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Modulation of Voltage-Dependent K^+ Channel Current in Vascular Smooth Muscle Cells from Rat Mesenteric Arteries

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Abstract. Voltage-dependent K^+ (Kv) channels were studied in smooth muscle cells (SMCs) freshly isolated from rat mesenteric arteries. A delayed outward rectifier Kv current (I_K) with a weak voltage dependence was identified. The amplitude of I_K, but not its inactivation kinetics, was inhibited by 4-aminopyridine (4-AP) (IC_{50}, 5.1 ± 0.9 mM). The inhibitory effect of 4-AP was not use-dependent, and the unbinding of 4-AP from I_K channels was complete in the absence of depolarization stimuli, suggesting the binding of 4-AP to the closed state of Kv channels. There was no change in the steady-state inactivation, but the steady-state activation curve of I_K was shifted in the presence of 4-AP by +6 mV. Including 4-AP in pipette solution instantly inhibited I_K upon the rupture of cell membrane, indicating that 4-AP bound to the inner mouth of Kv channel pores. Several Kv channel proteins encoding the native I_K-type Kv channels, but not the transient outward A-type Kv channels, were identified. Among the identified I_K-encoding gene transcripts, the expression of Kv1.5 was the most abundant. Our results elucidate the modulating mechanisms for the 4-AP-induced I_K inhibition in rat mesenteric artery SMCs and suggest that the unique properties of Kv channels in these cells might be related to the heteromeric expression of the I_K-encoding genes with Kv1.5 subunit playing an important role.

Key words: 4-Aminopyridine — Delayed outward rectifier K^+ current — Kv1.5 — Mesenteric artery — Patch-clamp — Tetraethylammonium

Introduction

At least four types of K^+ channel currents have been identified in excitable cells, i.e., voltage-dependent outward Kv channel, calcium-activated K_{Ca} channel, ATP-sensitive K_{ATP} channel, and inward rectifier Kir channel. All of these K^+ channels have been identified in peripheral vascular smooth muscle cells (SMCs). The opening of K^+ channels generally results in K^+ influx, which causes membrane hyperpolarization, the inhibition of voltage-dependent Ca^{2+} channels, and the relaxation of vascular SMCs. It is well known that the mechanisms of contractility and its regulation vary among different types of vascular SMCs. Cell type-specific expression and modulation of different types of K^+ channels are among the mechanisms responsible for this diversity in vascular SMC functions. This realization has promoted an extensive investigation of the properties of different K^+ channels in different types of vascular SMCs. For instance, both K_{ATP} and K_{Ca} channels have been found to contribute to the membrane hyperpolarization of SMCs from rat mesenteric artery (Criddle, Greenwood & Weston, 1994; Weidelt, Bolt & Markwardt, 1997). K_{ATP} channels with altered properties have been demonstrated in mesenteric artery SMCs from spontaneously hypertensive rats and have been related to the pathogenesis of hypertension (Ohya et al., 1996).

Kv channels are important for the regulation of the resting membrane potential of vascular SMCs (Smirnov & Aaronson, 1992), and by doing so affect the resting tone of peripheral blood vessels. Rat mesenteric artery is a widely used model for studying the regulation of peripheral vascular resistance under different conditions, such as hypertension or the hypoxic vascular response (Yuan et al., 1990). Functional Kv channels have been found in rat mesenteric artery SMCs (Criddle et al., 1994). However, our understanding of Kv currents in rat mesenteric artery SMCs is very limited. For instance, the profile of steady-state activation and inactivation of Kv currents in this cell preparation is unknown. The mechanisms for the 4-aminopyridine (4-AP)-induced inhibition of I_K in these cells, including the concentration-

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dependence, voltage-dependence, the channel state dependence, and the binding sites of 4-AP, are not clear. This lack of knowledge impedes the identification of the molecular basis of Kv currents in these peripheral vascular SMCs, and generates difficulties for our understanding of the functional role of Kv channels in the regulation of vascular contractility under physiological or pathophysiological conditions. Furthermore, comparison of Kv currents in rat mesenteric artery SMCs with their counterparts in other types of vascular SMCs cannot be made without a comprehensive knowledge of their fundamental properties. Although Kv currents were also reported in mesenteric artery SMCs from guinea pig (Ohya et al., 1997) and human (Smirnov & Aaronson, 1992), the composition of Kv channel currents as well as the general electrophysiological properties of these cells were not identical to those in rat mesenteric artery SMCs. Two subtypes of Kv channels (a transient outward current, I_A, and a sustained delayed outward rectifier current, I_K) were found in human mesenteric artery SMCs (Smirnov & Aaronson, 1992). Only one type of Kv channels (I_K) was recorded in guinea pig mesenteric artery SMCs (Ohya et al., 1997). In rat mesenteric artery SMCs, however, either only I_K (Smirnov, Knock & Aaronson, 1998) or three types of Kv channels (Yuan et al., 1993) were reported. In addition, rat mesenteric artery SMCs had a smaller membrane input resistance (2 $G\Omega$, Yuan et al., 1993) as compared to human mesenteric artery SMCs (14 $G\Omega$, Smirnov & Aaronson, 1992).

In the present study, the whole-cell patch-clamp technique was applied on freshly isolated single SMCs from rat mesenteric arteries. The voltage-dependence and time-dependence of Kv currents as well as their steady-state activation and inactivation were examined. The effect of 4-AP on Kv currents and the underlying mechanisms were specifically studied. The motivation for carrying out these experiments lies in the fact that 4-AP has been extensively used in studying the pharmacological responsiveness of Kv currents in different types of cells. Determination of the profile of 4-AP action would shed light on the tissue-specific features of Kv currents and on the molecular basis of Kv channels in peripheral vascular SMCs. To further define the pharmacological regulation of Kv channels in rat mesenteric artery SMCs, the effect of tetraethylammonium (TEA), β-dendrotoxin, charybdotoxin, iberiotoxin, and 4-acetamido-4′-isothiocyanatostilbene -2,2′-disulfonic acid (SITS) were also determined. Finally, in order to elucidate the molecular basis of Kv channels in rat mesenteric artery SMCs, the abundant levels of different I_K-encoding Kv gene transcripts were determined using RNAse protection assay. Western blot technique was applied to detect the expression of different Kv channel proteins. Characterization of Kv channels in rat mesenteric artery SMCs, using electrophysiological, pharmacological, and molecular biology techniques, will pave the way for a better understanding of the diversity and functional role of Kv currents in the regulation of peripheral vascular tone.

Materials and Methods

Cell Preparation

Single smooth muscle cells were isolated and identified according to our established method with modifications (Wang, Karpinsky & Pang, 1989). Male Sprague-Dawley rats (150–200 g) were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). Small mesenteric arteries below the second branch off the main mesenteric artery were dissected out. Only the small arteries and arterioles were kept in ice-cold physiological salt solution (PSS) containing (in mM) NaCl 137, KCl 5.6, NaH_2PO_4 0.44, Na_2HPO_4 0.42, $NaHCO_3$ 4.17, $MgCl_2$ 1, $CaCl_2$ 2.6, Hepes 10, and glucose 5 with pH adjusted to 7.4 with NaOH. The vascular tissues were cut into 5 mm-long pieces, and incubated at 37°C in low-Ca^{2+} PSS (0.1 mM $CaCl_2$) containing 1 mg/ml albumin, 0.5 mg/ml papain, and 1.0 mg/ml dithioerythritol for 30 min, and for another 20 min in the nominally Ca^{2+}-free PSS containing 1 mg/ml albumin, 0.8 mg/ml collagenase, and 0.8 mg/ml hyaluronidase. Single cells were released by gentle trituration through a Pasteur pipette, stored in the nominally Ca^{2+}-free PSS at 4°C, and used the same day.

Animal experimental protocols were approved by the University Committee on Animal Care and Supply of the University of Saskatchewan.

Recording of Whole-Cell K^+ Channel Currents

The whole-cell patch-clamp technique was used to record Kv currents in the voltage-clamp mode (Tang, Hanna & Wang, 1999). Briefly, 2–3 drops of the cell suspension were added to the perfusion chamber inside a Petri dish that was mounted on the stage of an inverted phase contrast microscope (Olympus IX70). Cells were left to stick to the glass coverslip in the recording chamber for 15–20 min before starting an experiment. Pipettes were pulled from soft microhematocrit capillary tubes (Fisher, Nepean, ON) with tip resistances of 2–4 MΩ when filled with the pipette solution. Currents were recorded with an Axopatch 1-D amplifier (Axon Instruments, Foster City, CA), controlled by a Digidata 1200 interface and a pClamp software (version 6.02, Axon Instruments). Membrane currents were filtered at 1 kHz with a 4-pole Bessel filter, digitized and stored. At the beginning of each experiment, junctional potential between pipette solution and bath solution was electronically adjusted to zero (Wu et al., 1996, 1997; Wang, Wu & Wang, 1997). No leakage subtraction was performed to the original recordings, and all cells with visible changes in leakage currents during the course of study were excluded from further analysis. Test pulses were made at 10 mV increments from −50 to +50 mV. The holding potential was set at −80 mV, a potential at which voltage-dependent K^+ channels were not inactivated. *I-V* curves were constructed using the current amplitude measured between the 300–600 msec of the test pulses when the current amplitude became sustained. The bath solution contained (in mM): NaCl 140, KCl 5.4, $MgCl_2$ 1.2, Hepes 10, EGTA 1, glucose 10 (pH adjusted to 7.3 with NaOH). The pipette solution was composed of (in mM): KCl 140, $MgCl_2$ 1, EGTA 10, Hepes 10, glucose 5, Na_2-ATP 2 (pH adjusted to 7.3 with KOH). When the steady-state activation curve of I_K was determined, the components of bath solution

were changed to (in mM): KCl 140, $MgCl_2$ 1.2, Hepes 10, glucose 10 (pH adjusted to 7.3 with KOH). The symmetrical potassium concentration across cell membrane sets the equilibrium potential for K^+ ions at 0 mV. Unless otherwise indicated, cells were continuously superfused with bath solution containing the tested chemicals at desired final concentrations. The time required for a complete solution change from the onset of a drug application was estimated to be 5 sec, as described before (Diarra et al., 1994). Data were collected and processed using a software of pClamp (version 6.02) from Axon Instruments. All experiments were conducted at room temperature (20–22°C).

RNAse Protection Assay (RPA)

The cDNA templates for preparing [α-^{32}P] UTP radiolabeled antisense cRNA probes (MAXIscript, Ambion) were generated by subcloning small fragments of the cDNAs into PGEM-T vector (Promega) or by ligating the cDNA fragments with an oligonucleotide that had a T7 promoter. These cDNA fragments were amplified by the RT-PCR technique using isoform-specific primers for the selected Kv channel genes, and confirmed by sequencing. To differentiate between the specifically protected region and the remaining regions of the probe, all probes were designed to contain regions of the plasmid sequence at both ends of the inserted cDNA fragments. All cRNA probes were purified before use over a 5% polyacrylamide/8 M/L urea gel. RPA were performed using the RPA II kit (Ambion). Briefly, hybridization of the cRNA probes for the selected Kv channel genes with 10 μg total RNA from mesenteric artery tissues or brain tissues were carried out at 42°C for 16 hr, followed by digestion with RNAse A and T1 at 37°C for 30 min. The protected fragments were purified by ethanol precipitation and visualized by autoradiography after electrophoresis on a 5% polyacrylamide/8 M/L urea gel. Quantitative evaluation of mRNA levels of the selected Kv channel subunit genes, with the simultaneously determined mRNA level of β-actin as control, were carried out using scanning densitometric analysis.

Membrane Protein Preparation and Immunoblotting

Membrane proteins were prepared as previously described (Xu et al., 1999). Briefly, mesenteric artery was homogenized with a polytron homogenizer in 3 ml of Tris-buffered saline containing protease inhibitor mixture. The homogenate was centrifuged at 6,000 × *g* for 15 min at 4°C to remove nuclei and undisrupted cells. The supernatant was further centrifuged at 40,000 × *g* for 1 hr at 4°C. The resulting pellets were then washed and re-suspended with the same Tris-buffered saline without sucrose. Protein concentration was determined using Bio-Rad protein assay solution with bovine serum albumin as the standard. For Western blot, membrane proteins were loaded and run on standard 7.5% SDS-polyacrylamide gel in Tris-glycine electrophoresis buffer. Proteins were transferred onto nitrocellulose membrane at 100 Volt for 1.5 hr in a water-cooled transfer apparatus. The membrane was blocked in a blocking buffer, phosphate buffered saline (PBS) containing 3% nonfat milk, at room temperature for 1 hr. The membrane was then probed overnight at 4°C with either monoclonal antibodies against Kv1.2 and Kv2.1 (Upstate Biotechnology) or the affinity-purified polyclonal antibodies against Kv1.5 (Upstate Biotechnology), Kv1.3, Kv 1.4, Kv3.2, Kv3.4, Kv4.2, and Kv4.3 (Alomone Labs). The membrane was subsequently incubated with either goat anti-mouse IgG or goat anti-rabbit IgG for different Kv subunits conjugated with horseradish peroxidase for 2 hr at room temperature. Bound antibodies were detected using a chemiluminescent substrate kit (NEN™ Life Science Products).

Chemicals and Data Analysis

Tetraethylammonium (TEA), 4-aminopyridine (4-AP), 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS), and other chemicals were purchased from Sigma Chemical (St. Louis, USA). Charybdotoxin (ChTX), β-dendrotoxin, and iberiotoxin were from Alomone labs (Jerusalem, Israel). Papain was from Calbiochem Corporation (La Jolla, CA). A stock solution of β-dendrotoxin (5 mM) was prepared in distilled water. This stock solution was directly added to the static bath with a fixed volume to achieve the final concentration of β-dendrotoxin. The stock solution of 4-AP (1 M) was freshly prepared daily in distilled water with pH adjusted to 7.3 with NaOH. The cells were superfused with bath solutions containing the desired concentrations of 4-AP or TEA with equimolar reduction in the amount of NaCl. Therefore, both pH and osmolality of the 4-AP- or TEA-containing solutions did not differ from the regular solutions.

Data were expressed as means ± SEM. Concentration-response curves were fitted to a Hill equation using a computerized curve fitting software (Microcal Origin, version 5.0, Microcal Software, Northampton, MA) to obtain the concentration at which half-maximal inhibition occurred, IC_{50}. Data were analyzed using Student's paired *t*-test, and analysis of variance in conjunction with the Newman-Keuls test where applicable. Differences were considered statistically significant at the level of $P < 0.05$.

Results

Electrophysiological Properties of Kv Currents in Mesenteric Artery SMCs

Kv currents in rat mesenteric artery SMCs were characterized by their delayed activation, relative lack of inactivation during 600 msec depolarization pulses, and outward rectification. Kv currents became obvious at membrane potentials positive to −20 mV (Fig. 1). In a total of 59 cells, the outward Kv currents either slowly activated without an obvious peak or reached the peak and then slowly decayed following exponential inactivation kinetics. Since all these outward Kv currents exhibited the same pharmacological responses to different agents, data obtained were pooled and these currents were referred to as the predominant I_K. No transient outward K^+ currents (I_A) were recorded in freshly isolated rat mesenteric artery SMCs.

The recorded I_K was not contaminated by other ion conductances. In all experiments, the activity of K_{Ca} channels was minimized by using 10 and 1 mM EGTA in the calcium-free pipette solution and the calcium-free external solutions, respectively. Furthermore, the outward Kv currents were not affected by the K_{Ca} channel blockers iberiotoxin and ChTX. Iberiotoxin or ChTX at different concentrations had no effect on the amplitude or kinetics of the outward K^+ current ($P > 0.05$) (Table). ATP (2 mM) in the pipette solution minimized the contribution of K_{ATP} channels to the whole-cell outward K^+ current. In addition, the outward K^+ current was not affected by a Cl^- channel blocker, SITS. Ten min after

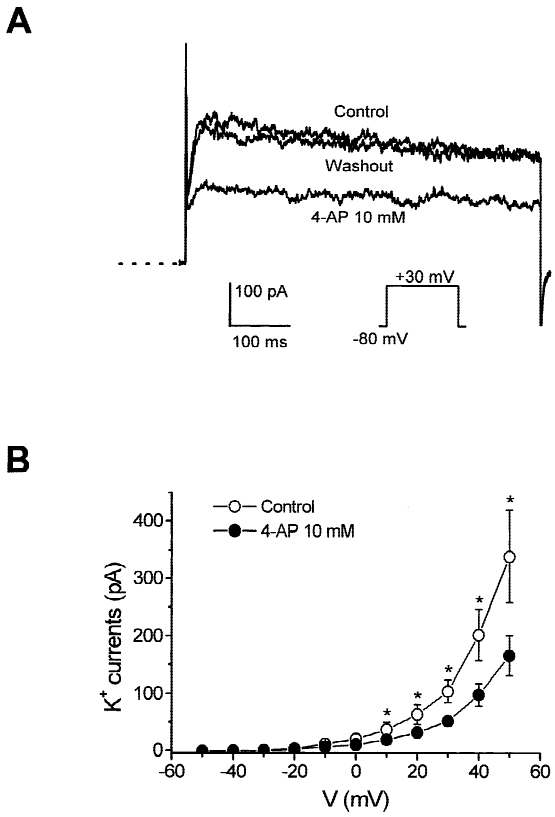


Fig. 1. Delayed rectifier K^+ channel current (I_K) in freshly isolated rat mesenteric artery SMCs. (*A*) Representative original traces of I_K recorded during 600 msec depolarizing pulses (0.1 Hz) from a holding potential of −80 to +30 mV. I_K was inhibited by 4-AP (10 mM), and this inhibition was completely reversed after washing out 4-AP from the bath solution. The dashed line denotes the zero current level. (*B*) Current-voltage relationships of I_K in the absence (○) and then presence (●) of 10 mM 4-AP ($n = 6$). *$P < 0.05$.

Table. Effects of iberiotoxin and charybdotoxin (ChTX) on I_K(pA)

Control	Iberiotoxin (50 nM)	Control	Iberiotoxin (100 nM)
160.4 ± 23.5	154.9 ± 19.9(5)	212.6 ± 51.4	199.8 ± 48.6 (4)
Control	ChTX (25 nM)	Control	ChTX (250 nM)
315.2 ± 27.3	310.2 ± 22.9(6)	263.5 ± 6.7	272.5 ± 12.4 (3)

* No significant difference between control and treatment groups. Numbers in parentheses indicate cell number. HP, −80 mV; Test potential, +30 mV.

application of SITS (1 mM), the outward K^+ current remained unchanged (476 ± 79 versus 476 ± 66 pA at +30 mV, $n = 5$, $P > 0.05$).

The steady-state activation properties of I_K in rat mesenteric artery SMCs were examined. A standard double-pulse stimulation protocol was used, which was

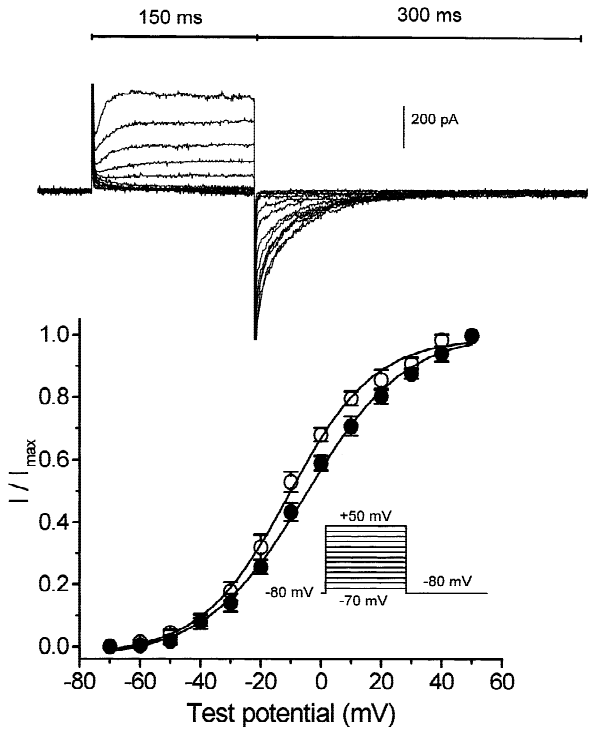


Fig. 2. The steady-state activation of I_K in the absence (○, control) and then presence (●) of 4-AP (5 mM) in freshly isolated rat mesenteric artery SMCs ($n = 5$). The double-pulse protocol is shown in the inset at the bottom right corner, and the representative outward I_K and tail current traces at the top. A rightward shift of the steady-state activation curve of I_K, fitted with a Boltzmann equation, was observed in the presence of 4-AP. The slope factor of the steady-state activation curve of I_K was unchanged in the presence of 4-AP.

composed of different test potentials (−70 to +50 mV, 150 msec duration) from a holding potential of −80 mV. Since K^+ concentrations of bath solution and pipette solution were equal (140 mM), repolarization of membrane potential to −80 mV instantly elicited a train of inward tail currents (Fig. 2). The amplitudes of the tail currents were measured by using a monoexponential fit extrapolated to $t = 0$. Given that the driving force for K^+ ions was fixed at −80 mV, the amplitude of these tail currents reflected the extent of voltage-gated activation of Kv channels in rat mesenteric artery SMCs. Tail currents became detectable at potentials more positive than −50 mV, and were near the maximum at +50 mV. The steady-state activation curves were constructed as the relative peak tail current amplitudes against test potentials, and were fitted to a Boltzmann function of the form $I/I_{max} = \{1 + \exp[(V_{1/2} - V)/k]\}^{-1}$, where I was the current amplitude elicited at different test potentials (V), I_{max} the maximally achieved current amplitude, $V_{1/2}$ the half-activation potential, and k the slope factor. As shown in Fig. 2, $V_{1/2}$ of I_K in rat mesenteric artery SMCs is −11 ± 2 mV. The slope factor (k) was 15 ± 2 mV, indicating a relative weak voltage-dependence of I_K.

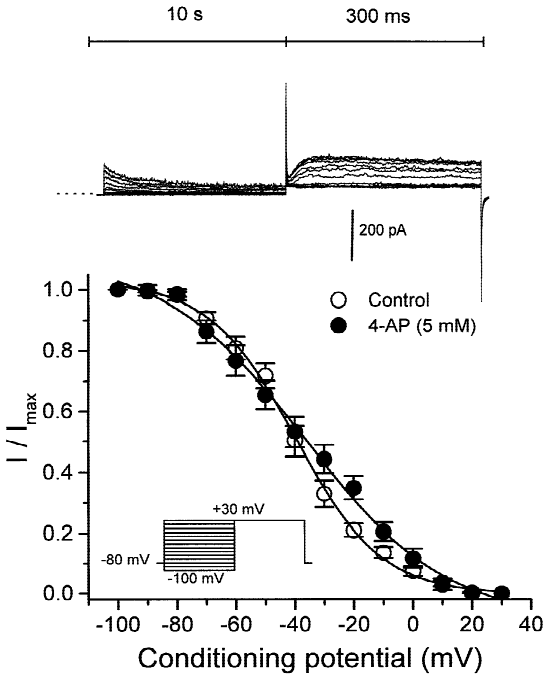


Fig. 3. The steady-state inactivation of I_K in the absence (○, control) and then presence (●) of 4-AP (5 mM) in freshly isolated rat mesenteric artery SMCs ($n = 7$). The double-pulse protocol is shown in the inset at the bottom left corner, and the representative outward I_K traces at the top. The dashed line denotes the zero current level. The outward K^+ currents elicited at different conditioning potentials of 10 sec duration were sampled at 0.1 Hz, and the sampling rate for the K^+ currents during the 300 msec test potential of +30 mV was 0.1 Hz. No parallel shift of the steady-state inactivation curve of I_K, fitted with a Boltzmann equation, was observed in the presence of 4-AP. However, the slope factor of the steady-state inactivation curve of I_K was increased by 4-AP.

The relative availability of I_K was examined by comparing the steady-state inactivation curves of I_K under different conditions. With the holding potential set at −80 mV, conditioning potentials (−100 to +30 mV in 10 mV increments) of 10 sec duration, which was sufficient to reach a steady-state inactivation of Kv channels, were followed by a 300 msec test potential of +30 mV (Fig. 3). The relative amplitudes of the elicited outward currents were plotted as functions of the conditioning potentials. The derived steady-state inactivation curves were fitted to a Boltzmann function of the form $I/I_{max} = \{1 + \exp[(V - V_{1/2}/k]\}^{-1}$, where I was the current amplitude elicited at +30 mV from certain conditioning potentials (V), I_{max} the maximally achieved current amplitude, $V_{1/2}$ the half-inactivation potential, and k the slope factor. Mean data pooled from 7 cells are plotted in Fig. 3 along with the best-fitted Boltzmann distribution curves (solid curves). I_K was completely inactivated at membrane potentials equal to or more positive than +20 mV. $V_{1/2}$ of I_K was −40 ± 3 mV and the slope factor (k) was 14 ± 1 mV.

The Effects of 4-AP on I_K in Mesenteric Artery SMCs

Two protocols were used to study the effect of 4-AP on I_K. The first protocol involved a one-step depolarization train from a holding potential of −80 to +30 mV every 10 sec. After I_K became stable as indicated by consecutive current traces with unaltered amplitude and kinetics for at least 5 min, the normal bath solution was changed to one containing 4-AP. The 4-AP-induced changes in I_K were monitored continuously until a stable inhibition was achieved. Thereafter, a normal bath solution was used to wash away 4-AP under the same recording conditions. The results from this protocol are shown in Fig. 1*A*. The inhibition of I_K by 4-AP in mesenteric artery SMCs was significant and the reversal of the 4-AP effect by the washout procedure was complete. The second protocol consisted of constructing *I-V* relationships of I_K before and after the application of 4-AP. Test pulses from −50 to +50 mV were applied every 10 sec in 10 mV increments from a holding potential of −80 mV. After two consecutive *I-V* relationships became unchanged (stable control), 4-AP was applied to the cell. The stable inhibitory effect of 4-AP was not registered until another two consecutive *I-V* relationships in the presence of 4-AP became unchanged. The results from this protocol are shown in Fig. 1*B*. I_K was inhibited by 4-AP over the entire voltage range studied ($n = 6$). The concentration-dependence of the inhibition of I_K by 4-AP is shown in Fig. 4*A*. The inhibitory effects of 4-AP on I_K became significant at 0.3 mM. The IC_{50} of 4-AP was 5.1 ± 0.9 mM. Figure 4*B* shows the percentage change of I_K as a function of the membrane potential. No significant change was observed in the effects of 4-AP at 0.3, 1, 3, and 10 mM on I_K at different test potentials. The only exception was with 30 mM of 4-AP that caused significantly lesser inhibition of I_K at more positive potential levels ($P < 0.05$ between the amplitude of I_K at +50 mV and those at −10 to +10 mV).

The effect of 4-AP on the time course of I_K was studied by examining the inactivation kinetics of I_K in the absence and then presence of 4-AP. The inactivation of I_K (holding potential, −80 mV; test potential, +30 mV; duration, 10 sec) was best fitted by exponential decay of secondary order. The fast time constant (τ_f) and the slow time constant (τ_s) of I_K inactivation were 371 ± 81 msec and 2884 ± 656 msec ($n = 4$), respectively. The fast inactivated component of I_K accounts for 45 ± 8% of the total I_K. In the presence of 4-AP, τ_f (540 ± 96 msec) and τs (3219 ± 675 msec) remained unchanged ($P > 0.05$, $n = 4$). These results are in agreement with the observation on rabbit coronary artery SMCs (Remillard & Leblanc, 1996).

Whether the inhibitory effects of 4-AP were dependent on specific functional states of I_K channels was studied. After I_K became stable, cells were kept at −80

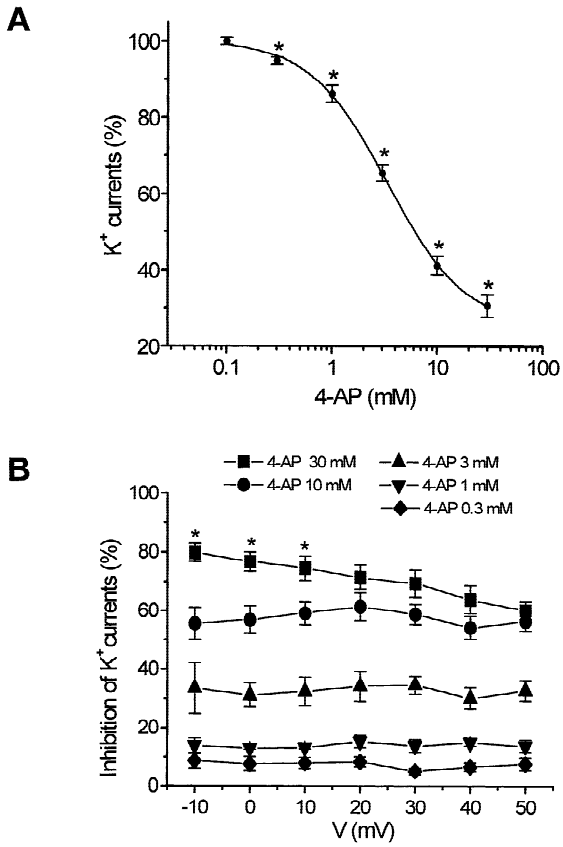


Fig. 4. The concentration-dependence and voltage-dependence of the inhibitory effect of 4-AP on I_K in freshly isolated rat mesenteric artery SMCs. (*A*) I_K, measured at a test potential of +30 mV, was inhibited by 4-AP in a concentration-dependent manner. Each data points on the dose-response curves represent results from 6 to 8 cells. The solid line represents the fitted curve. (*B*) I_K, measured at different membrane potentials (*V*), was inhibited by 4-AP in a voltage-independent manner. A depolarization-relieved effect was observed as an exception when the cells exposed to a high concentration of 4-AP (30 mM). Each data points on the voltage-response curves represent results from 5 to 8 cells. *$P < 0.05$.

mV for 5 min either in the absence or presence of 4-AP (5 mM). Thereafter, the cells were repetitively depolarized to +30 mV at a frequency of 0.2 Hz. As shown in Fig. 5*A*, I_K was not changed during the 5 min incubation at rest or during the repetitive depolarization of 45 sec. In contrast, I_K was inhibited by 4-AP during the 5 min incubation at rest, as the first depolarization pulse revealed a significant decrease of the amplitude of I_K. This inhibition had reached a stable level as no further decrease of I_K was developed during the following depolarization train. These results demonstrated that after a stable inhibition was achieved, repetitive depolarizations did not further potentiate, or relieve the 4-AP effect. The unbinding of 4-AP from Kv channels was studied by washing out of 4-AP from the bath solution while keeping cells at −80 mV. After incubation of the cells with the 4-AP-free solution for 5 min, the cells were repetitively depolarized to open Kv channels. Under these conditions, I_K fully recovered from the 4-AP blockade at rest (Fig. 5*A*) as the amplitude of I_K during the first depolarization pulse after resting incubation was identical to that before 4-AP application. These results suggest that 4-AP may not be trapped in the channel protein and the separation of 4-AP from Kv channels does not need the opening of the channel in rat mesenteric artery SMCs.

It is possible that 4-AP binds to both the closed state and the open state of Kv channels. The closed-state binding would inhibit I_K whereas the open-state binding may potentiate (use-dependent) or reduce (reverse use-dependent) the 4-AP-induced I_K inhibition. In the experiments shown in Fig. 5*B*, the cells were subject to consecutive one-step depolarization pulses (from a holding potential of −80 mV to +30 mV) with three different frequencies (0.05, 0.1, and 0.2 Hz) until I_K became stable. Then, 4-AP was applied to the cells. Clearly, depolarization frequency did not alter the time course of the 4-AP-induced inhibition of I_K. After the application of 4-AP (5 mM) for about 60 sec, the inhibition of I_K became stable regardless of the depolarization frequency. These results indicate that 4-AP does not bind to the open-state of Kv channels.

If the inhibitory effect of 4-AP was favored by the binding of 4-AP to the closed-state of Kv channels, the steady-state activation of I_K would have been affected by 4-AP. This hypothesis was supported by results shown in Fig. 2. A rightward shift of the steady-state activation curve of I_K was observed in the presence of 4-AP (5 mM) with $V_{1/2}$ changed from the control value of −11 ± 2 to −5 ± 2 mV ($n = 5$, $P < 0.05$). However, the slope factor was not altered by 4-AP (15 ± 2 versus 17 ± 1 mV, $P > 0.05$).

The effect of 4-AP on the steady-state inactivation of I_K is shown in Fig. 3. The $V_{1/2}$ of inactivation was not changed by 4-AP (−40 ± 3 versus −39 ± 5 mV, $P > 0.05$). However, the slope factor of the steady-state inactivation curves was increased from 14 ± 1 mV to 24 ± 2 mV by 4-AP ($P < 0.05$), indicating that 4-AP dissipated the voltage-dependence of the steady-state inactivation of I_K in rat mesenteric artery SMCs.

The time-dependent effect of 4-AP may reflect the time course of 4-AP binding to Kv channels rather than the time course of bath solution exchange, which would have completed within 5 sec, as stated in Materials and Methods. To investigate whether the binding site of 4-AP is located on the inner or outer surface of the cell membrane, 4-AP (5 mM) was included in pipette solution to directly perfuse the inner membrane. In the absence of 4-AP in pipette solution, the density of I_K gradually increased initially and reached a plateau phase about 30 sec after formation of whole-cell recording configuration

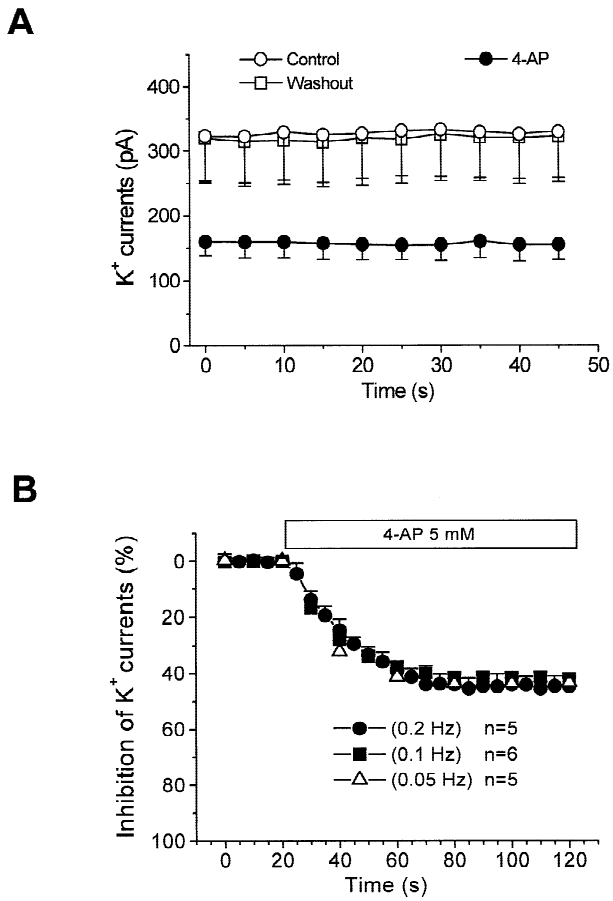


Fig. 5. I_K in freshly isolated rat mesenteric artery SMCs were inhibited by 4-AP that bound to the channels in the closed-state. (*A*) Consecutive recordings of I_K to examine the binding and unbinding of 4-AP (5 mM) to I_K channels in the same mesenteric artery SMCs (n = 6). The cells were kept at rest (−80 mV) for 5 min and then repetitively depolarized to +30 mV (duration of 600 msec) at a frequency of 0.2 Hz for a total of 45 sec. Under these conditions, I_K was recorded as control (○). Subsequently, these cells were kept at −80 mV and exposed to 4-AP for 5 min and then repetitively depolarized (■) following the same protocol as used in control. Finally, 4-AP was washed out from the bath solution for 5 min while the cells were kept at rest (−80 mV), and then the same cells were repetitively depolarized (●) following the same protocol as used in control. (*B*) The frequency-dependent inhibition of Kv channels by 4-AP. The cells were repetitively depolarized from a holding potential of −80 to +30 mV (600 msec test pulse) at different frequencies (● 0.2 Hz—a pulse every 5 sec; ■ 0.1 Hz—a pulse every 10 sec; △ 0.05 Hz—a pulse every 20 sec). The consecutive depolarization trains were delivered in the presence of 4-AP until a stable inhibition of Kv channels by 4-AP was achieved. At least 5 cells were tested with each depolarization frequencies.

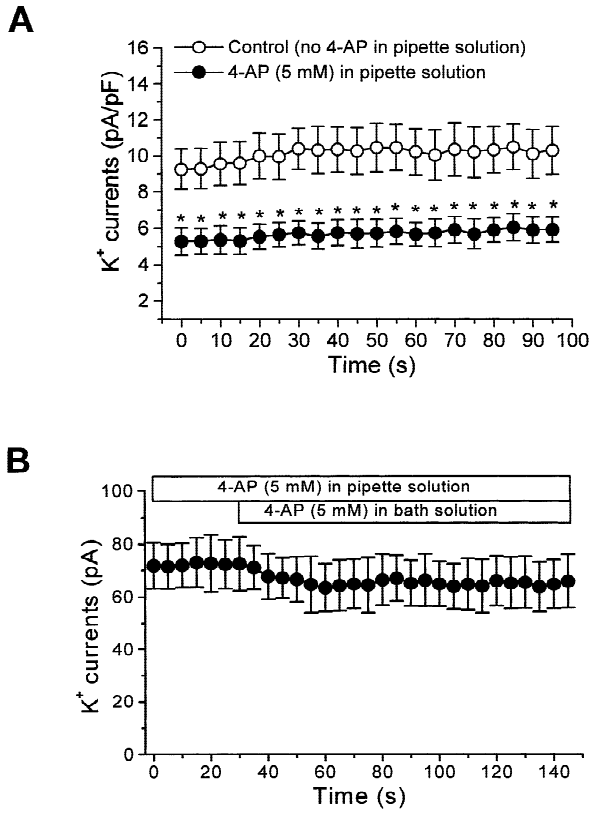


Fig. 6. The inhibition of I_K by intracellularly applied 4-AP (5 mM). (*A*) Changes in the densities of I_K recorded in two groups of rat mesenteric artery SMCs. I_K densities were continuously recorded after the formation of whole-cell recording configuration in the absence of (○, control) or the presence of 4-AP in pipette solution (●). The cells were repetitively depolarized to +30 mV (holding potential, −80 mV) with duration of 600 msec at 0.2 Hz (n = 17 for both groups). (*B*) The effects of 4-AP, applied both intracellularly and extracellularly, on I_K. The extracellularly applied 4-AP failed to inhibit I_K in the presence of the same concentration of 4-AP in pipette solution (n = 6).

(Fig. 6*A*). With 4-AP included in pipette solution, the initial increase phase of I_K density was abolished and the stable level of I_K density was decreased by 42.5% as compared to the stable level of I_K density in control cells (Fig. 6*A*). The inhibitory effect of I_K by intracellularly applied 4-AP is similar to that of extracellularly applied 4-AP (48.6%, *see* Fig. 5*B*). Furthermore, in the presence of 4-AP (5 mM) in pipette solution, extracellularly applied 4-AP at the same concentration failed to inhibit I_K (Fig. 6*B*).

The Effects of TEA on I_K in Mesenteric Artery SMCs

Following the same experimental protocols used in the 4-AP experiments depicted in Fig. 1*A* and *B*, the effect of TEA on I_K was studied. I_K in mesenteric artery SMCs was inhibited by bath-applied TEA in a reversible manner (Fig. 7*A*). Figure 7*B* shows the current-voltage relationships of I_K in the absence and then presence of TEA (10 mM, n = 6). TEA inhibited I_K in a concentration-dependent manner with an IC_{50} of 10 ± 1 mM (n = 6) (Fig. 8*A*). TEA at each concentration inhibited I_K

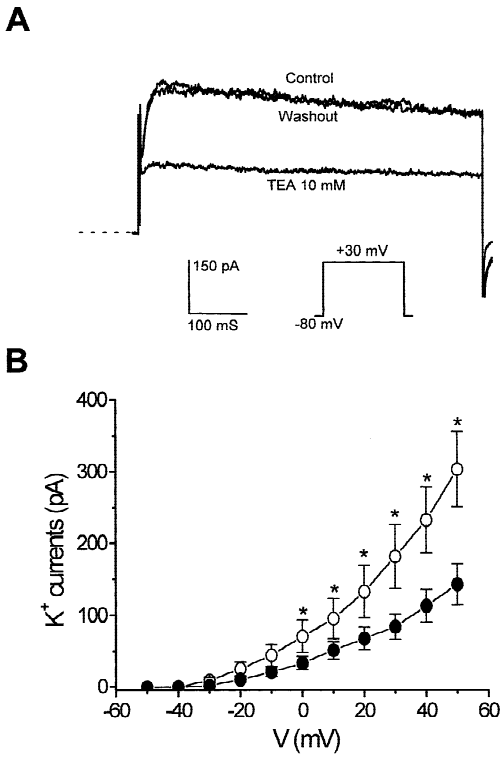


Fig. 7. The inhibition of I_K by TEA in freshly isolated rat mesenteric artery SMCs. (*A*) Representative original traces of I_K recorded during 600 msec depolarizing pulses (0.1 Hz) to +30 mV (holding potential, −80 mV). I_K was inhibited by TEA (10 mM), and this inhibition was completely reversed after washing out TEA from the bath solution. The dashed line denotes the zero current level. (*B*) Current-voltage relationships of I_K in the absence (○) and then presence (●) of TEA (n = 6). *P < 0.05.

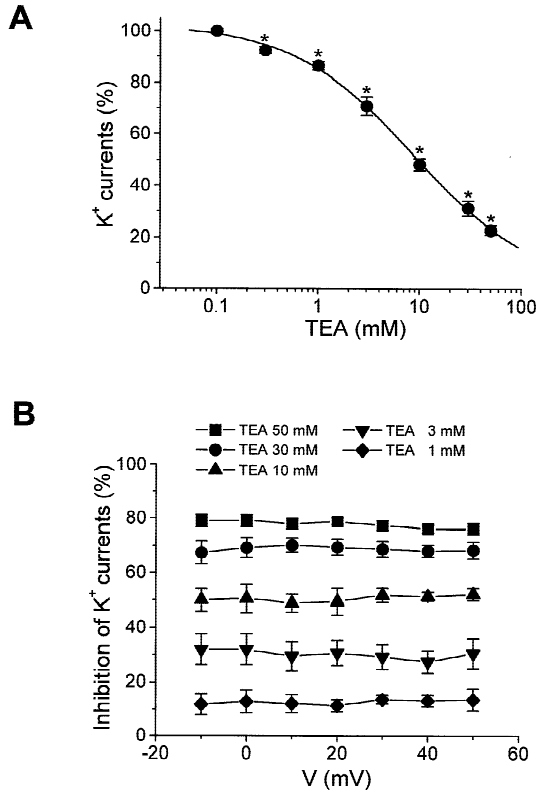


Fig. 8. The concentration-dependence and voltage-dependence of the inhibitory effect of TEA on I_K in freshly isolated rat mesenteric artery SMCs. (*A*) I_K, measured at a test potential of +30 mV, was inhibited by TEA in a concentration-dependent manner. Each data point on the dose-response curves represent results from 5 to 8 cells. The solid line represents the fitted curve. (*B*) I_K, measured at different membrane potentials (*V*), was inhibited by TEA in a voltage-independent manner. Each data points on the voltage-response curves represent results from 5 cells. *P < 0.05 versus the current amplitudes before the application of TEA.

equally within the entire test potential range (Fig. 8*B*), indicating the lack of a voltage-dependent inhibition mechanism. The inactivation kinetics of I_K was also not affected by TEA (*not shown*).

The Effect of β-Dendrotoxin on I_K in Mesenteric Artery SMCs

Following the same experimental protocols used in the 4-AP experiments depicted in Fig. 1*A* and *B*, the effect of β-dendrotoxin on I_K was studied. At different concentrations (0.5 μM and 1 μM), β-dendrotoxin did not affect the current amplitude or *I-V* relationships of I_K (*not shown*). For example, I_K at +30 mV had an amplitude of 210 ± 11 pA under control conditions and 208 ± 9 pA in the presence of 0.5 μM β-dendrotoxin (n = 3, P > 0.05).

Quantitative Comparison of the Expression Levels of the I_K-Encoding Kv mRNA

Determination of the expression levels of different Kv genes in rat mesenteric artery SMCs is important for the understanding of the molecular basis of the native Kv currents. Using RT-PCR technique, we have previously identified the expression of different I_K-encoding Kv subunit mRNA, including Kv1.2, Kv1.3, Kv1.4, Kv1.5, Kv2.1, Kv2.2, Kv3.2, Kv3.3, Kv3.4, Kv4.1, Kv4.2, and Kv4.3, in mesenteric artery tissues as well as in the purified single SMCs (Xu et al., 1999). In the present study, the quantities of the particular I_K-encoding Kv subunit mRNA were determined (Fig. 9). Kv1.2, Kv1.5, Kv2.1, Kvβ1, and Kvβ3 were quantified using the RNAse protection assay, while other Kv subunits were not detectable with this technique. The relative abundant level of Kv1.5 in mesenteric artery as compared to the expression level of β-actin was 15.7 ± 1.2%, which was significantly higher than the levels of Kv1.2 (4.1 ± 0.6%, P < 0.05) and Kv2.1 (3.6 ± 0.3%, P < 0.05) (n = 3).

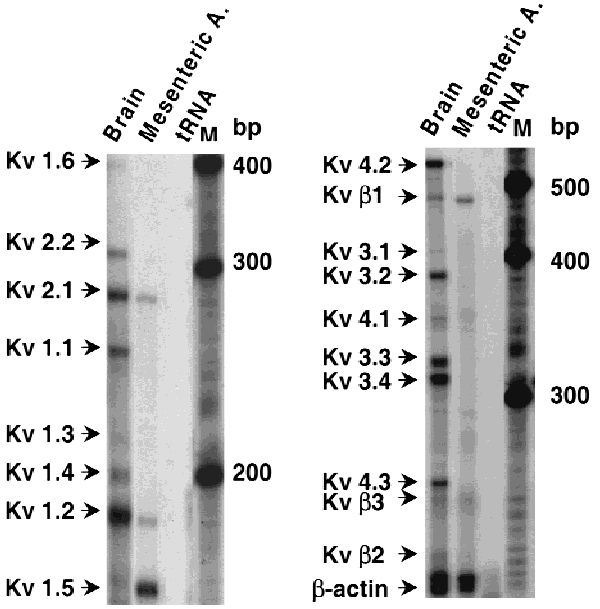


Fig. 9. The transcriptional expression of different Kv subunits in rat brain and mesenteric artery detected using an RNAse protection assay. The mRNAs of Kv2.1, Kv1.2, Kv1.5, Kvβ1, and Kvβ3 were quantified. Transfer RNA (tRNA) was included (10 μg/lane) as a negative control. M, Marker. bp, base pairs. A., artery. The data shown are representative of three independent experiments.

Western Blot Analysis of the Expression of I_A-Encoding Kv Subunits

The transcriptional expression of many Kv subunits that encode either I_K or the transient outward A-type Kv current (I_A) has been demonstrated in rat mesenteric artery SMCs in our previous study (Xu et al., 1999). Among the I_K-encoding Kv genes, Kv1.2, Kv1.3, Kv1.5, and Kv 2.1 were identified in rat mesenteric artery SMCs at the protein level (Xu et al., 1999). Whether the I_A-encoding Kv genes were expressed at the protein level was examined in the present study. Western blot analysis recognized Kv1.4 (96 kD), Kv3.4 (120 kD), Kv4.2 (74 kD), and Kv4.3 (66 kD) proteins in rat brain tissues. However, these Kv proteins were not detectable in rat mesenteric artery tissues (Fig. 10). A broad band ranging from 130 to 190 kD in rat mesenteric artery tissues was found when the anti-Kv3.4 antibody was applied (Fig. 10*D*). After incubating mesenteric artery tissues or brain tissues with excessive inhibiting peptide for Kv3.4 (Alomone Labs) for 1 hr, the Kv3.4 band of 120 kD in brain vanished but the broad band in rat mesenteric artery tissues remained (Fig. 10*E*). The nature of this nonspecific band detected by anti-Kv3.4 antibody was not clear yet. Our results indicate that the lack of expression of the specific Kv proteins corresponding to I_A underlined the lack of detectable I_A in rat mesenteric artery SMCs.

Discussion

Under resting conditions and with physiological concentration of intracellular calcium, Kv channels play a major role in determining the resting membrane potential of vascular SMCs (Okabe, Kitamura & Kuriyama, 1987; Smirnov et al., 1994; Archer et al., 1998), and consequently affect the basal vascular tone. Although the important functional role of Kv channels in other types of vascular SMCs has been shown (Okabe et al., 1987; Smirnov et al., 1994; Archer et al., 1998), the electrophysiological and pharmacological characteristics of Kv channels in peripheral vascular SMCs, such as mesenteric artery SMCs, have not been systematically studied. A careful examination of the literature reveals that in the limited available studies, Kv channels in mesenteric artery SMCs were merely mentioned as a background current or used as a comparison to other ion channels of interest. The steady-state activation and inactivation of I_K were not analyzed. The sensitivities of Kv currents in mesenteric artery SMCs to 4-AP or TEA were concluded in most cases only based on one concentration. The blocking mechanisms and the binding sites of 4-AP for Kv channels have not been clarified. A comparison of the Kv current in mesenteric artery SMCs to that in other types of vascular SMCs was not performed. The quantitative analysis of the expression of Kv channel genes in this tissue preparation was not completed. These gaps in our knowledge are being filled by the results reported here.

Delayed Rectifier I_K in Rat Mesenteric Artery SMCs

I_K in rat mesenteric artery SMCs observed in the present study shares many characteristics with its counterpart in mesenteric artery SMCs from other species, including guinea pig (Ohya et al., 1997) and human (Smirnov & Aaronson, 1992). These similarities include: (i) activation at membrane potentials positive to −20 mV, (ii) decrease in the time-to-peak with increasing voltage steps; (iii) slow activation kinetics; and (iv) that it is relatively insensitive to TEA but sensitive to 4-AP. For example, the sustained outward rectifier I_K in human mesenteric artery SMCs was inhibited by 50% by 4-AP at concentrations around 5 mM and by TEA with IC_{50}s equal to or greater than 10 mM (Smirnov & Aaronson, 1992), which echo our results from rat mesenteric artery SMCs. Indeed, I_K in freshly isolated rat mesenteric artery SMCs exhibit some unique traits. Pharmacologically speaking, I_K in rabbit coronary artery SMCs were inhibited by charybdotoxin (50–100 nM) (Leblanc, Wan & Leung, 1994), but those in rat mesenteric artery SMCs were not. I_K in rat mesenteric artery SMCs were not

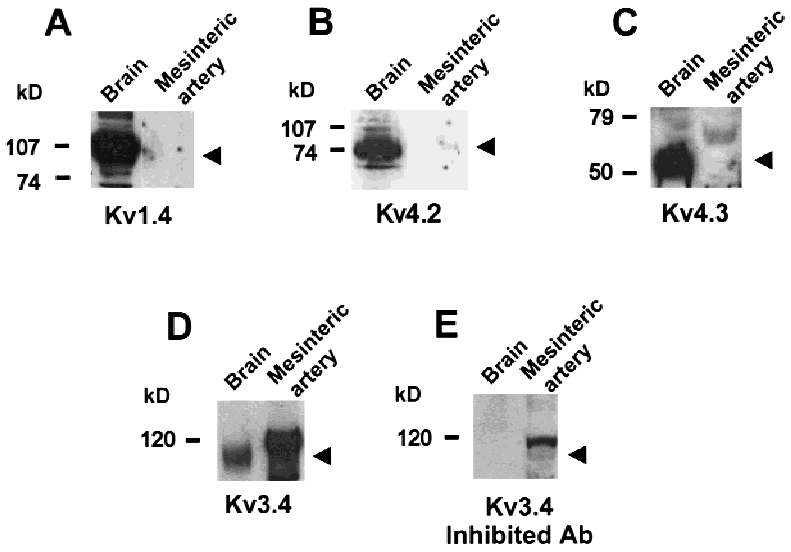


Fig. 10. Western blotting analysis of the expression of the I_A-encoding Kv genes in rat brain and mesenteric artery tissues. Immunoblots of rat brain and mesenteric artery membrane proteins (20–25 μg/lane) were incubated with affinity-purified antibodies against Kv1.4 (*A*), Kv4.2 (*B*), Kv4.3 (*C*), and Kv3.4 (*D*), respectively. The tissues shown in (*E*) were pre-incubated with the inhibiting peptide for Kv3.4 for 1 hr before the application of anti-Kv3.4 antibody. Protein markers were shown on the left side of each panel in kilodaltons (kD). The data shown are representative of six independent experiments.

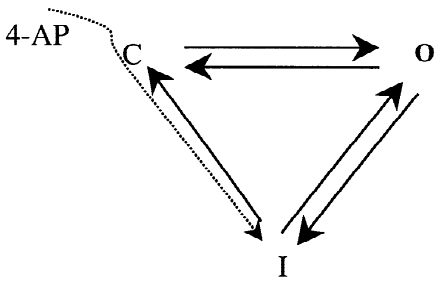
affected by β-dendrotoxin, a polypeptide component of Eastern Green Mamba snake venom. This toxin, however, inhibited I_K in freshly isolated (*unpublished observations*) or primary and subcultured rat tail artery SMCs (Ren, Karpinski, & Benishin, 1993). The mechanisms of the 4-AP-induced inhibition of I_K in mesenteric artery SMCs, as discussed in the following section, are also different from other types of cells. Functionally, I_K in rat mesenteric artery SMCs plays a more important role in setting the resting membrane potential than in other types of SMCs. The voltage-dependence of the steady-state activation of I_K is less steeper in rat mesenteric artery SMCs than in rabbit coronary artery SMCs (Remillard & Leblanc, 1996). The slope factors of the steady-state activation of I_K are 15 and 9 mV in rat mesenteric artery and rabbit coronary artery SMCs, respectively. While the activities of I_K in most other types of excitable cells are very low at resting membrane potential, a large window current through Kv channels in rat mesenteric artery SMCs is present around the reported resting membrane potential level (−40 mV) (Yuan et al., 1993) because of the significant overlap of the steady-state activation curve ($V_{1/2}$ of −11 mV) and the steady-state inactivation curve ($V_{1/2}$ of −40 mV).

The Mechanism of 4-AP Blockade of I_K

As the most specific blocker of I_K in SMCs, 4-AP has been extensively used. Interestingly, depending on the specific types of SMCs under investigation, the efficacy of 4-AP in blocking I_K, as well as the underlying mechanisms, are quite different. Different models have been proposed to explain the interaction of 4-AP and Kv channel proteins. One theory suggests that the binding sites for 4-AP lie in the inner vestibule of the Kv channel pore (Chandy & Gutman, 1995). The blockade of Kv channels may therefore require channel opening for 4-AP to reach its binding site. This open-state block, quite different from the closed-state block, required channel opening for the onset of block, and displayed use-dependent or reverse-use-dependent inhibition (Ogata & Tatebayashi, 1993). Chen and Fedida (1998) suggested that 4-AP-blocked Kv1.5 channels might exist in a partially open, nonconducting state. This is, in fact, a modified open-state binding model. The binding of 4-AP becomes possible only when Kv channel is open. Our observations that the onset of I_K blockade depended on how long the cell had been exposed to 4-AP, but was independent of whether the channel was open during this exposure period (Fig. 2*A*), that I_K recovered fully after 4-AP was washed out from the bath solution while cells were kept quiescent at −80 mV, and that a fully developed stable inhibition of I_K was achieved during 5 min incubation of cells with 4-AP at a constant membrane potential of −80 mV, are in discrepancy with the open-state binding hypothesis. Furthermore, repetitive channel openings at different stimulation frequencies did not alter the stable blockade of I_K by 4-AP. During the first 60 sec of application of 4-AP the same percentage of I_K was inhibited no matter whether Kv channels had been opened twice (0.05 Hz), 6 times (0.1 Hz) or 12 times (0.2 Hz) (Fig. 5*B*). These results, together with the rightward shift of the steady-state activation curve of I_K in the presence of 4-AP (Remillard & Leblanc, 1996), support the notion that 4-AP preferably bound to the closed-state of Kv channels in rat mesenteric artery SMCs.

A simplified kinetic model for the transition of Kv channels is proposed to explain the relatively voltage-independent interaction of 4-AP and Kv channels in rat

mesenteric artery SMCs. Similar to the model of Remillard & Leblanc (1996), the Kv channel undergoes 3 sequential states, i.e. closed (C), open (O) and inactivated (I). The difference is that upon 4-AP binding to the closed state, Kv channel enters a single non-conducting blocked state in rabbit coronary artery SMCs (Remillard & Leblanc, 1996), but Kv channel changes to the inactivated state in rat mesenteric artery SMCs. If the closed-



state binding of 4-AP and channel inactivation were mutually exclusive, one might also expect that the steady-state inactivation curve of Kv channels would be shifted by 4-AP (Thompson, 1982; Kehl, 1990; Ogata & Tatebayashi, 1993). Interestingly, in our study 4-AP did not affect the steady-state inactivation curve of the Kv channel in rat mesenteric artery SMCs. Although the exact mechanisms for this phenomenon cannot be delineated yet, the linkage of the closed state and inactivated state of the Kv channel in this cell type might be a plausible interpretation. The Kv channel is unique in rat mesenteric artery SMCs in that this channel has a weak voltage dependence, both the binding and unbinding of 4-AP are voltage-independent, and the amplitude but not the inactivation kinetics of I_K was inhibited by 4-AP. These features suggest that the binding of 4-AP may facilitate the transition of Kv channels from the closed state to inactivated state. As such, the relative availability curve of Kv channels would remain unchanged in the presence of 4-AP. This simple model, however, needs to be verified experimentally.

To reiterate, the mechanisms for the inhibitory effect of 4-AP on Kv channels are unique for rat mesenteric artery SMCs, compared to other types of vascular SMCs and excitable cells. (i) The closed-state binding of 4-AP in rat mesenteric artery SMCs was reminiscent of that in rabbit coronary artery SMCs (Remillard & Leblanc, 1996), but different from the open-state binding and use-dependent action in lymphocytes (Choquet & Korn, 1992). The unbinding of 4-AP from Kv channels was promoted by repetitive depolarization in SMCs of rabbit coronary artery (Remillard & Leblanc, 1996), but not in our study. (ii) The voltage-independent inhibitory effects of 4-AP in rat mesenteric artery SMCs disagreed with the voltage-dependent relief of inhibition of I_K in rabbit coronary artery SMCs (Remillard & Leblanc, 1996). (iii) The steady-state activation curves of I_K in both rat mesenteric artery SMCs and rabbit coronary artery SMCs (Remillard & Leblanc, 1996) were shifted rightward in the presence of 4-AP, but the slope factors were changed only in coronary artery SMCs. (iv) The $V_{1/2}$ of steady-state inactivation curves of I_K in rat mesenteric artery SMCs was not changed by 4-AP, but increased significantly in neurons (Ogata & Tatebayashi, 1993) and in rabbit coronary artery SMCs (Remillard & Leblanc, 1996). The slope factors of the steady-state inactivation curves were increased by approximately 10 mV by 4-AP in mesenteric artery SMCs, whereas virtually no change in slope factor was observed in coronary artery SMCs. (v) The instant blockade of I_K by intracellularly applied 4-AP strongly supports the notion that the chemical binds to the inner side of the Kv channel pore (Chandy & Gutman, 1995). However, the entry of 4-AP into the cytosol does not require Kv channel opening (Ogata & Tatebayashi, 1993). The delayed time course for the maximal development of the inhibitory effect of extracellularly applied 4-AP in the absence of Kv channel opening indicates that 4-AP penetrates the cell membrane by a still unknown mechanism to reach its binding site. Taken together, these features of 4-AP effects in freshly isolated rat mesenteric artery SMCs emphasize the tissue-specific mechanism underlying the interaction of Kv channels and 4-AP.

Molecular Basis of Kv Channels in Rat Mesenteric Artery SMCs

The molecular identity of Kv channels in peripheral vascular SMCs, including mesenteric artery SMCs, has been largely unknown. It has been suggested recently that Kv channels in smooth muscle cells may exist as heterotetramers, i.e., they could be assembled from different Kv gene products, giving rise to hybrid channels with a range of different kinetic and pharmacological properties. Our study showed that I_K in rat mesenteric artery SMCs has a shallow voltage-dependence of channel activation and inactivation. This might be interpreted as the presence of contaminating currents, which is in any rate not likely under our experimental conditions, as discussed before. Alternatively, the unique weak voltage-dependence of I_K may be caused by the functional expression of multiple Kv genes in these cells.

In portal vein SMCs (Beech & Bolton, 1989) or rabbit aortic SMCs (Halliday et al., 1995), I_K was not sensitive to extracellular TEA at low millimolar concentrations. In our study, I_K in rat mesenteric artery SMCs was inhibited by TEA with an IC_{50} of 9.9 mM. Our findings are in line with the observations made in rabbit

coronary artery SMCs (Volk, Matsuda & Shibata, 1991), and rabbit portal vein SMCs (Hume & Leblanc, 1989). Among the I_K-encoding Kv genes, Kv1.1, Kv3.1–Kv3.4 are extremely sensitive to external TEA (Kd, 0.1–1 mM); Kv1.6, Kv2.1, and Kv2.2 are moderately sensitive (Kd, 1–10 mM) (Chandy & Gutman, 1995). Therefore, these moderate TEA-sensitive Kv genes and 4-AP-sensitive Kv genes could make different contributions to the formation of native Kv channels in rat mesenteric artery SMCs. Our results from the experiments with β-dendrotoxin and charybdotoxin may help to further shorten the long candidate list of Kv genes. Kv1.1, Kv1.2, and Kv1.6 have been reported to be blocked by β-dendrotoxin. Kv1.2 and Kv1.3 are also sensitive to recombinant charybdotoxin (Chandy & Gutman, 1995). Moreover, the expressed Kv1.2 channel currents were blocked by 4-AP due to the open-state binding of the drug (Russell et al., 1994*a*). In rat mesenteric artery SMCs, I_K was not sensitive to either β-dendrotoxin or charybdotoxin, but inhibited by 4-AP bound to the closed-state of the channel. Therefore, our results suggest that the contribution of Kv1.1, 1.2, 1.3, and 1.6 to the native I_K in rat mesenteric artery SMCs may not be significant. However, it should be recognized that the possibility exists that the binding of a specific Kv channel blocker to its sensitive Kv subunits could be prevented by the presence of even one single insensitive subunit in the heterotetrameric structure of Kv channels (Russell, Overturf & Horowitz, 1994). Nevertheless, our electrophysiological and pharmacological data were supported by molecular biology data. Our western blot analysis confirmed the expression of many I_K-encoding, but not I_A-encoding, Kv subunits at protein level in rat mesenteric arteries. More importantly, the relative expression levels of I_K-encoding Kv subunit mRNA in rat mesenteric arteries were determined with an abundance order of Kv1.5, Kv2.1, and Kv1.2. Taken together, our results suggest that the 4-AP sensitive Kv channels in rat mesenteric artery SMCs are most likely the heterotetrameric products of multiple Kv subunits with Kv1.5 playing an important role.

In summary, our study provides the first comprehensive profile, to our knowledge, of Kv channels in freshly isolated rat mesenteric artery SMCs. The results support our hypothesis that the characteristics of Kv channels in these peripheral artery SMCs are different from other types of vascular SMCs. The shallow voltage-dependence of the steady-state activation of Kv channels endows them an important role in setting up resting membrane potential of mesenteric artery SMCs. The sensitivity of Kv channels in mesenteric artery SMCs to 4-AP is similar to other types of vascular SMCs. However, the mechanisms of the 4-AP effect are quite different. In particular, the inhibition of I_K was induced by 4-AP binding to the inner site of Kv channel pores in their closed state, and the unbinding of 4-AP was not affected by repetitive openings of the channel. While the steady-state inactivation of I_K was not affected, the steady-state activation curve of I_K was shifted rightward in the presence of 4-AP. Differences in the electrophysiological and pharmacological properties of I_K between freshly isolated rat mesenteric artery SMCs and other types of vascular SMCs, such as those from pulmonary artery or cerebral arteries, are essential for determining the basal function and responsiveness of various vasculatures to physiological or pathophysiological stimuli. The functional diversity of Kv channels can be explained by the tissue-specific expression and assembly of Kv subunit genes as well as different modulating mechanisms.

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