



Atypical antipsychotic olanzapine inhibits voltage-dependent K⁺ channels in coronary arterial smooth muscle cells

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Abstract

Background Olanzapine, an FDA-approved atypical antipsychotic, is widely used to treat schizophrenia and bipolar disorder. In this study, the inhibitory effect of olanzapine on voltage-dependent K⁺ (Kv) channels in rabbit coronary arterial smooth muscle cells was investigated.

Methods Electrophysiological recordings were performed in freshly isolated coronary arterial smooth muscle cells.

Results Olanzapine inhibited the Kv channels in a concentration-dependent manner with an IC₅₀ value of $7.76 \pm 1.80 \mu\text{M}$ and a Hill coefficient of 0.82 ± 0.09 . Although olanzapine did not change the steady-state activation curve, it shifted the inactivation curve to a more negative potential, suggesting that it inhibited Kv currents by affecting the voltage sensor of the Kv channel. Application of 1 or 2 Hz train pulses did not affect the olanzapine-induced inhibition of Kv channels, suggesting that its effect on Kv channels occurs in a use (state)-independent manner. Pretreatment with DPO-1 (Kv1.5 subtype inhibitor) reduced the olanzapine-induced inhibition of Kv currents. In addition, pretreatment with guangxitoxin (Kv2.1 subtype inhibitor) and linopirdine (Kv7 subtype inhibitor) partially decreased the degree of Kv current inhibition. Olanzapine induced membrane depolarization.

Conclusion From these results, we suggest that olanzapine inhibits the Kv channels in a concentration-dependent, but state-independent, manner by affecting the gating properties of Kv channels. The primary Kv channel target of olanzapine is the Kv1.5 subtype.

Keywords Atypical antipsychotic · Olanzapine · Vascular smooth muscle cell · Voltage-dependent K⁺ channels

Introduction

Antipsychotics are generally categorized into first-generation (typical) and second-generation (atypical) antipsychotics based on their specific neurotransmitter receptor affinity

and pharmacological activity. Both types are effective for the treatment of schizophrenia, bipolar mania, acute agitation, and some other psychiatric disorders, but atypical antipsychotics may offer some advantages in terms of both modestly greater efficacy and reduced probability of an involuntary movement disorder [1]. Currently, several atypical antipsychotics are FDA-approved and commercially available in the US, with olanzapine, quetiapine, risperidone, and aripiprazole being the most widely used [2]. Among these, olanzapine has been on the market since 1996 and is currently one of the leading treatments for schizophrenia and bipolar disorder in many countries [3, 4]. Compared to other antipsychotics, olanzapine has a low propensity to cause extrapyramidal symptoms or sustained increases in plasma prolactin concentrations [5]. Although its clinical effects are beneficial, several adverse effects including weight gain and movement disorders cannot be ignored [6, 7]. Regarding its adverse effects on ion channels, it inhibits

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human ether-a-go-go-related gene (hERG) K⁺ channels, a major molecular component of the delayed rectifier K⁺ current [8]. In fact, it prolongs action potential duration by blocking the rapid component of the delayed rectifier K⁺ current in guinea pig hearts [9]. However, previous studies have focused on only cardiac K⁺ channels, which generally serve to control cardiomyocyte excitability; side effects of olanzapine on vascular K⁺ channels, specifically, voltage-dependent K⁺ (K_v) channels, have not yet been investigated.

K⁺ channels in vascular smooth muscle cells are specifically important to determine cell membrane potential and therefore vessel diameter [10]. In vascular smooth muscle cells, four distinct K⁺ channels have been characterized: Ca²⁺-activated K⁺ (BK_{Ca}), inward rectifier K⁺ (K_{ir}), ATP-sensitive K⁺ (K_{ATP}), and K_v channels [11]. Among these, K_v channels are highly expressed in vascular smooth muscle cells and are considered a major determinant of resting membrane potential and vascular tone [10, 12, 13]. K_v channels are activated by membrane depolarization, which induces a return to the resting membrane potential [14]. In addition, K_v channels are an important regulatory factor of myogenic tone of vascular smooth muscle cells [14]. Therefore, K_v channel dysfunctions are closely related to pathological conditions such as hypercholesterolemia, atherosclerosis, hypertension, and metabolic diseases [15–17]. For these reasons, unexpected effects of drugs on vascular K_v channels should be clearly identified to avoid the misinterpretation of vascular functional studies.

In this study, we investigated the inhibitory effect of olanzapine on K_v channels using rabbit coronary arterial smooth muscle cells. We found clear evidence that olanzapine inhibits K_v channels in a concentration-dependent, but state-independent, manner independent of its own function as a receptor antagonist of serotonin and dopamine.

Materials and methods

Single-cell preparation

Single arterial smooth muscle cells were isolated from the hearts of male New Zealand White rabbits (9–10 weeks old, 1.8–2.2 kg). All of our experiments followed the guidelines of the National Institutes of Health and the Animal Care Institution of Kangwon National University. The rabbits were anesthetized with a mixture of sodium pentobarbital (50 mg/kg) and heparin (100 U/kg) through the ear vein, and the heart was rapidly removed and rinsed in normal Tyrode's solution. The left descending coronary artery was isolated from the heart using microscissors and microforceps, and the endothelium of the isolated arteries was removed by introducing air bubbles into the lumen. Single arterial smooth muscle cells were obtained using two-step

enzymatic procedures. First, the isolated arteries were rinsed in Ca²⁺-free normal Tyrode's solution containing papain (1.15 mg/mL), bovine serum albumin (BSA, 1.25 mg/mL), and dithiothreitol (DTT, 1.0 mg/mL) at 37 °C for 25.5 min. Then the arteries were rinsed in Ca²⁺-free normal Tyrode's solution containing collagenase (2.93 mg/mL), BSA (1.25 mg/mL), and DTT (1.5 mg/mL) at 37 °C for 18.5 min. The single smooth muscle cells were obtained by soft agitation in Kraft–Brühe solution [18]. The smooth muscle cells were kept at 4 °C and used within 6 h.

Solutions and drugs

The normal Tyrode's solution for K_v channel recordings contained (mM): 143 NaCl, 15.0 glucose, 4.6 KCl, 5.0 HEPES, 1.6 CaCl₂, 0.6 MgCl₂, 0.35 NaH₂PO₄, adjusted to pH 7.40 with NaOH. The pipette-filling solution (mM): 103 K-aspartate, 24.0 KCl, 8.0 EGTA, 10.0 HEPES, 4.3 NaCl, 4.0 Mg-ATP, 1.1 MgCl₂, adjusted to pH 7.28 with KOH. The Kraft–Brühe solution contained (mM): 73 KOH, 55 KCl, 53 L-glutamate, 17 KH₂PO₄, 10 glucose, 20 taurine, 10 HEPES, 0.5 EGTA, and 4.0 MgCl₂, adjusted to pH 7.3 with KOH. Olanzapine, DPO-1, guangxitoxin, and linopirdine were purchased from Tocris Cookson (Ellisville, MO, USA), which dissolved in dimethyl sulfoxide.

Electrophysiological recordings

K_v current recordings were performed using an EPC-8 patch-clamp amplifier (Medical System Corporation, Darmstadt, Germany) with a NIDAQ-7 digital interface (National Instruments, Union, CA, USA). The patch pipettes were manufactured from borosilicate glass (Clark Electromedical Instruments, Berks, UK) with a microelectrode puller (PP-830, Narishige Group, Tokyo, Japan) and had a resistance of 3–4 MΩ. The average cell capacitance was 17.22 ± 0.31 pF (*n* = 20). The series resistance (6.95 ± 0.23 MΩ, *n* = 20) was continuously monitored during K_v current recordings. The voltage error from the voltage drop at the series resistance was calculated as ~4 mV; no correction was performed for this error. Input resistance or passive leak conductance was 2.32 ± 0.19 GΩ (*n* = 20), and it was not changed during the recordings. The nystatin-perforated patch was conducted to measure membrane potential. The nystatin (140 μg/ml) was added to the internal solution. The success of the nystatin-perforated patch configuration was verified by the appearance of a slow capacitive current. All electrophysiological experiments were conducted at room temperature.

Data analyses

OriginPro 8 software (Microcal Software, Inc., Northampton, MA, USA) was used for data analyses. The

half-maximal inhibitory concentration (IC_{50}) value and Hill coefficient (n) were obtained by fitting concentration–response data using the Hill equation:

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

where f indicates the percentage of Kv current inhibition ($f = [1 - I_{\text{drug}} \div I_{\text{control}}] \times 100\%$) at each test potential, and $[D]$ indicates the olanzapine concentration.

The steady-state activation curve was determined from the tail currents and was obtained by returning the potential to -40 mV after a short (20–40 ms) depolarizing pulses of -80 to $+60$ mV in the absence and presence of olanzapine. Activation curves were fitted with the following Boltzmann equation:

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

where V , $V_{1/2}$, and k indicate the test potential, mid-point of activation, and gradient value, respectively.

The steady-state inactivation was determined from a two-step voltage protocol; it can be obtained by returning the potential to $+40$ mV after pre-conditioning pulses (7 s) of -80 to $+30$ mV in the absence and presence of olanzapine. Inactivation curves were fitted with another Boltzmann equation:

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

where V , $V_{1/2}$, and k indicate the test potential, mid-point of inactivation, and gradient value, respectively.

All results are expressed as mean \pm standard error of the mean (SEM) and median with ranges. The Mann–Whitney U test was applied to identify statistical significance, and p values < 0.05 were considered significant.

Results

The inhibitory effect of olanzapine on Kv channels in coronary arterial smooth muscle cells of rabbits

Whole-cell Kv currents were recorded using the patch-clamp technique to examine the effects of olanzapine on the Kv channels. Involvement with Kir channels, which are expressed in small-diameter arterioles, was excluded by using second-branch conduit arteries [19]. In addition, involvement with BKCa and K_{ATP} channels was excluded by the inclusion of EGTA (8 mM) and ATP (4 mM) in the internal solution. Kv currents were elicited by applying voltage steps from -80 mV to $+60$ mV in 10 mV increments from a holding potential of -80 mV. Figure 1A shows the typical Kv currents, which rapidly reached a peak and then showed slow and partial inactivation. Application of 10 μM

olanzapine inhibited the Kv current, and this inhibition reached steady state within 2 min (Fig. 1B). Current–voltage (I – V) relationships measured at steady state are shown in Fig. 1C [(mean \pm SEM) 0 mV: 7.05 (± 0.59) vs. 4.46 (± 1.18); 10 mV: 10.74 (± 0.68) vs. 6.65 (± 1.20); 20 mV: 14.47 (± 0.88) vs. 8.80 (± 1.56); 30 mV: 18.29 (± 1.73) vs. 11.71 (± 0.76); 40 mV: 23.08 (± 1.24) vs. 15.24 (± 0.64); 50 mV: 28.62 (± 1.28) vs. 18.6 (± 0.56); 60 mV: 33.99 (± 1.08) vs. 22.42 (± 0.79). (median with ranges) 0 mV: 7.28 (5.33–7.93) vs. 5.2 (1–6.32), $U = 2.5$, $n = 5$, $p = 0.04653$; 10 mV: 10.56 (9.33–12.61) vs. 7 (3.2–8.6), $U = 0$, $n = 5$, $p = 0.01219$; 20 mV: 14 (12.33–16.5) vs. 8 (4.8–11.92), $U = 0$, $n = 5$, $p = 0.01219$; 30 mV: 18.56 (13.33–21.06) vs. 11.56 (10.2–13.4), $U = 1$, $n = 5$, $p = 0.02157$; 40 mV: 22.64 (20.67–26.56) vs. 15.76 (13.8–16.73), $U = 0$, $n = 5$, $p = 0.01219$; 50 mV: 29 (26.64–32.11) vs. 18 (17.8–19.67), $U = 0$, $n = 5$, $p = 0.01219$; 60 mV: 32.5 (32.32–37.78) vs. 22 (20.67–24.2), $U = 0$, $n = 5$, $p = 0.01219$].

Olanzapine inhibits the Kv currents in a concentration-dependent manner

To evaluate the concentration dependence of olanzapine-induced inhibition of Kv currents, various concentrations of olanzapine (0.1, 1, 3, 10, 30, 50, and 100 μM) were applied to Kv currents. The Kv currents were elicited by a one-step depolarizing pulse at $+60$ mV from a holding potential of -80 mV. As shown in Fig. 2, the Kv current inhibition increased with increasing concentration of olanzapine. The steady-state Kv currents were normalized to the control (without olanzapine) and fitted to the Hill equation. From this fitting, we obtained an IC_{50} value of $7.76 \pm 1.8 \mu\text{M}$ and a Hill coefficient of 0.82 ± 0.09 . These results suggest that olanzapine inhibited the Kv current in a concentration-dependent manner.

Effect of olanzapine on steady-state activation and inactivation kinetics of the Kv channel

To explore whether the inhibitory effect of olanzapine was due to a shift in the activation and/or inactivation curve, the steady-state activation and inactivation kinetics of Kv channels in the absence and presence of 10 μM olanzapine was investigated. The steady-state activation curve was obtained from peak tail currents, and the results were analyzed using a Boltzmann equation, as described in “Materials and methods”. As shown in Fig. 3A, 10 μM olanzapine did not change the activation curve of the Kv channels. The half-maximal activation potential ($V_{1/2}$) under the control condition and in the presence of 10 μM olanzapine was -5.25 ± 1.72 and -1.56 ± 1.33 , respectively, and the slope value (k) under the same conditions was 27.09 ± 1.74 and 27.08 ± 1.55 , respectively.

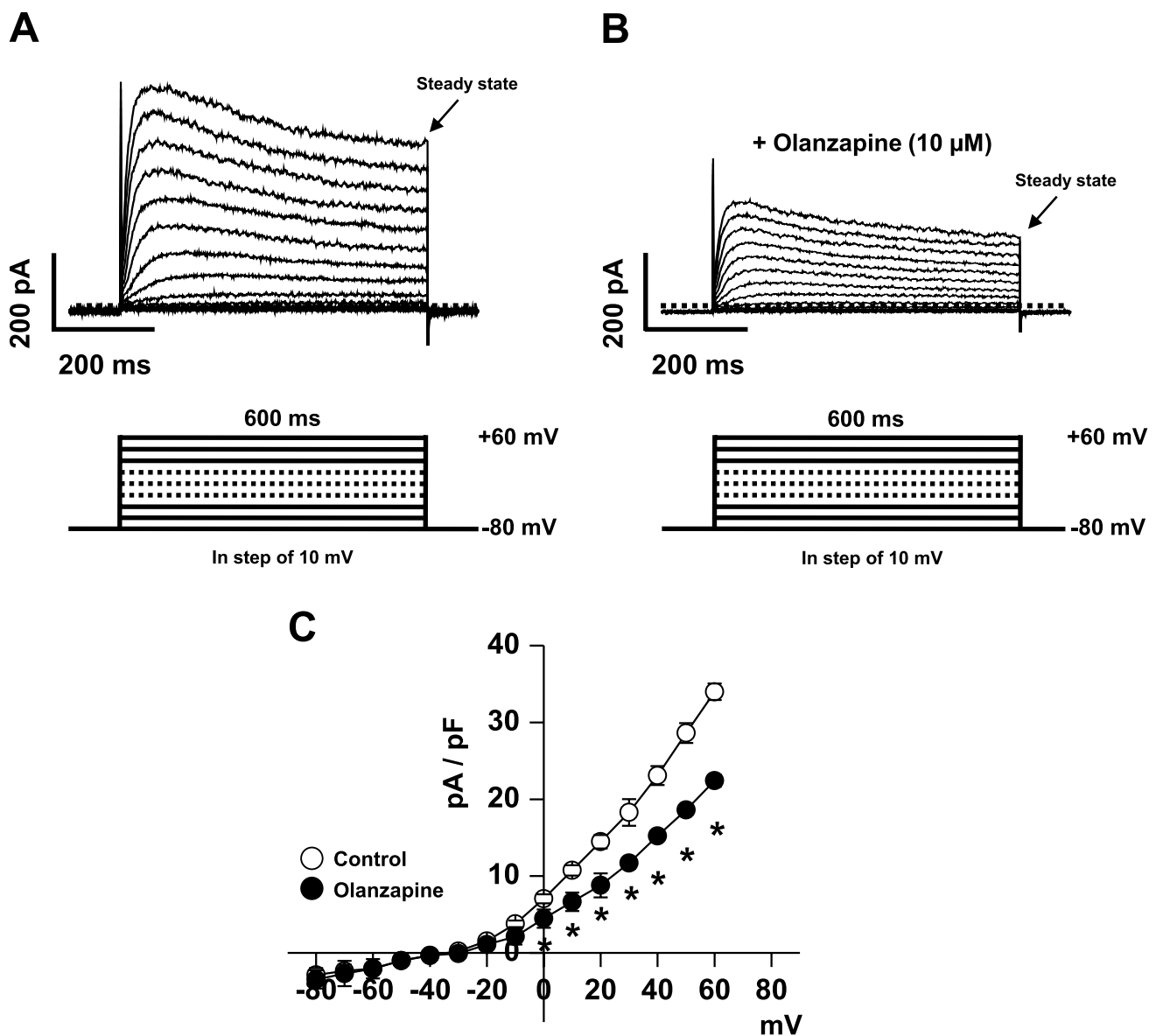


Fig. 1 Olanzapine-induced inhibition of Kv currents. Representative currents were activated by 600 ms step-depolarizing pulses from -80 mV to $+60$ mV at a holding potential of -80 mV in the absence (**A**) and presence (**B**) of $10 \mu\text{M}$ olanzapine. The time interval between voltage steps was 10 s. Olanzapine was applied for 3 min.

(**C**) The current–voltage (I – V) relationships at steady state in the absence (\circ) and presence (\bullet) of $10 \mu\text{M}$ olanzapine. $n=7$ (n means the number of cells isolated from 7 different rabbits). $*p<0.05$ (control vs. olanzapine, at each voltage by Mann–Whitney U test). Data are mean \pm SEM

The steady-state inactivation curve was obtained using a two-step voltage protocol, and the results were analyzed using a Boltzmann equation, as described in “[Materials and methods](#)”. Figure 3B shows that $10 \mu\text{M}$ olanzapine shifted the inactivation curve in a negative direction [(mean \pm SEM) -60 mV: $0.937 (\pm 0.004)$ vs. $0.870 (\pm 0.012)$; -50 mV: $0.819 (\pm 0.023)$ vs. $0.612 (\pm 0.029)$; -40 mV: $0.054 (\pm 0.046)$ vs. $0.363 (0.022)$; -30 mV: $0.257 (\pm 0.022)$ vs. $0.158 (\pm 0.002)$. (median with ranges) -60 mV: $0.934 (0.926–0.949)$ vs. $0.864 (0.840–0.898)$, $U=0$, $n=5$, $p=0.01219$; -50 mV: $0.804 (0.762–0.889)$

vs. $0.597 (0.571–0.667)$, $U=0$, $n=5$, $p=0.01219$; -40 mV: $0.536 (0.444–0.671)$ vs. $0.365 (0.337–0.406)$, $U=0$, $n=5$, $p=0.01219$; -30 mV: $0.278 (0.213–0.282)$ vs. $0.155 (0.154–0.161)$, $U=25$, $n=5$, $p=0.01219$]. The half-maximal inactivation potential ($V_{1/2}$) under the control condition and in the presence of $10 \mu\text{M}$ olanzapine was -38.13 ± 1.18 and -44.78 ± 1.43 , respectively, and the slope value (k) under the same conditions was 8.68 ± 0.61 and 8.51 ± 0.77 , respectively. These results suggest that olanzapine inhibited Kv currents by affecting the voltage sensor of the Kv channel.

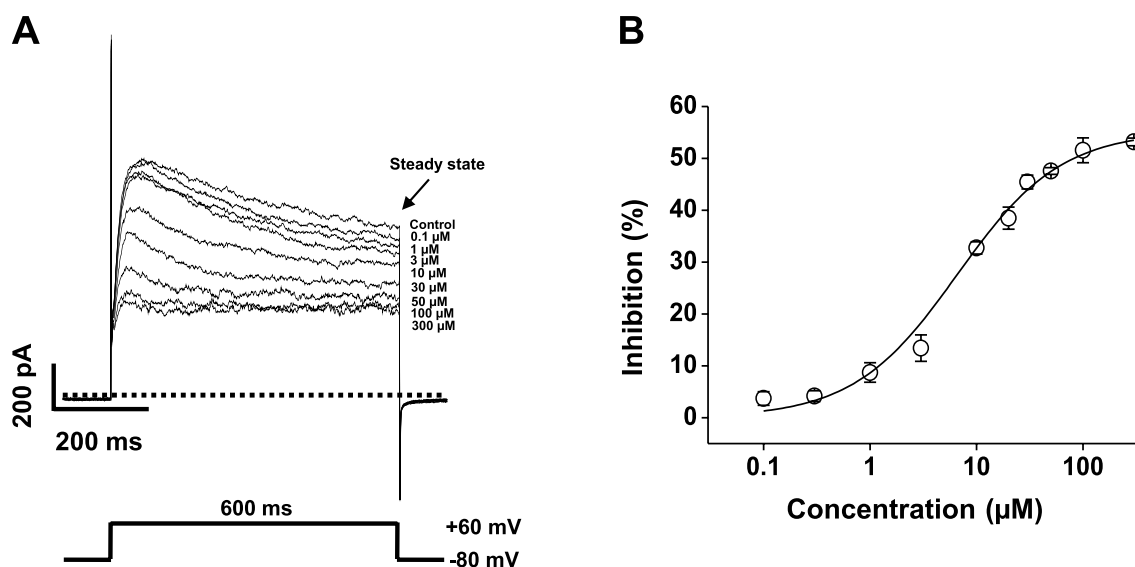


Fig. 2 Concentration dependence of olanzapine-induced inhibition of Kv currents. **A** Representative currents were recorded by 600 ms one-step depolarizing pulses from -80 mV to $+60$ mV at a holding potential of -80 mV in the presence of various concentrations of olanzapine. Olanzapine at each concentration was applied for 3 min.

B Concentration-dependent inhibition of Kv currents by olanzapine was measured at steady state (end of pulse). The smooth lines were obtained using the Hill equation. $n=5$ (n means the number of cells isolated from 5 different rabbits). Data are mean \pm SEM

Use (state) dependence of Kv current inhibition by olanzapine

To demonstrate the use (state)-dependent inhibition of the Kv channel by olanzapine, 20 repetitive depolarizing pulses were applied at frequencies of 1 or 2 Hz. As shown in Fig. 4, the application of train pulses at 1 (Fig. 4A) or 2 Hz (Fig. 4B) gradually reduced the Kv current. However, the application of train pulses at 1 or 2 Hz in the presence of $10 \mu\text{M}$ olanzapine did not induce further inhibition. These results suggest that Kv current inhibition by olanzapine does not depend on channel state.

Effect of olanzapine on Kv1.5, Kv2.1 and Kv7 subtypes

We investigated the specific Kv subtype involved in Kv channel inhibition by olanzapine. Vascular smooth muscle expresses various Kv subtypes. Among these, the Kv1.5, Kv2.1, and Kv7 subtypes are commonly expressed in vascular smooth muscle and are specifically important in the regulation of vascular tone [14]. Therefore, we evaluated the inhibitory effects of olanzapine on Kv current in the presence of the corresponding inhibitors. As shown in Fig. 5A, B, application of the Kv1.5 subtype inhibitor DPO-1 ($1 \mu\text{M}$) effectively reduced the Kv current. However, the subsequent application of $10 \mu\text{M}$ olanzapine did

not induce further inhibition. Application of the Kv2.1 subtype inhibitor guangxitoxin (30 nM) and the Kv7 subtype inhibitor linopirdine ($10 \mu\text{M}$) also reduced the Kv currents, and additional treatment with olanzapine led to further inhibition (Fig. 5C, E). However, the magnitude of Kv current inhibition by olanzapine was 24% (Fig. 5D; [(mean \pm SEM) $19.01 (\pm 0.86)$ vs. $14.45 (\pm 0.66)$]. (median with ranges) $18.56 (17.14\text{--}20.78)$ vs. $14.38 (13.33\text{--}16.33)$, $U=0$, $n=5$, $p=0.01219$) and 16% (Fig. 5F; (mean \pm SEM) $23.16 (\pm 1.16)$ vs. $20.25 (\pm 0.99)$. (median with ranges) $23.44 (21.03\text{--}25.01)$ vs. $19.90 (18.74\text{--}22.11)$, $U=2$, $n=5$, $p=0.03671$) in the presence of guangxitoxin and linopirdine, respectively, both lower than the 33% inhibition rate induced by olanzapine alone. To further confirm the involvement with Kv2.1 and Kv7 subtype on olanzapine-induced inhibition of Kv current, we simultaneously pre-treated with guangxitoxin and linopirdine prior to olanzapine exposure, which inhibited the Kv current by 11%, compared with 24% and 16% by guangxitoxin and linopirdine alone, respectively (Fig. 5G, H; (mean \pm SEM) $18.47 (\pm 0.65)$ vs. $16.02 (\pm 0.64)$. (median with ranges) $18.98 (14.82\text{--}20.08)$ vs. $16.95 (13.07\text{--}17.84)$, $U=8$, $n=8$, $p=0.013587$). From these results, we concluded that the Kv1.5 subtype is the primary target of olanzapine. In addition, the Kv2.1 and Kv7 subtypes were partially associated with the inhibitory effect of olanzapine.

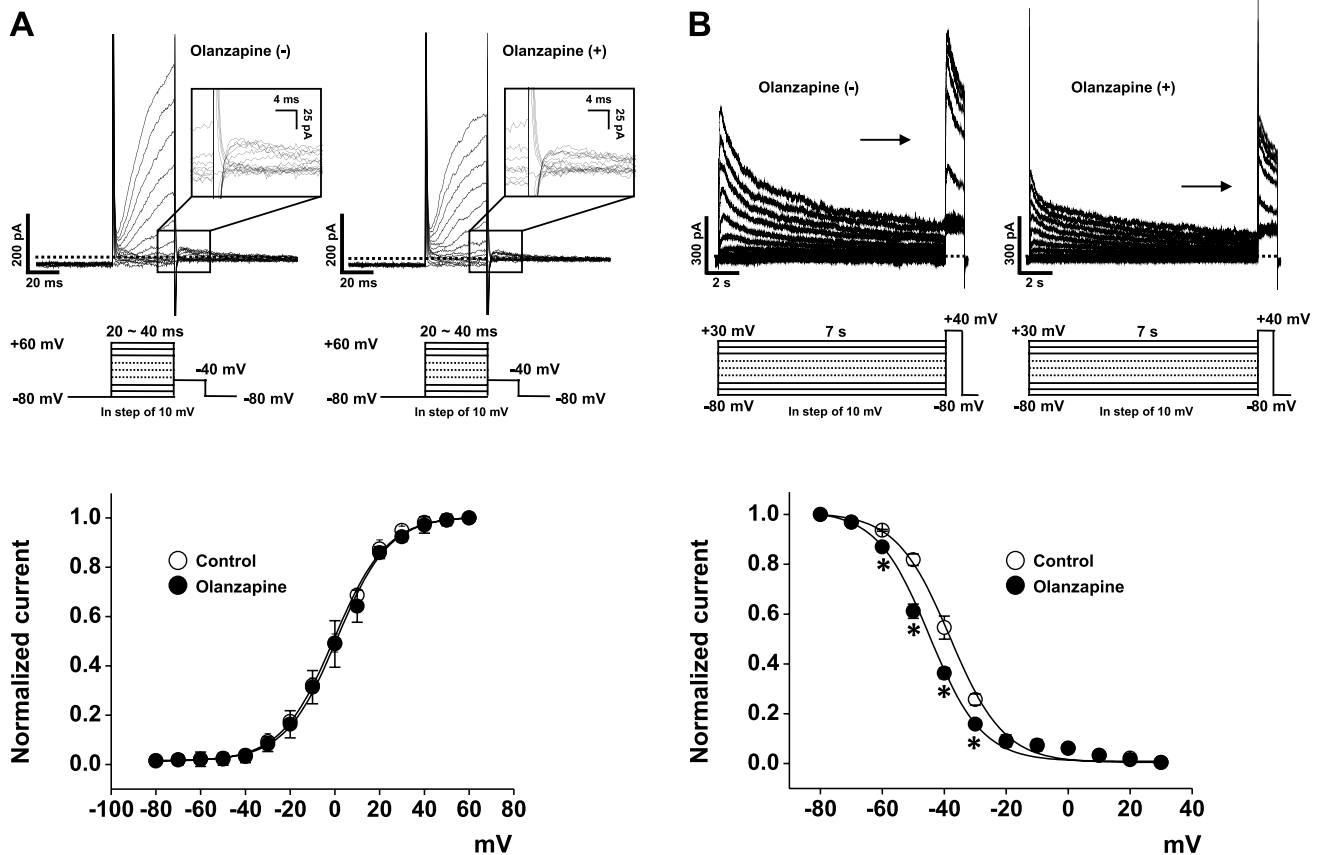


Fig. 3 Influence of olanzapine on the activation and inactivation curves. **A** Activation curves of Kv currents in the absence (○) and presence (●) of 10 μM olanzapine. The activation curve was calculated from tail currents, elicited by a returning voltage of -40 mV after short (20–40 ms) depolarizing pulses from -80 to +60 mV at a holding potential of -80 mV. Olanzapine was applied for 3 min. The recorded tail currents were normalized to the maximum peak value of the tail current. $n=7$ (n means the number of cells isolated from 7 different rabbits). Data are mean \pm SEM. **B** Inactivation curves of Kv

currents in the absence (○) and presence (●) of 10 μM olanzapine. The inactivation curve was obtained by applying a recorded voltage to +40 mV after 7 s preconditioning pulses from -80 to +30 mV. Olanzapine was applied for 3 min. The currents recorded at +40 mV were normalized to the peak current of the preconditioning pulses. $n=5$ (n means the number of cells isolated from 5 different rabbits). * $p<0.05$ (control vs. olanzapine, at each voltage by Mann–Whitney U test). Data are mean \pm SEM

Effect of olanzapine on resting membrane potential

To investigate whether the inhibition of the Kv channel by olanzapine affects the resting membrane potential, the membrane potential before and after olanzapine application was measured. As shown in Fig. 6A, B, 10 μM olanzapine induced the membrane depolarization by ~5 mV (Fig. 6B; [(mean \pm SEM) -34.92 (\pm 1.34) vs. -28.92 (\pm 0.82)]. (median with ranges) -34.27 (-38.65 to -32.64) vs. -28.82 (-31.15 to -27.17), $U=0$, $n=5$, $p=0.01219$).

Discussion

We demonstrated the inhibitory effect of olanzapine on Kv channels in rabbit coronary arterial smooth muscle cells. Olanzapine-induced inhibition of Kv channels occurred in

a concentration-dependent, but use-independent, manner. In addition, olanzapine shifted inactivation curves toward a more negative potential, indicating that olanzapine inhibited Kv currents by changing the gating properties of the channels.

The inhibitory effect of olanzapine is independent of its antagonistic action on serotonin and dopamine receptors, as evidenced by the following results. First, the Kv channel inhibition by olanzapine was rapid, reaching a steady-state within 2 min. This rapid action of olanzapine indicates that it acted directly rather than through a complicated intracellular signaling system. Second, the steady-state inactivation curve was shifted by olanzapine toward a more negative potential (Fig. 3B). This implies that olanzapine interacts near the Kv channel, therefore changing the voltage sensitivities independent of any antagonistic action on serotonin and dopamine receptors. Third, the IC₅₀ value of olanzapine for Kv

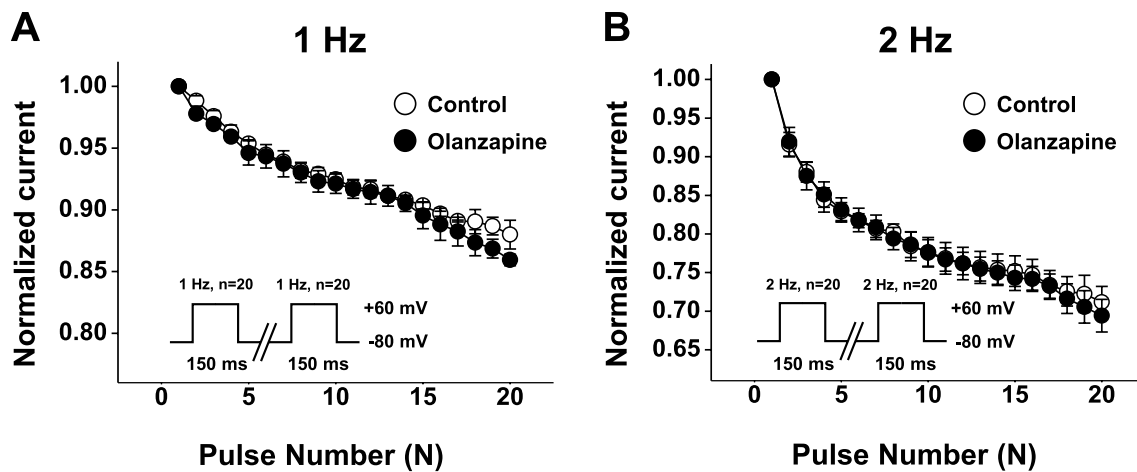


Fig. 4 Effects of olanzapine on use (state)-dependent inhibition of Kv currents. Twenty repeated 150 ms depolarizing pulses from -80 to $+60$ mV were applied at frequencies of 1 (**A**) and 2 (**B**) Hz in the absence (\circ) and presence (\bullet) of $10 \mu\text{M}$ olanzapine. Olanzapine was

applied for 3 min. The recorded currents were normalized to the first pulse-induced Kv current and plotted against the pulse number. $n=7$ (n means the number of cells isolated from 7 different rabbits). Data are mean \pm SEM

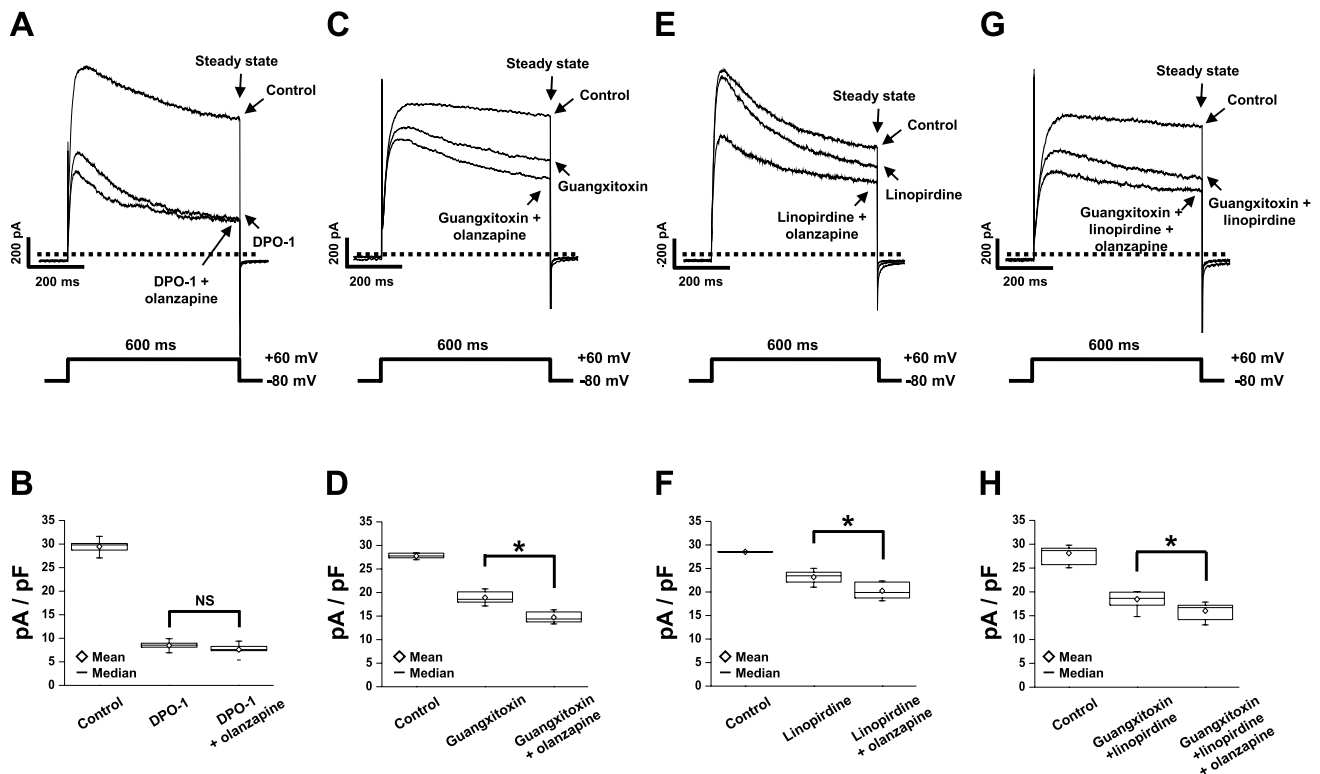


Fig. 5 Involvement of the Kv1.5, Kv2.1, and Kv7 subtypes in olanzapine-induced inhibition of Kv channels. Kv currents were evoked by a one-step depolarizing pulse from -80 mV to $+60$ mV. **A** Current traces under the control, in the presence of DPO-1, and in the presence of DPO-1 + olanzapine. **B** Summary of panel (**A**). $n=4$ (n means the number of cells isolated from 4 different rabbits). *NS* not significant (DPO-1 vs. DPO-1 + olanzapine). **C** Current traces under the control, in the presence of guangxitoxin, and in the presence of guangxitoxin + olanzapine. **D** Summary of panel (**C**). $n=4$ (n means the number of cells isolated from 4 different rabbits). $*p < 0.05$ (guangxitoxin vs. guangxitoxin + olanzapine by Mann–Whitney *U*

test). **E** Current traces under the control, in the presence of linopirdine, and in the presence of linopirdine + olanzapine. **F** Summary of panel (**E**). $n=4$ (n means the number of cells isolated from 4 different rabbits). $*p < 0.05$ (linopirdine vs. linopirdine + olanzapine by Mann–Whitney *U* test). **G** Current traces under the control, in the presence of guangxitoxin + linopirdine, and in the presence of guangxitoxin + linopirdine + olanzapine. **H** Summary of panel (**G**). $n=5$ (n means the number of cells isolated from 5 different rabbits). $*p < 0.05$ (guangxitoxin + linopirdine vs. guangxitoxin + linopirdine + olanzapine by Mann–Whitney *U* test). Pretreatment with all Kv subtype inhibitors was performed for 5 min prior to applying olanzapine

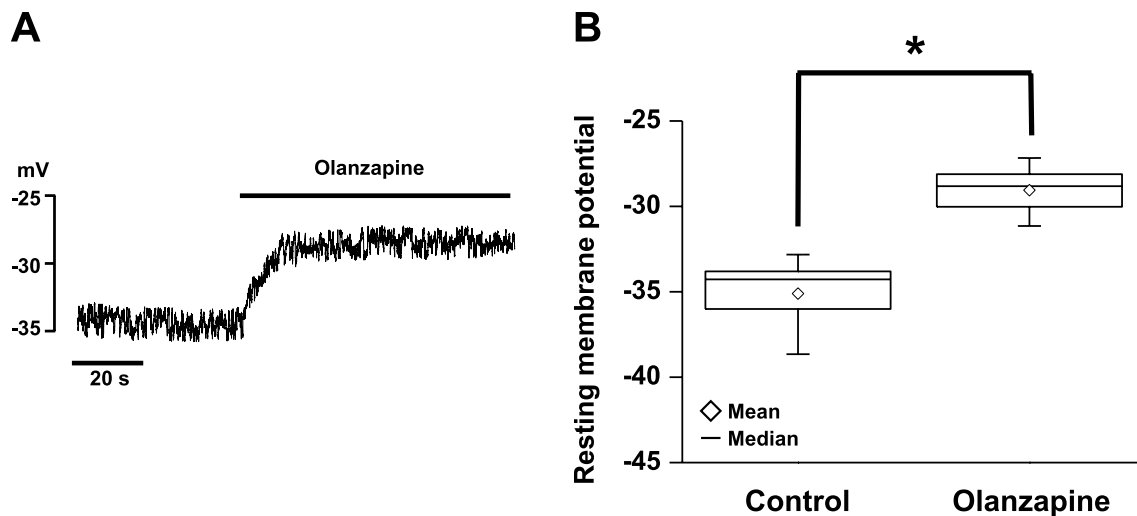


Fig. 6 The influence of olanzapine on resting membrane potential. **A** Effect of 10 μM olanzapine on resting membrane potential. **B** Summary of panel (A). $n=4$ (n means the number of cells isolated from 4 different rabbits). $*p < 0.05$ (control vs. olanzapine by Mann–Whitney U test)

channel inhibition is $7.76 \pm 1.8 \mu\text{M}$, which is higher than the serotonin (30–40 nM) or dopamine (404 nM) antagonistic concentration [20]. This inconsistency in values also supports the conclusion that olanzapine inhibits the Kv channels independent of serotonin and dopamine receptor antagonism. Fourth, serotonin inhibits Kv channels, inducing membrane depolarization and vasoconstriction [21, 22]. Although olanzapine can increase serotonin levels in the bloodstream, this increase could not be observed in our single-cell system. From these findings, we concluded that olanzapine-induced inhibition of Kv channels occurs independently of serotonin and dopamine receptor antagonism.

Olanzapine is an atypical antipsychotic agent primarily used to treat schizophrenia and manic or mixed episodes associated with bipolar disorder [3]. A number of neurotransmitter receptors have relatively high affinity with olanzapine, and like other atypical antipsychotic agents, it has greater affinity for serotonin 5-HT_{2A}, muscarinic, and histaminic receptors than for dopamine D2 receptors [18, 23]. These pharmacological actions indicate that olanzapine has a low propensity to cause extrapyramidal side effects and has less effect on plasma prolactin concentration [5]. However, several studies have reported that olanzapine also had negative effects on ion channels. Indeed, olanzapine inhibits human cardiac K⁺ channels (hERG channel), with an IC₅₀ value of 6 μM , which can prolong the cardiac QT interval [8, 9]. In this study, we also provide information on the side effects of olanzapine on vascular Kv channels.

Kv channels are highly expressed in most vascular smooth muscle cells [14]. These vascular Kv channels are responsible for maintaining the resting membrane potential and controlling vascular tone in response to various pathophysiological changes [12, 14]. In fact, several pathological

conditions such as hypercholesterolemia, atherosclerosis, hypertension, and metabolic disease can induce changes in the activity of the Kv channels [15, 16]. Therefore, it is essential to study the side effects of drugs on the Kv channels in vascular ion channel studies or vascular functional studies. Recently, our group suggested that other atypical antipsychotics such as risperidone, iloperidone, and ziprasidone inhibit vascular Kv channels independent of serotonin and dopamine receptor antagonism. Risperidone inhibited the Kv currents in a time- and use (state)-dependent manner by altering both the activation and inactivation curves [24]. Similarly, iloperidone and ziprasidone inhibited Kv currents in a use (state)-dependent manner. However, the effects of iloperidone were not time dependent and had no influence on the inactivation curve [25, 26]. The present study suggests that olanzapine inhibits Kv currents in a use (state)-independent manner by altering the inactivation curve. We cannot address the observed differences among these inhibitory mechanisms, but they may be due to structural differences among the drugs. This issue remains to be elucidated in the near future.

The inhibitory effect of olanzapine on Kv channel currents is related primarily to a shift in the steady-state inactivation curve to the hyperpolarizing membrane potentials. Although the mechanism by which olanzapine shifts the inactivation curve in the hyperpolarizing direction is unclear, it is possible that olanzapine interacts with some (allosteric) sites on the Kv channels to enhance the interaction of the voltage sensor with inactivation gates, leading to sensitization of the inactivation gate and a left shift in the inactivation curve. This modulation of voltage sensor, inactivation gate, and/or its interaction with the inactivation gate by olanzapine, if any, does not require prior Kv channel

activation (opening) because the peak and quasi-steady-state Kv currents were similarly inhibited (Figs. 1 and 2), and no use-dependency (Fig. 4) for Kv current inhibition by olanzapine was observed. The precise mechanism by which olanzapine shifts the steady-state curve could be determined using molecular biology combined with patch-clamp techniques, such as substitution of the amino acids of the voltage sensor or inactivation gate domains. However, that is beyond the scope of this study.

Various Kv subtypes have been identified in vascular smooth muscle. Based on their molecular structure and function, Kv channels are classified into 12 subtypes (Kv1–12). However, most studies of the expression and/or function of Kv subtypes have been performed using rat, mouse, and human samples. Therefore, the exact Kv subtypes expressed in rabbit arteries are not known. To date, however, Kv1.5 and Kv2.1 subtypes have been identified in rabbit arteries [27, 28]. Furthermore, the Kv7 subtype is emerging as an important factor in the regulation of membrane potential and thereby resting tone [29]. Indeed, our results show that application of the Kv1.5 inhibitor DOP-1, the Kv2.1 inhibitor guangxitoxin, and the Kv7 inhibitor linopirdine decreased the Kv current amplitude, indicating that these subtypes are actually expressed in rabbit coronary arterial smooth muscle cells (Fig. 5). Our results suggest that the Kv1.5 subtype is the primary target of olanzapine. In addition, the Kv2.1 and Kv7 subtypes are partially associated with the inhibitory effect of olanzapine. Information on the expression of Kv subtypes in rabbit arterial smooth muscle cells and the development of specific Kv subtype inhibitors is limited. Therefore, it is necessary to investigate the exact subtypes involved in the effects of olanzapine using specific subtype expression systems.

Clinically, olanzapine is administered at a dose of 17.5 mg/day and reached maximal plasma concentration, up to 173 nM [30]. In this study, olanzapine inhibited Kv channels with an IC_{50} value of 7.76 ± 1.8 μ M, which is higher than the maximal concentration in plasma. However, our results show that a low concentration of olanzapine, such as 100 nM, slightly reduced the Kv current (Fig. 2). Considering that vascular smooth muscle cells have high input resistance, such small changes in K^+ conductance can trigger changes in vascular tone and coronary blood flow. Furthermore, abuse or overdose of olanzapine can increase the concentration of olanzapine in plasma. Therefore, strict restrictions are required when olanzapine is used in patients with cardiovascular diseases.

In summary, we investigated the inhibitory effect of olanzapine on Kv channels using freshly isolated coronary arterial smooth muscle cells from rabbits. We found that olanzapine inhibits the vascular Kv channel (mainly the Kv1.5 subtype) in a concentration-dependent, but state-independent, manner by changing the inactivation curve.

This inhibition occurs independently of its own function as a receptor antagonist of serotonin and dopamine.

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

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