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Blockade of voltage-dependent K^+ current in rabbit coronary arterial smooth muscle cells by the tricyclic antidepressant clomipramine

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ABSTRACT

We investigated the effect of the tricyclic antidepressant clomipramine on voltage-dependent K^+ (Kv) channels in native rabbit coronary arterial smooth muscle cells. Our results showed that clomipramine inhibited vascular Kv channels in a concentration-dependent manner, with an IC_{50} value of 8.61 ± 4.86 μM and a Hill coefficient (n) of 0.58 ± 0.07. The application of 10 μM clomipramine did not affect the activation curves of the Kv channels; however, the inactivation curves of the Kv channels were shifted toward a more negative potential. The clomipramine-induced inhibition of Kv currents was not changed by the application of train pulses (1 or 2 Hz), which demonstrated that clomipramine inhibited Kv current in a state (use)-independent manner. Pretreatment with the Kv1.5 and Kv2.1 inhibitors, DPO-1 and guangxitoxin, respectively, partially reduced the clomipramine-induced inhibition of Kv currents. Therefore, we concluded that clomipramine inhibited vascular Kv channels in a concentration-dependent, but state (use)-independent manner, regardless of its own function.

1. Introduction

For decades, tricyclic antidepressants (TCAs) have been used for the treatment of depression and neuropathic pain.[1] Clomipramine, a representative TCA, is effective for many central nervous system disorders, such as obsessive-compulsive disorder, major depressive disorder, panic disorder, and depersonalization disorder.[2] However, common side effects of clomipramine, including dry mouth, loss of appetite, constipation, sleepiness, and weight gain, have been reported.[3] The side effects of clomipramine on ion channels also have been reported. For example, clomipramine inhibits human *ether-a-go-go*-related gene (hERG) channels expression in oocytes,[4] and Na^+/K^+-ATPase activity in the cerebral cortex.[5] In addition to these side effects, clomipramine has been reported to effect the cardiovascular system, for example it can prolong the QT interval.[6] However, the molecular mechanisms for this has not been investigated. Furthermore, the effect of clomipramine on vascular ion channels, specifically Kv channels, has been neglected.

In vascular smooth muscle cells, the resting membrane potential is mainly determined by K^+ efflux through plasma membrane K^+ channels. To date, four main types of K^+ channels, the ATP-sensitive K^+ (K_{ATP}), inwardly rectifying K^+ (Kir), big-conductance Ca^{2+}-activated K^+ (BK_{Ca}), and voltage-dependent K^+ (Kv) channels, were found to be expressed in vascular smooth muscle cells.[7,8] Among these, Kv channels are highly expressed in most vascular smooth muscle cells, which play an important role in the regulation of the contraction of vascular smooth muscle cells through effects on the

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membrane potential and modulation of voltage-gated Ca^{2+} channel activity.[7] Indeed, the application of the Kv channel inhibitor 4-aminopyridine induced constriction in response to increased intraluminal pressure.[9] Consequently, changes in vascular Kv channel activity and/or expression are closely associated with many vascular dysfunctions. For example, the loss of Kv1.5 function may cause microvascular dysfunction in coronary artery disease or other vascular diseases,[10] and decreased expression of Kv7 subtypes is closely related to hypertension and diabetes.[11,12]

To evaluate the therapeutic efficacy of clomipramine for the treatment of psychiatric disorders and the pathophysiological importance of Kv channels, the effect of clomipramine on vascular Kv channels should be clearly identified to ensure the accurate prescription of clomipramine to patients with diabetes or cardiovascular disease.

In our present study, we investigated the effect of clomipramine on vascular Kv channels using freshly isolated rabbit coronary arterial smooth muscle cells. We clearly demonstrated that clomipramine inhibited vascular Kv channels in a concentration-dependent, but state (use)-independent, manner separate from its own function.

2. Materials and methods

2.1. Single cell isolation

Male New Zealand White rabbits were used in experiments. All animal experiments were approved by the Committee for Animal Experiments of Kangwon National University. The rabbits were sacrificed by the simultaneous injection of sodium pentobarbital (50 mg/kg) and heparin (100 U/kg) into the ear vein. The second to third order branches of left descending coronary artery were carefully isolated from the heart and the connective tissue was cleaned in normal Tyrode's solution under a stereomicroscope. The endothelium of the isolated arteries was eliminated by passing an air bubble through the arteries. The arteries were cut into lengths of 10 mm and then transferred to 1 mL Ca^{2+}-free normal Tyrode's solution containing papain (1.0 mg/mL), BSA (2.0 mg/mL), and DDT (1.5 mg/mL) for 25 min. Next, the arteries were transferred to 1 mL Ca^{2+}-free normal Tyrode's solution containing collagenase (2.8 mg/mL), BSA (2.0 mg/mL), and DDT (1.5 mg/mL) for 18–20 min at 37 °C. Single cells were separated by gentle agitation in Kraft-Brühe (KB) solution with a fire-polished glass pipette. The isolated cells were stored at 4 °C and used within 10 h.

2.2. Solutions and chemicals

The constituents of normal Tyrode's solution were (in mM): 138 NaCl, 5.3 KCl, 0.35 NaH_2PO_4, 5 HEPES, 15 glucose, adjusted to pH 7.4 with NaOH. The KB solution contained (in mM): 75 KOH, 52 L-glutamate, 18 KH_2PO_4, 50 KCl, 20 taurine, 2.5 $MgCl_2$, 15 glucose, 10 HEPES, 1 EGTA, adjusted to pH 7.3 with KOH. The pipette-filling (internal) solution contained (in mM): 115 K-aspartate, 20 KCl, 5 NaCl, 1.5 $MgCl_2$, 4 Mg-ATP, 10 EGTA, 10 HEPES, adjusted to pH 7.2 with KOH. Clomipramine, DPO-1, and guangxitoxin were purchased from Tocris Cookson (Ellisville, MO) and dissolved in dimethyl sulfoxide (DMSO).

2.3. Electrophysiology and data analysis

A conventional whole-cell configuration was applied to record the Kv currents using PatchPro software, a digital interface (NI-DAQ-7; National Instruments, Union, CA, USA), and amplifier (EPC-8; Medical system Corp., Darmstadt, Germany). Recording pipettes were made from borosilicate capillary tubes (Clark Electromedical Instruments, Pangbourne, UK) using a vertical puller (PP-830, Narishige Scientific Instrument Laboratory, Tokyo, Japan). When filled with the internal solution, the resistance of patch pipettes was 3–4 MΩ. The average cell capacitances were 12.74 ± 0.63 pF ($n = 17$) and the series resistance was 8.62 ± 0.72 MΩ ($n = 13$). The input resistance was stably maintained at 2.51 ± 0.42 GΩ ($n = 10$) and not significantly altered by the application of clomipramine.

Data analysis was conducted using Origin 7.5 software (Microcal Software, Inc., Northampton, MA, USA). The channel–drug interaction kinetics were expressed as a first-order blocking scheme, as previously described.[13] The half-inhibition concentration (IC_{50}) value and the Hill coefficients (n) were calculated based on the concentration-dependent data fitted to the following Hill equation:

$$f = 1/\{1 + (IC_{50}/[D])^n\}$$

where f indicates the fractional current inhibition ($f = 1 - I_{drug}/I_{control}$) at the test potential, and [D] indicates drug concentration.

The steady-state activation kinetics were deduced from tail currents induced by a return potential to −40 mV after depolarizing pulses from −80 to +60 mV in 10 mV increments. The recorded tail currents were normalized to the maximal tail current and fitted to the Boltzmann equation, as described below:

$$y = 1/\{1 + \exp(-(V - V_{1/2})/k)\}$$

where V indicates the test potential, $V_{1/2}$ represents the mid-point of activation, and k indicates the slope factor.

The steady-state inactivation kinetics were obtained by using a two-pulse voltage protocol. The currents were elicited by the application of test potential to +40 mV after 7-s of preconditioning pulses in the range from −80 to +30 mV. The steady-state inactivation curve was fitted to another Boltzmann equation as follows:

$$y = 1/[1 + \exp\{(V - V_{1/2})/k\}]$$

where V indicates the preconditioning potential, $V_{1/2}$ indicates the potential of the half-maximal of inactivation point, and k represents the slope value.

All results are expressed as the mean ± standard error of the mean (S.E.M). Student's t-test was applied to determine significance and values of $P < 0.05$ were regarded as statistically significant.

3. Results

3.1. Clomipramine-induced inhibition of Kv currents in coronary arterial smooth muscle cells

We investigated the effect of clomipramine on Kv currents in freshly isolated coronary arterial smooth muscle cells by using patch clamp techniques. Control Kv currents were generated by 600-ms step-depolarizing pulses between −80 and + 60 mV from a holding potential of −80 mV (Fig. 1A). To record the Kv currents, other K^+ channels expressed in arterial smooth muscle were suppressed by the addition of 10 mM EGTA (to block the BK_{Ca} channels) and 4 mM ATP (to block the K_{ATP} channels) in the pipette solution. The effects of clomipramine on Kv currents are shown in Fig. 2B. The application of 10 μM clomipramine rapidly blocked Kv currents and reached a steady-state within 2 min. This inhibition was partially washed out (~70%). The steady-state current–voltage (I-V) relationships of Kv channels in the absence and presence of clomipramine are presented in Fig. 1C. The application of clomipramine reduced the Kv currents by 53% when measured at +10 mV.