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Inhibitory Effect of Tricyclic Antidepressant Doxepin on Voltage-Dependent K^+ Channels in Rabbit Coronary Arterial Smooth Muscle Cells

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Abstract

Doxepin, tricyclic antidepressant, is widely used for the treatment of depressive disorders. Our present study determined the inhibitory effect of doxepin on voltage-dependent K^+ (Kv) channels in freshly isolated rabbit coronary arterial smooth muscle cells using a whole-cell patch clamp technique. Vascular Kv currents were inhibited by doxepin in a concentration-dependent manner, with a half-maximal inhibitory concentration (IC_{50}) value of 6.52 ± 1.35 μM and a Hill coefficient of 0.72 ± 0.03. Doxepin did not change the steady-state activation curve or inactivation curve, suggesting that doxepin does not alter the gating properties of Kv channels. Application of train pulses (1 or 2 Hz) slightly reduced the amplitude of Kv currents. However, the inhibition of Kv channels by train pulses were not changed in the presence of doxepin. Pretreatment with Kv1.5 inhibitor, DPO-1, effectively reduced the doxepin-induced inhibition of the Kv current. However, pretreatment with Kv2.1 inhibitor (guangxitoxin) or Kv7 inhibitor (linopirdine) did not change the inhibitory effect of doxepin on Kv currents. Inhibition of Kv channels by doxepin caused vasoconstriction and membrane depolarization. Therefore, our present study suggests that doxepin inhibits Kv channels in a concentration-dependent, but not use-, and state-dependent manners, irrespective of its own function.

Keywords Doxepin · Voltage-dependent K^+ channels · Coronary artery · Smooth muscle

Introduction

An increasing number of people presently are experiencing depression, owing to complicated environmental factors [1]. To counter this, numerous pharmaceutical companies have developed a variety of antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), serotonin–norepinephrine reuptake inhibitors (SNRIs), monoamine oxidase inhibitors (MAOIs), reversible inhibitors of monoamine oxidase A (RIMAs), and tricyclic antidepressants (TCA) [2]. Among

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these, TCAs are prescribed primarily in the clinical treatment of mood disorders such as dysthymia and major depressive disorders [3]. Doxepin is a dibenzoxepin-derivative TCA, structurally similar to amitriptyline and imipramine [4]. It is effective in the treatment of major depressive disorder, anxiety disorders, and neuropathic pain [5, 6]. In recent years, several studies suggested that low-dose doxepin was also effective in the treatment of insomnia [7, 8]. However, some common adverse effects of doxepin are inevitable, such as orthostatic, hypotensive, cardiovascular toxicity, and anticholinergic effects (e.g., dry mouth, constipation, urinary retention) [9–11]. Adverse effects of doxepin on ion channels such as human-ether-a-go-go-related gene (hERG), Na^+, and store-operated Ca^{2+} channels also have been studied [12–14]. However, there is little available information for the effect of doxepin on vascular Kv channels.

Although numerous factors have been involved in the regulation of vascular tone, K^+ channels are the most important ones in the regulation of resting membrane potential and thereby vascular tone [15, 16]. There are four types of K^+ channels in the vascular smooth muscle, namely big-conductance Ca^{2+}-activated K^+ (BK_{Ca}), ATP-sensitive K^+ (K_{ATP}), inwardly rectifying K^+ (Kir), and voltage-dependent K^+ (Kv) channels, and each of them plays a unique physiological role to maintain homeostasis [15, 17, 18]. Among the K^+ channels, Kv channels are mostly found in vascular smooth muscles, and openings of Kv channels restrict further membrane depolarization [15]. Moreover, Kv channels regulate resting membrane potential and vascular tone in some arteries. In fact, application of general Kv channel inhibitor 4-aminopyridine induced the membrane depolarization and thereby vasoconstriction [19, 20]. Furthermore, many cellular signaling pathways such as protein kinase A, protein kinase G, protein kinase C, and nitric oxide were closely related with the Kv channel activity [16, 21]. Numerous studies have also shown that changes in Kv channel function and/or expression were identified in cardiovascular and metabolic disease conditions [21–23]. Therefore, the unexpected effects of some drugs on vascular Kv channels should be clearly identified to avoid toxic effects on vasculature.

In our present study, we demonstrate the effect of doxepin on vascular Kv channels using freshly isolated rabbit coronary arterial smooth muscle cells. Our results suggest that doxepin inhibits vascular Kv channels in a concentration-dependent, but not in use- and state-dependent manners.

Materials and Methods

Isolation of Coronary Arterial Smooth Muscle Cells

Single coronary arterial smooth muscle cells were obtained from the hearts of New Zealand White rabbits (2.0–2.5 kg). All animal experiments were performed according to the guidelines of the Committee for Animal Experiments of Kangwon National University. Coronary arteries were dissected to be free of connective tissue. The endothelium was removed by pouring an air bubble into the arteries. The isolated arteries were enzymatically digested for 25 min at 37 °C in Ca^{2+}-free normal Tyrode's solution containing papain (1.4 mg/ml), bovine serum albumin (BSA, 1.3 mg/ml), and dithiothreitol (DTT, 1.3 mg/ml). Then, the arteries were transferred into Ca^{2+}-free normal Tyrode's solution containing collagenase (2.5 mg/ml), BSA (1.3 mg/ml), and DTT (1.3 mg/ml) for 21–22 min at 37 °C. After digestion, single smooth muscle cells were dispersed by gentle agitation in Kraft–Brühe (KB) solution with a fire-polished glass pipette. Cells were stored at 4 °C and used within 10 h.

Solutions and Chemicals

The composition of normal Tyrode's solution was (in mM) as follows: NaCl, 136; KCl, 5.7; $CaCl_2$, 1.7; $MgCl_2$, 1.3; NaH_2PO_4, 0.45; HEPES, 5.5; glucose, 15.5; adjusted to pH 7.4 with NaOH. The composition of the KB solution was (in mM) as follows: KOH, 72; KCl, 53; L-glutamate, 55; KH_2PO_4, 22; taurine, 15; $MgCl_2$, 2; glucose, 20; HEPES, 10; EGTA, 1; adjusted to pH 7.3 with KOH. The composition of the pipette solution for the recording of Kv channels was (in mM) as follows: K-aspartate, 112; KCl, 23; NaCl, 5.5; $MgCl_2$, 1.5; Mg-ATP, 4.5; EGTA, 10; HEPES, 10; adjusted to pH 7.2 with KOH. Physiological salt solution (PSS) for tension measurement contained (mM) KCl 4.4, NaCl 122, $CaCl_2$ 1.7, $MgSO_4$ 1.2, $NaHCO_3$ 25.5, KH_2PO_4 1.35, and glucose 15 and was adjusted to pH 7.4 with NaOH. Doxepin, DPO-1, guangxitoxin, and linopirdine were purchased from Tocris Cookson (Ellisville, MO, USA) and dissolved in dimethyl sulfoxide (DMSO).

Electrophysiology and Data Analysis

Membrane currents were measured by using EPC-8 patch amplifier (Medical system Corp, Darmstadt, Germany) under voltage-clamp mode. Recording pipettes were pulled from borosilicate capillaries (Clark Electromedical Instruments, Pangbourne, UK) using PP-830 vertical puller (Narishige Scientific Instrument Laboratory, Tokyo, Japan). Tip resistance of patch pipette was maintained at 3–4 MΩ when filled with the internal solution. Membrane potential was measured using the perforated-patch clamp technique. The pipette solution was supplemented with 140 μg/ml of nystatin. The success of the perforated-patch configuration was determined by the appearance of slow capacitive currents.

Origin 7.5 software (Microcal Software, Inc., Northampton, MA, USA) was used for data analysis. A first-order blocking scheme was used for description of the kinetics of doxepin-channel interactions [24]. The half-maximal inhibitory (IC_{50})

value and the Hill coefficient (n) were obtained from concentration–response relations fitting to the following Hill equation:

$$f = 1/\left\{1 + \left(\mathrm{IC}_{50}/[D]\right)^{n}\right\}$$

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

A steady-state activation curve was conventionally obtained from deactivating tail currents. Tail currents were induced by returning to a potential of − 40 mV after depolarization of the cell from − 80 to + 60 mV in 10 mV increments. All recorded tail currents were normalized to the maximal tail current. The activation curve was fitted with the Boltzmann equation described below:

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

where $V_{1/2}$ represents the voltage at which the conductance was mid-maximal, V indicates the test potential, and k indicates the slope factor.

The steady-state inactivation curves were acquired using a double-pulse voltage protocol: 7-s preconditioning pulses were varied from − 80 to + 30 mV in the absence and the presence of doxepin, and then returned to + 40 mV. Steady-state inactivation curves were fitted with another Boltzmann equation as follows:

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

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

Results are presented as mean ± standard error of the mean (SEM). Statistical significance was calculated by Student's t test and Mann–Whitney U test, and was considered at $P < 0.05$.

Tension Measurement

The isolated coronary artery was cut to a length of 10 mm and mounted on two wire hooks in an organ chamber system containing PSS oxygenated with 95% O_2 and 5% CO_2 for at least 2 h and maintained at 37 °C. The endothelium was eliminated by the injection of air bubbles through the lumen of the intact arteries. Prior to the experiments, the coronary arteries were exposed to high K^+-PSS solution (80 mM) to test arterial viability.

Results

Doxepin Inhibits Kv Current in Smooth Muscle Cells from Rabbit Coronary Arteries

Doxepin-induced inhibition of Kv current was tested using freshly isolated rabbit coronary arterial smooth muscle cells. EGTA (10 mM) and ATP (4 mM) in the pipette solution were used for inhibiting the BK_{Ca} and K_{ATP} channels, respectively. Large-diameter coronary arteries were used for excluding the Kir channel activity since this channel is not expressed in large-diameter coronary arteries [25]. We also confirmed that doxepin had no effect on Ca^{2+} currents (Supplementary Fig. 1). Kv currents were produced by 600-ms depolarizing steps from a holding potential of − 80 mV in steps of 10 mV. As shown in Fig. 1a, Kv currents arose rapidly to a peak and then were inactivated partially and slowly (intrinsic inactivation). Treatment of 10 μM doxepin reduced the Kv currents immediately (within 1 min) (Fig. 1b). The current–voltage (I–V) relationships measured at steady-state are shown in Fig. 1c. Application of 10 μM doxepin inhibited the steady-state current by 48.05% ± 2.5% at + 60 mV.

Concentration-Dependent Kv Channel Block by Doxepin

Various concentrations (0.1, 0.3, 1, 3, 10, 30, and 100 μM) of doxepin were applied to determine whether the doxepin-induced inhibition of Kv current was concentration dependent. Superimposed Kv currents were elicited by 600-ms depolarizing one-step pulses to + 60 mV from a holding potential of − 80 mV. As shown in Fig. 2a, doxepin-induced inhibition was increased with the increasing doxepin concentrations. Analysis of the data using the Hill equation showed that IC_{50} value was 6.52 ± 1.35 μM and a Hill coefficient was 0.72 ± 0.03 (Fig. 2b).

Doxepin-Induced Influence on Steady-State Activation and Inactivation Curves

To test whether doxepin-induced inhibitions of Kv channels were associated with the changes in gating properties of Kv channels, we evaluated the effect of doxepin on steady-state activation and inactivation kinetics of Kv channels. The steady-state activation curve was obtained using a deactivating tail current, which was described in detail in the Materials and Methods section. As shown in Fig. 3a, the activation curve was not affected by applying 10 μM doxepin. The half-activation potential ($V_{1/2}$) and slope value (k) were − 14.16 ± 1.62 mV and 9.71 ± 1.20 under control conditions, and − 12.32 ± 2.23 and 9.73 ± 1.61 in the presence of 10 μM doxepin, respectively.

The steady-state inactivation curve was obtained by using a double-pulse voltage protocol as described in the Materials and Methods section. As shown in Fig. 3b, application of 10 μM doxepin did not move the steady-state inactivation

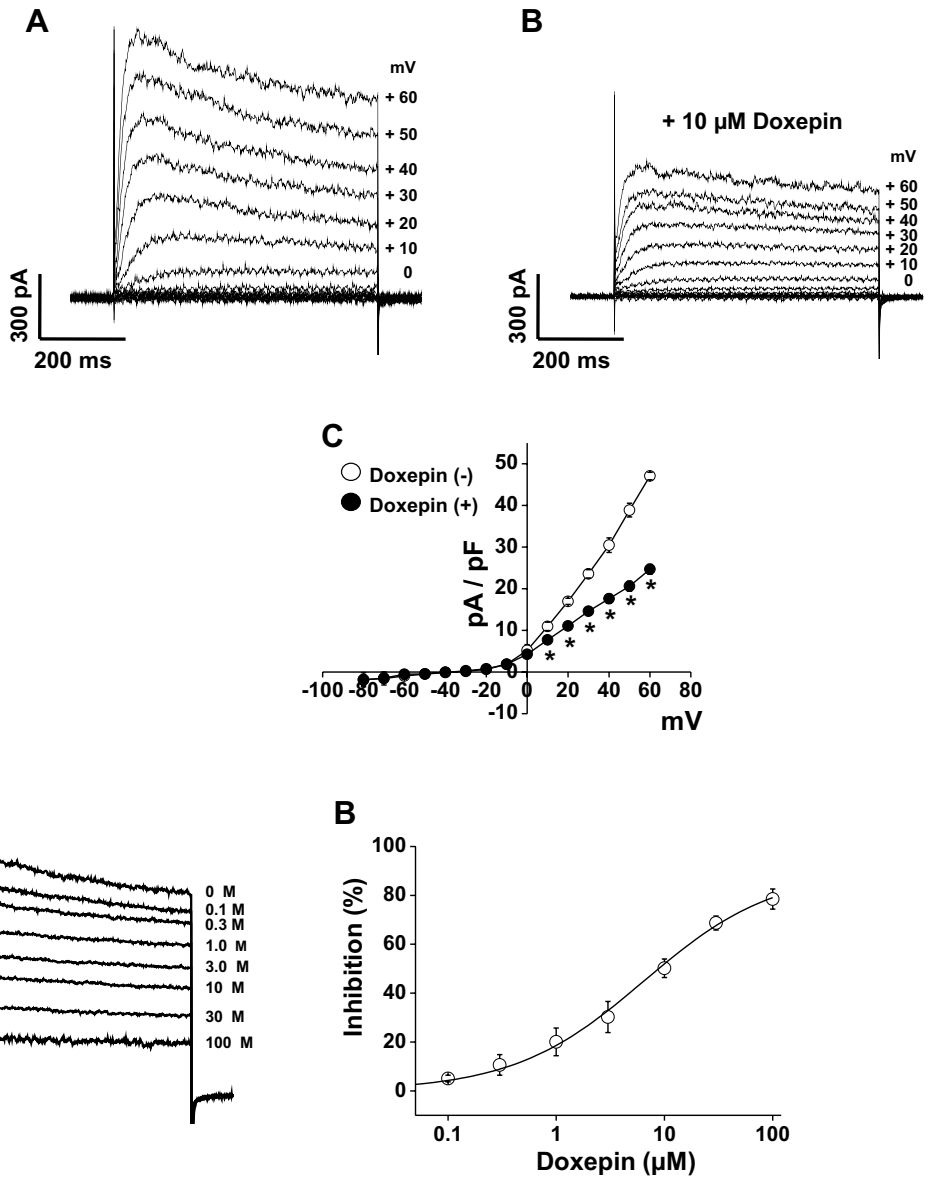

Fig. 1 Reduction of Kv currents by doxepin. Kv currents were elicited by 600-ms depolarizing pulses from − 80 to + 60 mV at − 80 mV holding potential in steps of 10 mV under control conditions (**a**) and in the presence of 10 μM doxepin (**b**). **c** Current–voltage (*I–V*) relationship at the steady-state Kv current in the control (open circle) and in the presence of 10 μM doxepin (filled circle). $n=6$. $^*P<0.05$

Fig. 2 Concentration dependence of doxepin-induced blocking of Kv currents. **a** Representative Kv current traces were obtained by 600-ms depolarizing pulses of + 60 mV from a holding potential of − 80 mV with application of 0, 0.1, 0.3, 1, 3, 10, 30, and 100 μM doxepin. **b** Analytical curve for concentration-dependent inhibition of Kv currents by doxepin. The percentage inhibitions of Kv currents at various concentrations of doxepin were normalized to the control current (without application of doxepin). The smooth line is fitted to the data points by the Hill equation. $n=6$

curve of Kv channels. The half-activation potential ($V_{1/2}$) and slope value (k) were − 42.49 ± 0.61 mV and 5.87 ± 0.4 under control conditions, and − 44.01 ± 0.71 and 5.64 ± 0.66 in the presence of 10 μM doxepin, respectively. These results suggest that doxepin inhibits Kv currents without affecting the gating properties of Kv channels.

Use-Dependent Effects of Doxepin on Kv Currents

The increase in block induced by repetitive depolarizing pulses has been termed "use-dependent inhibition," which usually indicates that the drug is likely to interact preferentially with specific state(s) of the channels. Doxepin was reported to inhibit neuronal voltage-dependent Na^+ channels in a use-dependent manner; the inhibition of Na^+ currents by doxepin was potentiated as repetitive

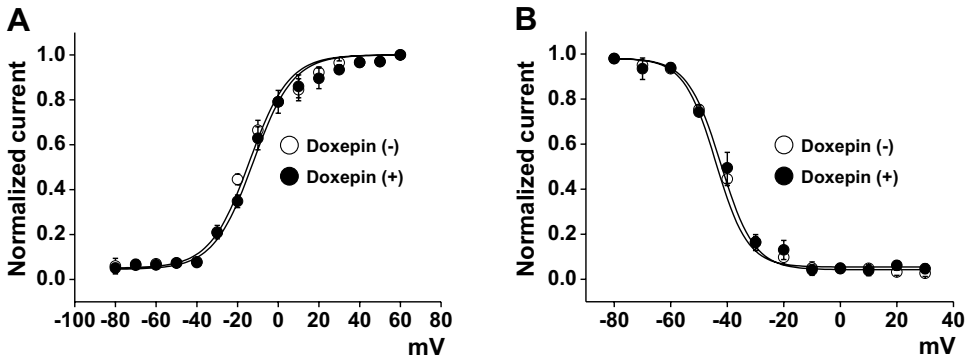

Fig. 3 Effect of doxepin on steady-state activation and inactivation curves of Kv channels. **a** Activation curve under the control conditions (open circle) and in the presence of 10 μM doxepin (filled circle). Activation curves were elicited by applying short depolarizing (20–50 ms) step pulses between − 80 and + 60 mV in steps of 10 mV at − 80 mV holding potential with a subsequent returning pulse to − 40 mV. $n=5$. **b** Inactivation curve under the control conditions (open circle) and in the presence of 10 μM doxepin (filled circle). Inactivation curves were elicited by applying a test step to + 40 mV after 7-s preconditioning pulses from − 80 to + 30 mV. $n=5$

depolarizations were applied [13], and the steady-state inactivation curve was shifted to the hyperpolarizing direction in the presence of doxepin. To investigate whether the inhibition of Kv currents by doxepin is also use dependent and/or state dependent, we applied repeated 20 train pulses at frequencies of 1 and 2 Hz. This experiment was frequently used to evaluate the use- and/or state-dependent inhibitions of Kv channels. As shown in Fig. 4a and b, application of repetitive train pulses reduced the Kv current by 25% and 33% at 1 and 2 Hz, respectively. However, this progressive inhibition of Kv current was not altered the by application of 10 μM doxepin. In fact, Kv current reduced during repetitive train pulses, by 25% and 35%, respectively, in the presence of doxepin. These results suggest that doxepin inhibited Kv currents in a use-independent manner.

Involvement of Kv1.5, Kv2.1, and Kv7 Subtypes on Doxepin-Induced Inhibition of Kv Channels

Kv1.5, Kv2.1, and Kv7 are known to be major subtypes expressed in arteries [26]. To investigate which Kv channel subtypes were involved in doxepin-induced inhibition, we checked the effect of pretreatment with the Kv1.5 inhibitor (DPO-1, 1 μM), Kv2.1 inhibitor (guangxitoxin, 100 nM), or Kv7 inhibitor (linopirdine, 10 μM) on doxepin-induced inhibition of Kv currents. As shown in Fig. 5a, Kv current was rapidly inhibited after application of DPO-1. However, additional application of doxepin induced the further inhibition of Kv currents by 12%, the value of which was smaller than inhibition by doxepin alone (48%) (Fig. 5b). Application of guangxitoxin also rapidly reduced Kv currents (Fig. 5c). However, the degree of doxepin-induced inhibition of Kv currents was not changed by pretreatment with guangxitoxin (Fig. 5d). Similar to the guangxitoxin results, pretreatment of linopirdine did not alter the degree of doxepin-induced inhibition of Kv currents (Fig. 5e and f). These results suggest that the inhibitory effect of doxepin on Kv channels is mainly manifested in the Kv1.5 subtype, but not in the Kv2.1 or Kv7 subtypes.

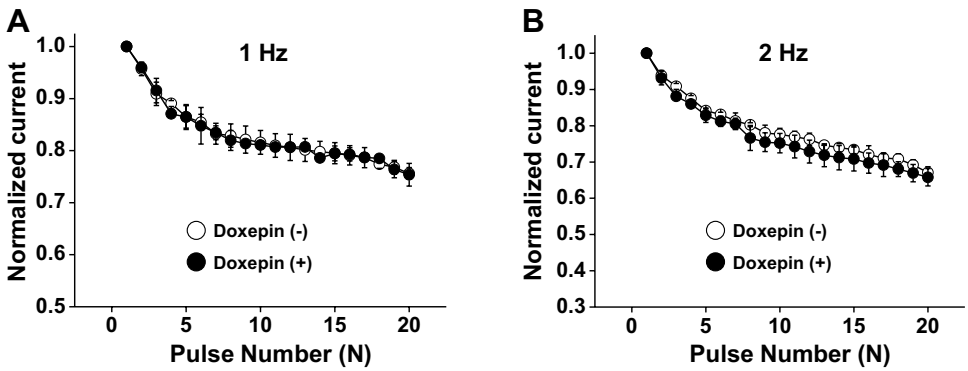

Fig. 4 Use-dependent inhibition of Kv currents by doxepin. Twenty repeated depolarizing pulses to + 60 mV from a holding potential of − 80 mV were applied at frequencies of 1 (**a**) and 2 Hz (**b**), in the absence (open circle) and the presence of doxepin (filled circle). The recorded Kv currents were normalized by the current evoked by the first pulse, and plotted against the pulse number. $n=5$

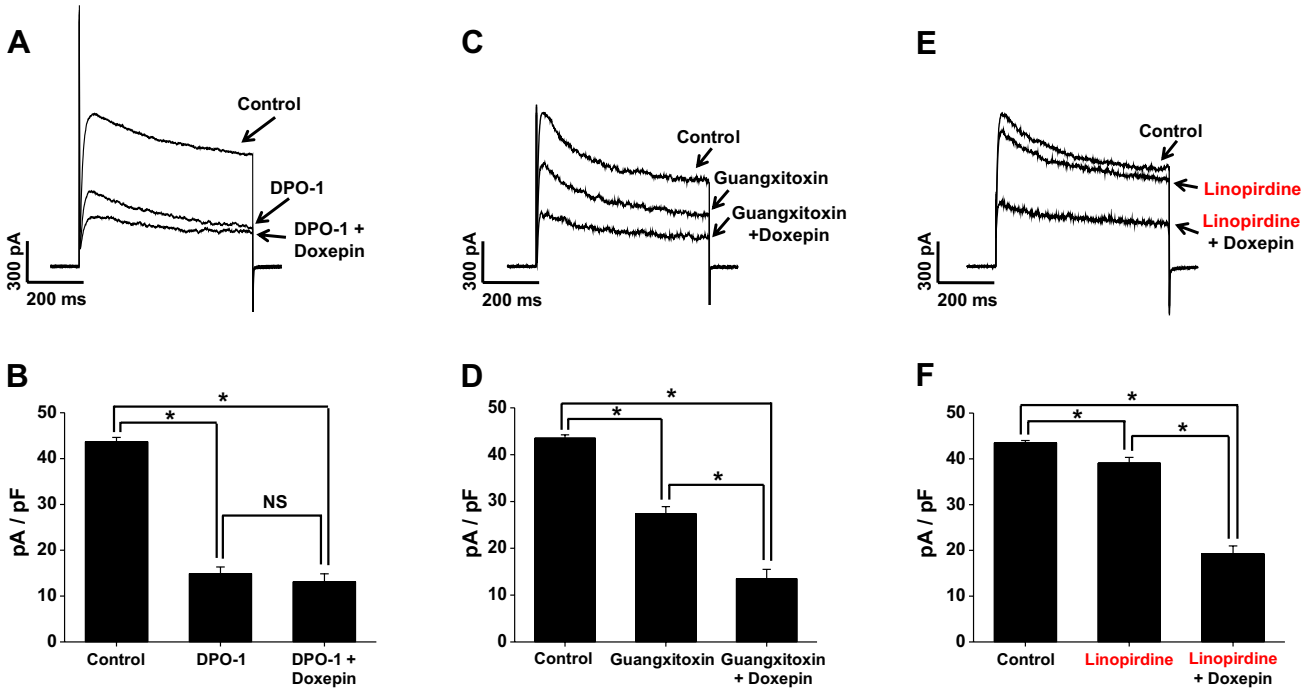

Fig. 5 Effects of pretreatment with Kv1.5, Kv2.1, and Kv7.1 subtype-specific inhibitors on the doxepin-induced inhibition of Kv currents. Representative Kv currents were obtained by 600-ms depolarizing pulses of + 60 mV from a holding potential of − 80 mV. **a** Superimposed current traces in the control condition, in the presence of DPO-1, and in the presence of DPO-1 + doxepin. **b** Summary of the results shown in panel **a**. $n = 5$. $^*P < 0.05$. *NS* not significant. **c** Superimposed current traces in the control condition, in the presence of guangxitoxin, and in the presence of guangxitoxin + doxepin. **d** Summary of the results shown in panel **c**. $n = 5$. $^*P < 0.05$. **e** Superimposed current traces in the control condition, in the presence of linopirdine, and in the presence of linopirdine + doxepin. **f** Summary of the results shown in panel **e**. $n = 5$. $^*P < 0.05$

Effects of Doxepin on Vascular Tone and Membrane Potential

To examine whether Kv channel inhibition by doxepin induces changes in vascular tone and membrane potential, we treated coronary arterial rings with 10 μM doxepin. Figure 6a shows that doxepin induced the vasoconstriction. At 10 μM, doxepin induced the vasoconstriction of the coronary arterial rings by 0.11 ± 0.04 g. Furthermore, the application of doxepin induced the membrane depolarization by ~ 4 mV (Fig. 6b and c).

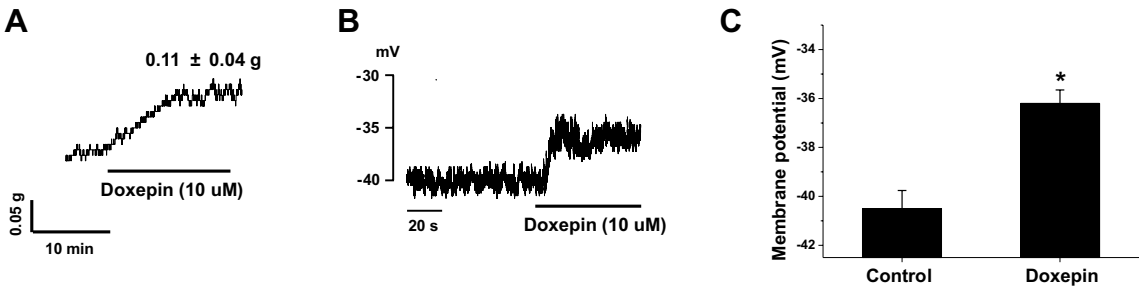

Fig. 6 Effects of doxepin on resting tone and resting membrane potential. **a** Effects of 10 μM doxepin on coronary arterial rings. $n = 4$. **b** Changes in resting membrane potential caused by 10 μM doxepin. **c** Summary of the effects of doxepin on resting membrane potential. $n = 4$. $^*P < 0.05$

Discussion

This study demonstrates the effect of doxepin on Kv channels in smooth muscle cells from rabbit coronary arteries. Doxepin inhibited the Kv channels in a concentration-dependent, but not in a use-dependent manner. Furthermore, doxepin has no significant effect on the activation or inactivation curves. It further shows that doxepin-induced inhibition of Kv channels mainly involves the Kv1.5 subtype.

According to our results, doxepin suppressed the Kv currents independent of the serotonin–norepinephrine reuptake inhibition. First, the IC_{50} value of doxepin for the Kv channel inhibition was 6.51 ± 1.35 μM. However, IC_{50} value of doxepin for serotonin–norepinephrine reuptake inhibition was 0.2 μM [27]. The difference in these values indicates that doxepin inhibits the Kv channels independent of serotonin–norepinephrine reuptake inhibition. Second, the Kv channel inhibition by doxepin occurs within 1 min. This rapid response suggests that doxepin interacts directly with the Kv channels without involving complicated signaling pathways such as serotonin–norepinephrine reuptake inhibition. Third, serotonin is known to suppress Kv channels in vascular smooth muscles [20]. Therefore, raising serotonin levels in blood could increase cardiovascular risk [28]. However, since we conducted the experiments using isolated single vascular smooth muscle cells, it is impossible to increase the serotonin levels by doxepin. Therefore, inhibitory effect of doxepin on Kv channels was completely independent of the doxepin-induced increase in serotonin levels. These evidences suggest that the inhibitory effect of doxepin on vascular Kv channels is not associated with serotonin–norepinephrine reuptake inhibition, but instead occurs directly through interaction between doxepin and the Kv channels.

Doxepin is a dibenzoxepin-derivative TCA that inhibits serotonin–norepinephrine reuptake [4]. As one of the most representative TCA drugs, doxepin is widely used in the treatment of major depressive disorder, anxiety disorders, and neuropathic pain [5, 6]. Apart from having common side effects like other antidepressants, doxepin has been reported to increase cardiovascular risk by inhibition of hERG channels [12]. This hERG channel blockade could lengthen the QT interval thereby increasing the risk of torsade de pointes [29]. Doxepin has also been reported to inhibit neuronal Na^+ channels. Doxepin-induced neuronal Na^+ channel inhibition occurs by shifting the steady-state inactivation curve by 15 mV, which slows the rate of recovery from the inactivated state. The state-dependent interaction of doxepin and Na^+ channels resulted in the use-dependent inhibition [13]. However, the results of the present study clearly indicate that the inhibition of Kv channels by doxepin was not use dependent or state dependent (Fig. 4). Contradictory reports about the changes in intracellular Ca^{2+} by doxepin have been published. For reports of increased intracellular Ca^{2+}, doxepin induced the increase of intracellular Ca^{2+} by evoking PLC-independent Ca^{2+} release from stores in PC3 human prostate cancer cells [14]. However, other reports suggested that doxepin treatment blunts the increase of intracellular Ca^{2+} in cultured neurons and human T-lymphocytes [30, 31]. To date, however, the effect of doxepin on vascular Kv channels has not been studied. This study was the first to examine the effect of doxepin on the Kv channels in coronary arterial smooth muscle cells. Therefore, caution is required when prescribing doxepin to patients with cardiovascular diseases.

K^+ channels in vascular smooth muscles play a crucial role in the regulation of vascular resistance and control of blood pressure [15, 16]. To date, several types of K^+ channels have been identified such as Kv channels, which are widely distributed on vascular smooth muscle cells and regulate vascular contractility by controlling membrane potential [18]. Considering pathophysiological relevance of Kv channels, such as, regulation of membrane potential, association with intracellular signaling pathways, and involvement in cardiovascular and metabolic diseases, the unexpected effects of some drugs on vascular Kv channels should be clearly elucidated. In fact, other TCAs such as, amitriptyline and nortriptyline, have been reported to inhibit vascular Kv channels by changing the voltage sensitivity of Kv channels. Amitriptyline inhibits vascular Kv channels by shifting the activation curve to more positive potentials [26], and nortriptyline inhibits Kv channels by shifting the inactivation curve to more negative potentials [32]. Furthermore, other types of antidepressants, SSRIs, also have a side effect on vascular Kv channels. In detail, sertraline inhibited Kv channels by shifting the inactivation curve to more negative potentials [33]. Dapoxetine has an effect on Kv channels in a concentration-, time-, and use (open state)-dependent manners [34]. Escitalopram also shows an inhibitory effect on Kv channels. Similar to the results of this study, Escitalopram inhibited Kv channels without alterations of steady-state activation or inactivation curve [35]. In the present study, although, our findings are limited to the effect of doxepin on the vascular Kv channels, given the importance of the Kv channels, our study will provide valuable information when prescribing doxepin in patients with cardiovascular disease, or using doxepin for vascular functional studies.

Kv channels are divided into 12 families and regulated by ~ 40 different genes [18]. The exact expression of Kv subtypes is still controversial, but at least Kv1.1, Kv1.2, Kv1.5, Kv2.1, Kv7, and Kv9.3 subtypes are thought to be expressed in most arteries [15, 18, 21]. Since the studies

for the expression of Kv subtypes were mainly performed in arteries of human, rat, and mouse, it is difficult to know exactly which Kv subtypes are expressed in rabbits. However, it is known that Kv1.5, Kv2.1, and Kv7 subtypes are mostly expressed in arteries, and their specific inhibitors have been developed. For this reason, we tested the involvement of Kv subtypes on doxepin-induced inhibition of Kv channels using these inhibitors. Our results show that the inhibitory effect of doxepin is closely related to the inhibition of Kv1.5 subtype, but not to that of Kv2.1 and Kv7. Although inhibition of Kv1.5 subtype effectively reduced the inhibitory effect of doxepin on the Kv channels, it did not eliminate the inhibitory effect of doxepin on the Kv channels completely. Therefore, other subtypes besides the Kv1.5 subtype may be involved, and this issue should be addressed in future studies.

Doxepin as an antidepressant is administered in 25 mg doses, three times a day, to treat depressive disorders. After taking doxepin, the plasma concentration of doxepin could reach an average value of 276.23 nM [36]. Our present study suggests that doxepin inhibits Kv channels with an IC_{50} value of 6.52 ± 1.35 μM, which is higher than the plasma maximum concentration of doxepin. However, doxepin effectively inhibits Kv channels at low concentrations, such as 0.1 and 0.3 μM. Since the blood vessel has high input resistance, small changes in current can also significantly change the vascular tone. In addition, overdose or abuse of doxepin may increase the plasma concentration of doxepin to more than 276 nM. Therefore, strict control of doxepin dosage is needed, specifically in patients with cardiovascular disease.

Although our study did not address the mechanisms of direct interaction between doxepin and Kv channels, a structural analysis could identify some possible mechanisms. The structure of doxepin consists of a dibenzoxepin containing aromatic rings and a tertiary amine connected with a carbon chain. It is possible that the aromatic rings in dibenzoxepin form π–π bonds with aromatic rings, such as phenylalanine, tyrosine, and tryptophan, exposed on the inner side of the Kv channels. However, previous studies have shown that this inhibition may not be the main mechanism, as compounds rich in aromatic residues, such as staurosporine, shifted the steady-state activation curve [37]. Another possibility is that tertiary amines, one of the doxepin structures, may be closely related to the inhibition of Kv channels. Indeed, it has been reported that the amine structure of K^+ channel inhibitors plays an important role in K^+ channel inhibition, and the inhibition becomes stronger as the number of carbons attached to the amine increases [38, 39]. It is also known that drugs containing amine structures, such as NNC 55-0396, escitalopram, and Y-27632, inhibit Kv channels without affecting steady-state activation and inactivation curves [35, 40, 41]. Therefore, although we cannot exclude the possibility of an interaction between aromatic rings in dibenzoxepin and Kv channels, the interaction between tertiary amines in doxepin and Kv channels is more feasible.

In conclusion, we demonstrated the inhibitory effect of doxepin on Kv channels using freshly isolated rabbit coronary arterial smooth muscle cells. Our results indicate that doxepin inhibits Kv channels in a concentration-dependent manner, but not in use- and/or state-dependent manners, regardless of serotonin–norepinephrine reuptake inhibition.

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Compliance with Ethical Standards

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

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