEA. On the right, the current was recorded before (A) and after (B) addition of 5 mmol·L^{-1} EGTA

2.2 [Ca^{2+}]i measuring

In the HEK-293 cells, the resting [Ca^{2+}]i was (23.9±1.3) nmol·L^{-1}. 50 mmol·L^{-1} KCl did not increase [Ca^{2+}]i. In Ca^{2+}-free medium, 1 μmol·L^{-1} adrenaline evoked a transient increase in [Ca^{2+}]i, which was due to the intracellular calcium release, from (23.9±1.3) nmol·L^{-1} to (293.3±85.4) nmol·L^{-1} (n = 4, P < 0.01). Subsequent addition of $CaCl_2$ (the final concentration was 1.5 mmol·L^{-1} in the medium) further induced an increase in [Ca^{2+}]i (due to the extracellular Ca^{2+} entry) to (456.6±52.6) nmol·L^{-1}, then gradually decreased to a sustained level of (156.8±29.7) nmol·L^{-1} (n = 4). 1 mmol·L^{-1} nifedipine did not reduce the [Ca^{2+}]i induced by addition of $CaCl_2$, whereas 1 mmol·L^{-1} $LaCl_3$ significantly decreased [Ca^{2+}]i from (156.8±29.7) nmol·L^{-1} to (84.9±4.6) nmol·L^{-1} (n = 1, P < 0.01; Fig. 4).

Table 1 The effects of nifedipine, CEC, EGTA and Mn^{2+} on the adrenalin-induced open probability of K^+ channel

Drugs	n	Pretreatment	Treatment
Nifedipine	4	0.057±0.002	0.058±0.001
CEC	3	0.061±0.010	0.009±0.001
TEA	4	0.051±0.012	0.010±0.005
EGTA	4	0.049±0.005	0 .0
Mn^{2+}	3	0.065±0.008	0. 0

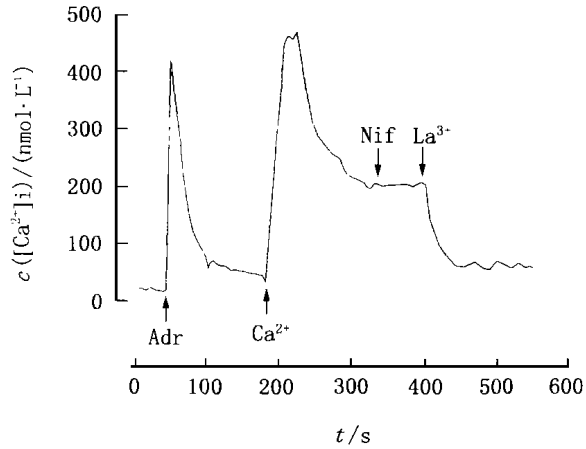

Fig. 4 Adrenaline (Adr) induced a transient increase in [Ca^{2+}]i under condition of Ca^{2+}-free medium

Subsequent addition of $CaCl_2$ further increased the [Ca^{2+}]i; 1 μmol·L^{-1} nifedipinde (Nif) did not reduced [Ca^{2+}]i whereas $LaCl_3$ significantly decreased it

Addition of EGTA (the concentration in medium was 3 mmol·L^{-1}) completely blocked the Ca^{2+} entry and made the [Ca^{2+}]i turn to resting level.

3 Discussion

Recently, the HEK-293 cell line was used to express cDNA of ATP-sensitive potassium channels for studying the characterization of these channels[7,8]. In

this study, although we did not transfect any cDNA of potassium channels in the α_{1B}-HEK-293 cells, activation of α_{1B}-adrenoceptor in HEK-293 cells evoked an outward K^+-current with a conductance of 160 pS. It indicates that HEK-293 cells can express some kind of K^+ channel. Because the EGTA blocked the current, this K^+-current was thought to be dependent on the extracellular Ca^{2+} entry. Nifedipine, a *L*-subtype voltage-dependent Ca^{2+} channel (VDC) blocker, did not change this current. In the experiments of fura-2 probe, it was shown that nifedipie did not decrease the elevation of $[Ca^{2+}]$ i induced by activation of α_{1B}-subtype, whereas, $LaCl_3$ significantly inhibited the increase in $[Ca^{2+}]$ i induced by Ca^{2+} entry. This data is consistent with the results from single channel recording experiments, and suggests that the Ca^{2+} entry induced by α_{1B}-adrenoceptor subtype in HEK-293 cell be not mediated by *L*-subtype of VDC.

The present results indicate that activation of α_{1B}-adrenoceptor in HEK-293 cells produces a Ca^{2+} entry through nifedipine-resistant Ca^{2+} channel, elevates $[Ca^{2+}]$ i, and then, evokes an outward K^+-current.

We have noted the data from other laboratories are distinct from ours. It was reported that the Ca^{2+} entry induced by α_{1B}-adrenoceptor activation in rat medullary thyroid carcinoma 6-23 cells[9], Cos-1 cells[10] and rat irideal blood vessels[11] was voltage dependent. Nifedipine markedly inhibited this Ca^{2+} entry.

All results submit a proof for the fact that the mechanisms of Ca^{2+} movement induced by α_{1B}-adrenoceptor are different from cell line to cell line. It appears that these differences are dependent on the nature of cells.

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