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Two Types of Voltage-dependent Outward Potassium Currents in Smooth Muscle Cells of Rabbit Basilar Atery

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We have investigated the two types of voltage-dependent outward potassium (K) currents, i.e. delayed rectifier K current ($I_{K(V)}$) and 'A-like' transient outward K current (I_{to}) with patch-clamp technique in single smooth muscle cells (SMCs) isolated from rabbit basilar artery, and investigated the characteristics of them. The time-courses of activation were well fitted by exponential function raised to second power (n^2) in $I_{K(V)}$ and fourth power (n^4) in I_{to}. The activation, inactivation and recovery time courses of I_{to} were much faster than that of $I_{K(V)}$. The steady-state activation and inactivation of $I_{K(V)}$ was at the more hyperpolarized range than that of I_{to} contrary to the reports in other vascular SMCs. Tetraethylammonium chloride (TEA; 10 mM) markedly inhibited $I_{K(V)}$ but little affected I_{to}. 4-Aminopyridine (4-AP) had similar inhibitory potency on both currents. While a low concentration of Cd^{2+} (0.5 mM) shifted the current- voltage relationship of I_{to} to the positive direction without change of maximum conductance, Cd^{2+} did not cause any appreciable change for $I_{K(V)}$.

Key Words: Basilar artery, Delayed rectifier K current, Transient outward K current, 4-AP, TEA

INTRODUCTION

The electrical and pharmacological characteristics of cerebral arteries differ from many other peripheral arteries. Harder (1980) demonstrated a significantly different resting membrane potential (E_m) in the middle cerebral artery as compared with the mesenteric and coronary artery in cat, and suggested that potassium conductance was high in the middle cerebral artery. Thus, to identify and characterize the K channels present in arterial smooth muscle cells (SMCs) would be important for understanding the physiological roles of cerebral arteries.

Various K channels have been characterized in many types of SMCs. Among them, voltage-dependent K channels (K_V) and large conductance Ca^{2+}-activated K channel (BK_{Ca} or maxi-K) are ubiquitous in vascular and visceral SMCs (Beech & Bolton, 1989a; Gelbland & Hume, 1992; Smirnov & Aaronson, 1992; Gordienko et al, 1994). Kv current in smooth muscles include the delayed rectifier K current ($I_{K(V)}$) and the A-type K current ($I_{K(A)}$) (Beech & Bolton, 1989a; Beech & Bolton, 1989b; Smirnov & Aaronson, 1992). These two voltage-dependent K currents are activated by membrane depolarization and are not by intracellular Ca^{2+}. The term 'delayed rectifier' was used originally by Hodgkin and Huxley (1952) in the squid giant axon. When single SMCs where internal Ca^{2+} was strongly buffered are subjected to a step depolarization under voltage-clamp, Ca^{2+}-insensitive, voltage-gated outward rectifying K current develops after a short delay. In general, this current is referred to as the delayed rectifier K current ($I_{K(V)}$) in SMCs. $I_{K(V)}$ is probably responsible for a large part of the voltage-activated K current and contributes membrane repolarization in smooth muscle. In neurones, $I_{K(A)}$ is distinguished from the $I_{K(V)}$ because it can be activated only from very negative voltages and it inactivates rapidly, making a transient appearance (Rudy, 1988). The $I_{K(A)}$ is blocked readily and reversibly by

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4-aminopyridine (4-AP) but not by external tetraethylammonium chloride (TEA). Potassium current in the A-type category also exists in SMCs (Beech & Bolton, 1989b; Lang, 1989; Imaizumi et al, 1990). In SMCs of rabbit portal vein, $I_{K(A)}$ resembles neuronal A-current very closely (Beech & Bolton, 1989b).

In cerebral SMCs, various K current were identified by using patch-clamp techniques. Stockbridge et al (1992) already showed that there were $I_{K(V)}$ and Ca^{2+}-activated K current ($I_{K(Ca)}$) in rat basilar artery. Bonnet et al (1991) characterized $I_{K(V)}$ and showed that reduction of pH from 7.43 to 7.20 increased peak outward current in SMCs of cat cerebral artery. There is no reports, at present, that transient outward K current as well as delayed rectifier K current is activated as depolarizing pulses are applied in SMCs of cerebral or basilar artery. In this study, we identified and characterized the outward K currents in SMCs of rabbit basilar artery. We have found that there are two types of voltage-dependent K current as well as $I_{K(Ca)}$. Contrary to other vascular SMCs, in rabbit basilar artery, 'A-like' transient outward K current (I_{to}) is activated and inactivated at more depolarized potential range than $I_{K(V)}$.

METHODS

Single cell isolation

Single SMCs were enzymatically isolated from rabbit basilar artery. Rabbits (New Zealand white rabbit, 1.5~2.0 kg) were anesthetized with sodium pentobarbital (40 mg/kg i.v.) and exsanguinated. The basilar artery was dissected along with brain stem and isolated in a Ca^{2+}-free phosphate-buffered Tyrode's solution of the following composition (in mM) : NaCl 147, KCl 4, $MgCl_2$ 2, NaH_2PO_4 0.42, Na_2HPO_4 1.81, glucose 5.5, pH 7.35. Tunica adventitia, surrounding connective tissue and side branches were removed gently under a stereomicroscope. The artery was longitudinally dissected and rinsed to remove the residual blood in vessel. The artery was first moved to a nominally Ca^{2+}-free Tyrode's solution of the following composition (in mM) : NaCl 138.5, KCl 6, glucose 10, $MgCl_2$ 0.5, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) 5, (pH adjusted to 7.35 with Tris), and cut into small pieces and then the segments were stored at 4°C for 15 minutes. Collagenase (1.5~1.8 mg; Wako, Japan), bovine serum albumin (2 mg; essentially fatty acid free, Sigma, USA) and dithioerythreitol (1~1.5 mg; Sigma, USA) were dissolved into 1 ml of Ca^{2+}-free Tyrode's solution. Then the arterial segments were incubated in this enzyme solution at 35°C for 13~15 minutes. After collagenase treatment, segments were transferred to Kraft-Bruehe (KB) solution of the following composition (in mM) : L-glutamic acid (free acid) 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, HEPES 10, ethyleneglycol-bis((-aminoethylether) N, N,N',N'-tetraacetic acid (EGTA) 0.5 (pH adjusted to 7.35 with KOH), and single SMCs were dispersed by gentle agitation with fire-polished glass pipette. Isolated single cells were stored at 4°C until use.

An aliquot of single SMCs of basilar artery in suspension was added to the recording chamber (0.5 ml) mounted on an inverted microscope (IMT-2, Olympus, Japan). Solutions were superfused through the chamber by gravity at a rate of 2~3 ml/min. Experiments were performed at room temperature.

Electrophysiological recordings

Whole-cell membrane currents (voltage-clamp configuration) and membrane potential (current-clamp configuration) were measured using standard patch-clamp technique. Patch pipettes were connected to the head stage of an patch-clamp amplifier (Axopatch-1C, Axon Instruments, USA). The liquid junction potentials were corrected with an offset circuit before each experiment. Whole-cell currents were filtered at 5.0 kHz (-3dB frequency) by a four-pole low-pass Bessel filter. Data were digitized on-line with an analogue-to-digital interface (Labmaster DMA interface, Scientific Solutions, USA) and stored on an IBM-AT compatible computer. All data analysis was performed with pCLAMP 5.5.1 software (Axon Instruments) and Sigmaplot. Fire-polished patch pipettes filled with intracellular solution had free-tip resistance of 3~5 MΩ. To measure whole-cell currents after gigaseal formation, access to the cell interior was obtained by rupturing the membrane at the tip of the pipette with additional negative pressure. The cell capacitative transients were not compensated, and series resistance compensation was not introduced. To analyze activation kinetics, however, the capacitative transients were subtracted digitally using hyperpolar-

izing pulses. The values given in the text are the means ± S.E.M. with n number of the sample size.

Solutions

Normal Tyrode's solution for potassium currents recording contained (in mM) : NaCl 138.5, KCl 6.3, $CaCl_2$ 1.8, glucose 10, $MgCl_2$ 0.5, HEPES 5, adjusted to pH 7.35 with Tris. The internal solution for patch electorde was composed of following composition (in mM): aspartic acid 110, Mg-ATP 5, HEPES 5, $MgCl_2$ 1, KCl 20, EGTA 0.1 or 10, creatine phosphate (ditris 2.5 / disodium 2.5) 5, KOH 110 (adjusted to pH 7.35 with KOH).

Tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP), and EGTA were all purchased from Sigma Chemicals (St. Louis, MO, USA). When 4-aminopyridine was treated, pH of the bath solution was adjusted by HCl.

RESULTS

SMCs isolated from rabbit basilar arteries were relaxed spindle-shap and the length of cells varied but not more than 150 μm long, 10 μm wide. Under current- clamp the isolated cells have a resting membrane potential of -29 ± 1.2 mV (n=16) with a pipette solution containing K-aspartate with 10 mM EGTA. At the same conditions the membrane input resistance and cell capacitance were measured by using 10 mV hyperpolarizing step pulse and ramp pulse (1 V/sec) at holding potential of -60 mV, respectively. The measured cell capacitance (C_m) was

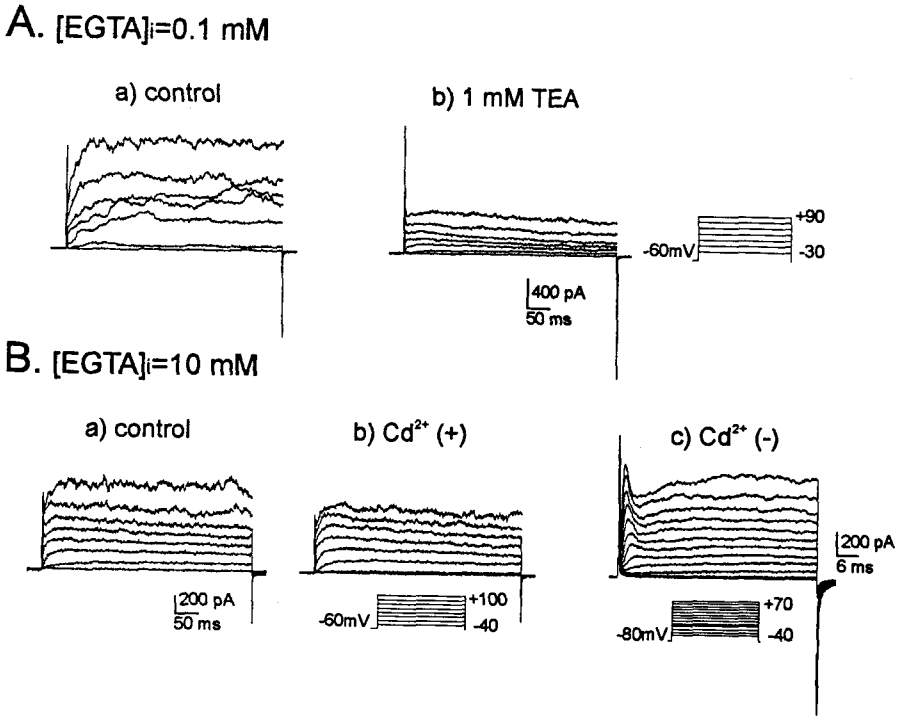

Fig. 1. Three different types of outward potassium currents.
A. Outward K currents recorded in weakly Ca^{2+}-buffered (0.1 mM EGTA) cells. The fluctuating outward K currents were activated by depolarizing step pulses (a), and they were markedly suppressed by the treatment of 1 mM TEA (b). B. Outward K currents recorded in strongly Ca^{2+}-buffered (10 mM EGTA) cells. Low-noise outward K currents were dominant, and rapid activating and inactivating transient outward current was superimposed on the slowly activating outward current. Cd^{2+}-sensitive transient K current was well demonstrated in c (compare the time-scale bars in a and c). In a Ca^{2+}-free bath solution containing Cd^{2+} (1 mM), the current amplitude was reduced and the transient outward K current was abolished (b).

19.2±0.65 pF (n=21) and input resistances (R_{input}) was 2.04±0.12 GΩ (n=12). Assuming a value of 1 μF/cm^2 for specific membrane capacitance, the estimated mean cell surface area corresponded to 1.92 ×10^{-5} cm^2. Using these values, the calculated specific membrane resistance (R_m) is about 39.2 kΩ cm^2. Using following equation, $\lambda = (dR_m/4R_i)^{1/2}$, a space constant ($\lambda$) of 0.153 cm was determined on the basis of the measured cell diameter (d) of 6 μm and assuming a specific internal resistivity (R_i) of 250 Ωcm (Abe & Tomita, 1968). Such a value of the space constant predicts that the entire membrane of a single rabbit basilar arterial cell would be virtually isopotential both in the resting state and during activation of ionic channels.

When cells were dialysed with a pipette solution containing low Ca^{2+} buffer (0.1mM EGTA without Ca^{2+}), dominant current was a fluctuating outward current and has a threshold potential of −30 mV (Fig. 1A). This current was markedly suppressed in cells dialysed with a pipette solution containing high Ca^{2+} buffer (10 mM EGTA without Ca^{2+}) (Fig. 1B), and it was sensitive to external TEA (Fig. 1Ab). These properties suggested that it is mainly carried by $I_{K(Ca)}$. In inside-out patches, we recorded the large-conductance $K_{(Ca)}$ current which is sensitive to $[Ca^{2+}]_i$. The slope conductance was 197±17 pS (n=4) and the open probability increased as the membrane potential was depolarized (> +50 mV at pCa 6.5).

When the membrane potential was held at −60 mV and serially depolarized, two types of outward K current were elicited in Ca^{2+} buffered cells: a transient outward K current superimposed on a slowly activating and sustaining K current. The representative current traces for transient outward current are shown in Fig. 1Bc at time scale expanded. The two components were seperated on the basis of voltage- and time-dependence of activation and inactivation, and pharmacological sensitivity to external TEA and Cd^{2+}. We refer transient outward K current as I_{to} and slowly activated outward K current as $I_{K(V)}$.

Delayed rectifier K current ($I_{K(V)}$)

We first performed classical kinetic analyses on $I_{K(V)}$. In a bathing solution containing 1 mM Cd^{2+} to block both the inward Ca and I_{to} current (see Figs. 1B and 12A), voltage dependence of the current was studied using a depolarizing voltage steps for 400 ms to various potentials (from −60 mV to +60 mV) from a holding potential of −60 mV. Potentials less than +60 mV were used to eliminated the possible contamination of $I_{K(Ca)}$ which might be activate at strong depolarization even in the presence of internal

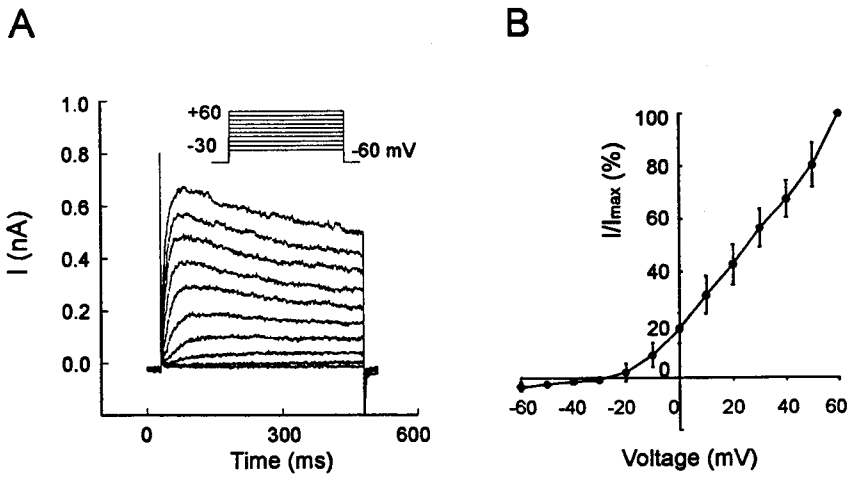

Fig. 2. The current-voltage relationship of delayed rectifier K current ($I_{K(V)}$). A. Representative current traces of $I_{K(V)}$. The bath solution contained 1 mM Cd^{2+} and the pipette solution contained 10 mM EGTA without Ca^{2+}. B. Mean current-voltage relationship. The current amplitudes were normalized to that of the current at +60 mV (n=17).

high Ca^{2+} buffer. The original current traces and current-voltage (I-V) relationship are shown in Fig 2. The first detectable outward current activated between −30 mV and −20 mV, and slow inactivation of the current could be seen. The I-V curve rectified outwardly in shape and the current increased in a nearly linear fashion with increasing voltage steps (Fig. 2B).

In order to identify the major charge carrier of this current, we measured the reversal potential (E_{rev}) of the tail currents at different external K ion concentrations ($[K^+]_o$; 6.3, 25, and 50 mM). Deactivating tail currents were recorded at various repolarizing potentials in 10 mV increments after a 100 ms prepulse voltage step to +50 mV (Fig. 3A). Measured E_{rev} were −62.5±2.5 at 6.3 mM $[K^+]_o$ (n=8), −30.0± 3.5 at 25 mM $[K^+]_o$ (n=4) and −13.8±4.1 mV at 50 mM $[K^+]_o$ (n=4), respectively. At each $[K^+]_o$, E_{rev} did not correspond to the calculated E_K from Nernst equation, −76.9, −43.4, and −25.9 mV for each $[K^+]_o$, respectively. The difference is likely in part due to a junction potential that develops between the pipette tip (containing K-aspartate) and the bathing solution (containing mainly NaCl). The measured junction potential was −7 mV with the pipette solution in the external 6.3 mM K^+. The slope per decade change of $[K^+]_o$, however, was 50.4 mV per ten-fold change of $[K^+]_o$ (Fig. 3B) and this is similar to the value predicted by the Nernst relation, 57 mV. From the above results we concluded that this outward current in rabbit basilar artery was largely carried by an activation of K-selective channel.

The voltage- and time-dependent behaviours of $I_{K(V)}$ were analyzed. Close examination of the current traces revealed a sigmoid onset of current (Fig. 4, *inset*). The time course of activation was well fitted by exponential function raised to second power (n^2). The superimposed exponential function fitted the current records very well from 0 to +50 mV. However the fit exhibited deviations at depolarizing potentials more than +60 mV. In these cases (> +60 mV) the

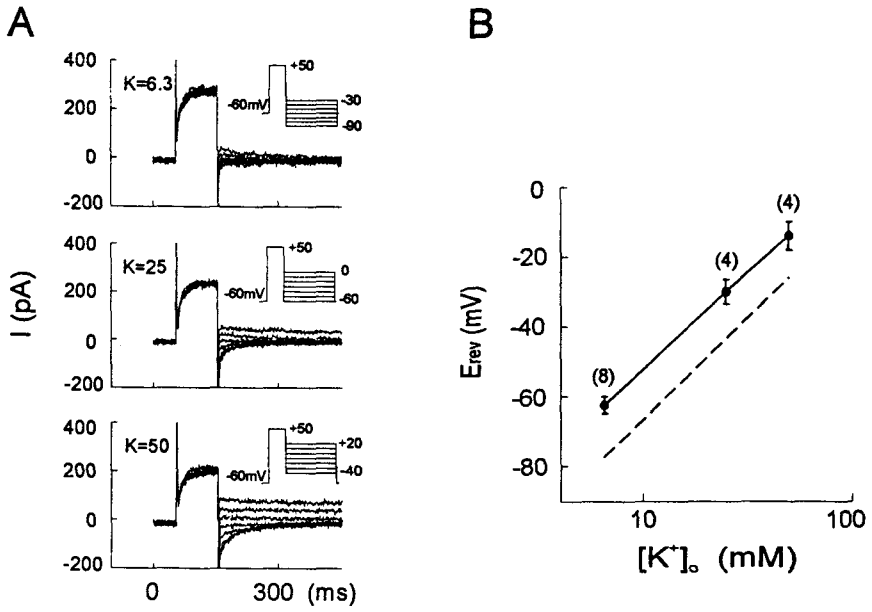

Fig. 3. Reversal potentials measured at various extracellular potassium concentrations ($[K^+]_o$).

A. Each panel represents a family of tail currents elicited by repolarization pulses following 100 ms voltage step to +50 mV from a holding potential of −60 mV. The repolarization steps were in 10 mV increments. Tail currents were recorded from the same cell in $[K^+]_o$ of 6.3, 25 and 50 mM, respectively. Equimolar NaCl was replaced with KCl in the presence of external Cd^{2+} (1 mM). B. Obtained E_{rev} were plotted against $[K^+]_o$. The lower dotted line represents the theoretical E_K calculated from Nernst equation. Obtained data have a slope of 50.4 mV per 10-fold change of $[K^+]_o$ (n=4~8).

currents were rather well fitted by single exponentials. The possible explanation for this deviation is the contamination of $I_{K(Ca)}$ and I_{to}. At high membrane potentials the current activated rapidly; activation time constants were 50 ± 3.7 ms at -10 mV and 8.9 ± 0.96 ms at 40 mV, respectively (Fig. 4).

The voltage dependence of steady-state activation was determined by using a double-pulse protocol. Currents were elicited by 50 ms depolarizing pulses to a various test potentials (-30 to $+40$ mV, 5 mV increments) from a holding potential of -60 mV, and then deactivating tail currents were recorded at a constant repolarizing potential (-30 mV) for 300 ms (Fig. 5A, upper panel). These tails were fitted by single exponential function, and the amplitude of instantaneous current (t_0) of each tails were calculated. The t_0 gives a measure of the instantaneous conductance of $I_{K(V)}$ activated during test pulses. Amplitudes of instantaneous tail currents were normalized to the largest tail current (I/I_{max}) and plotted against each test potential. Plotted data were well fitted by the following Boltzmann equation (Eq. 1), with a half activation voltage of -3.92 mV and a slope factor of -8.11 mV (Fig. 5B, open circles).

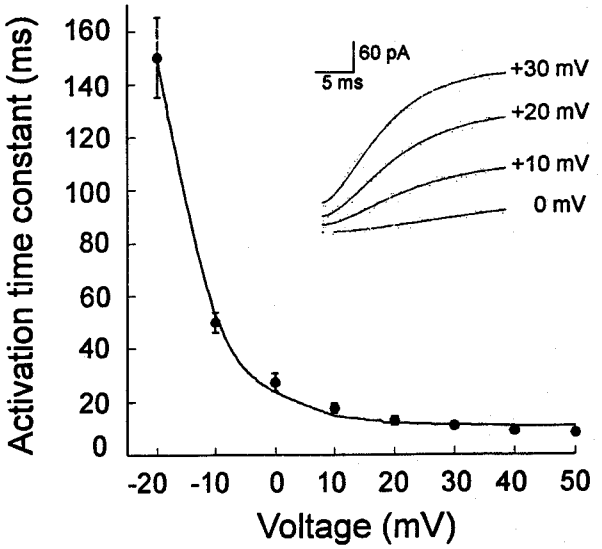

Fig. 4. Activation kinetics of delayed rectifier K current ($I_{K(V)}$).
Current traces (*dots*) from a representative cell were illustrated in *inset*. The continuous lines in *inset* are the best fit to the following equation : $n=n_\infty(1-\exp(-t/\tau)^2)+$ offset, where n is the probability, n_∞ the steady-state value, t the time, τ the voltage-dependent time constant. Individual values of activation time-constants are plotted against membrane potentials (n=12).

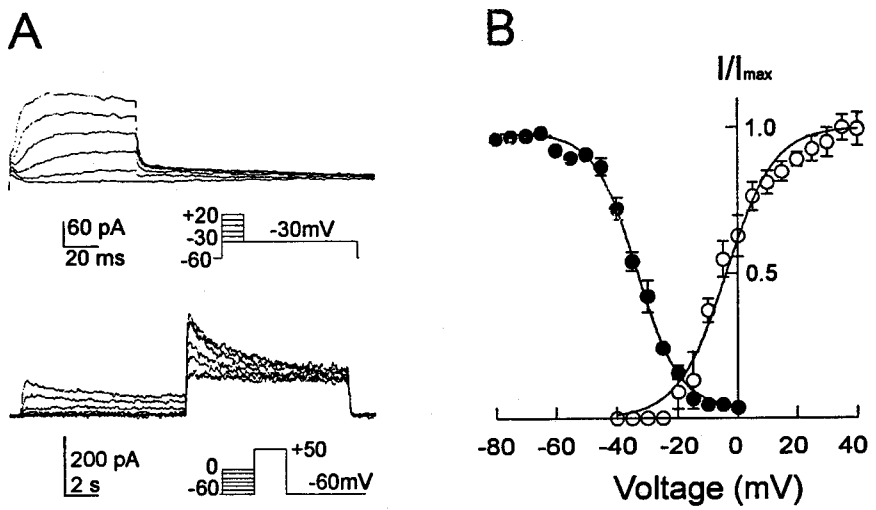

Fig. 5. Steady-state voltage dependence of delayed rectifier K current ($I_{K(V)}$).
The inactivation points (●) represent normalized peak current at $+50$ mV after holding at various potentials. The smooth line through these data points is the best fit to the Boltzmann function (Eq. 1), where $V_{1/2} = -33.48$ mV and $k_I = +6.76$ mV (n=6). The activation points (○) represent normalized instantaneous tail currents, where $V_{1/2} = -3.92$ mV and $k_I = -8.11$ mV (n=6).

$$I/I_{max} = 1/(1+\exp((V_t-V_{1/2})/k_1)) \quad \text{(Eq. 1)}$$

where $V_{1/2}$ is the half activation potential and k_1 is the slope factor of the curve. The curve saturated above +20 mV.

Steady-state inactivation curve was obtained by varying the potential of the conditioning prepulses (10 s) from −80 to 0 mV (5 mV increments) from a holding potential of −60 mV and by measuring the amplitude of the peak current at a constant test potential of +50 mV (10 s) (Fig. 5A, lower panel). During the 10 seconds prepulse the current was not completely inactivated to zero current level and stabilized at its new lower current level even at high membrane potentials. Thus the differences between the peak currents and the stabilized current elicited at test potential were obtained, and then they were normalized to the largest current (I/I_{max}) and plotted against each prepulse potential. Plotted data were well fitted by the Boltzmann equation, with a half inactivation voltage of −33.48 mV and a slope factor of +6.76 mV (Fig. 5B, closed circles).

The inactivation kinetics of $I_{K(V)}$ was analyzed. When depolarizing step pulses of long duration (7 s) were applied, $I_{K(V)}$ was slowly inactivated but hardly

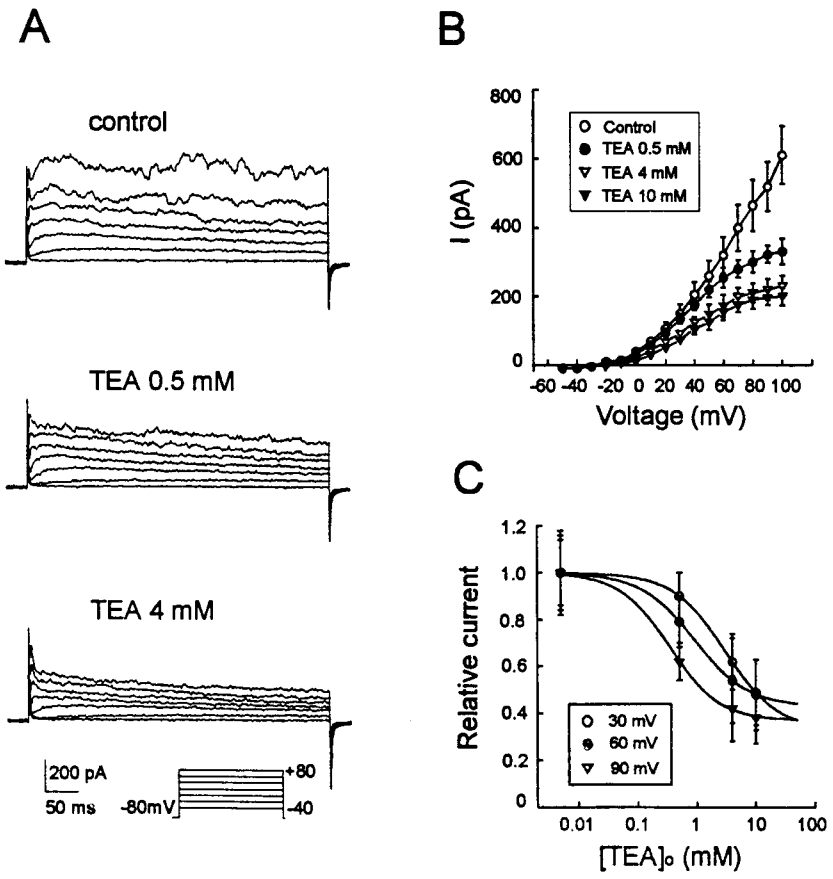

Fig. 6. Effect of TEA on outward currents in Ca^{2+}-buffered cells.
A. Recordings showing the concentration-dependent effect of TEA on the currents. Inhibitory effect of TEA was prominent to the later part of the current, and transient outward current could be hardly affected. B. I-V curves were compared at the end of 400 ms test pulses. Low concentration of TEA (0.5 mM) selectively decreased the noisy current elicited at strong depolarization. TEA (0.5~10 mM) dose-dependently reduced the current amplitude. C. Mean concentration-response curves at different potentials are illustrated. K_d values are 3.03, 0.89 and 0.34 mM at +30, +60 and +90 mV, respectively (n=5).

reach to a zero current level. The inactivation time courses were fitted by a double exponential function. Inactivation time constants of the fast and slow phase were 386±103 ms and 3596±655 ms (n=4) at +50 mV, respectively (data not shown). The kinetics of recovery from inactivation was measured using a double-pulse protocol. Both prepulses and testpulses were stepped to +50 mV for 7 s and 500 ms, respectively, from a holding potential of −60 mV, and the interpulse interval was progressively increased. Recovery was then determined as a function of interpulse interval. Recovery was well described by single exponential process and its time constant was 1.59±0.21 s (n=2) for a holding potential of −60 mV (data not shown).

Effects of representative K-channel blockers, TEA and 4-AP, on $I_{K(V)}$ were tested. TEA (0.5~10 mM) decreased the current amplitude in a dose-dependent manner (Fig. 6). Especially, low concentration of TEA (<0.5 mM) selectively decreased the noisy current elicited during the strong depolarization (> +50 mV). In contrast, currents elicited by moderate depolarization (< +50 mV) were relatively insensitive to this concentration of TEA. Higher doses of TEA (4~10 mM), however, inhibited the current at all membrane potentials (Fig. 6B). To quantitate the

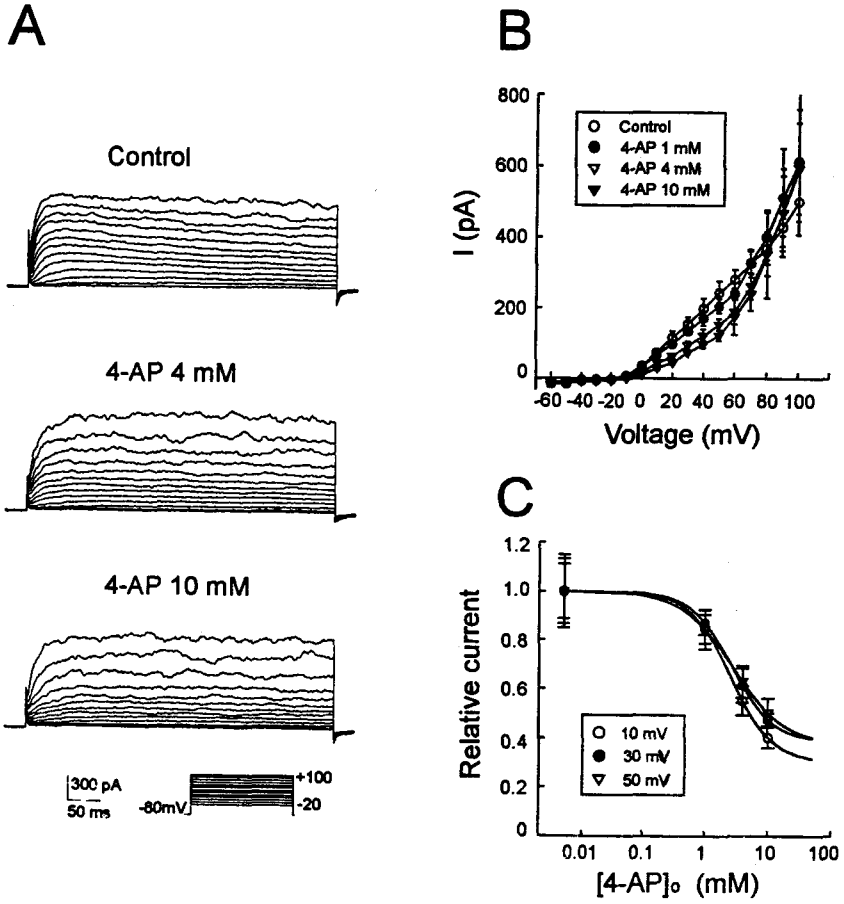

Fig. 7. Effect of 4-AP on delayed rectifier K current ($I_{K(V)}$).
A. Recordings showing the effect of 4-AP on the current. Inhibitory effect of 4-AP was prominent to the early part of the current. Note that the current elicited at strong depolarization was hardly affected by 4-AP even at high doses. B. I-V curves were compared to the peak current at each potential. 4-AP selectively decreased the current elicited at moderate depolarization. C. Mean concentration-response curves at +10, +30 and +50 mV were illustrated. K_d values are 2.57, 2.70 and 2.81 mM at each potential (n=6).

differential sensitivity of these two components to TEA, inhibitory effects were compared at each potential at the end of 400 ms step pulses. Mean concentration-responses curves at +30 mV, +60 mV and +90 mV are illustrated in Fig. 6C, and the data were well described by the following Langmuir equation (Eq. 2). The percentage of current in the presence of TEA as a fraction of its control value at three potentials was given by:

$$I/I_{max} = (1\text{-}C)/(1+(K_d/[TEA]_o)^N)+C \qquad \text{(Eq. 2)}$$

where I/I_{max} is the relative current amplitude, K_d the apparent dissociation constant, N the Hill coefficient, and C the fraction of TEA-insensitive current. The data points were well fit by this equation with a K_d of 3.03, 0.89 and 0.34 mM at +30 mV, +60 mV and +90 mV, respectively. Thus, TEA exhibited different blocking sensitivities at three membrane potentials, with that recorded at strong depolarized potentials being more sensitive to the compound. The Hill coefficients (N) and TEA-insensitive fractions were not significantly different at three test-potentials ; 0.97, 0.95, 1.03 for N, and 0.33, 0.43, 0.37 for C at +30 mV, +60 mV and +90 mV, respectively.

Inhibitory effects of 4-AP on K-channel currents were examined. In contrast to the effect of TEA, at all concentration used (1~10 mM), 4-AP appeared to preferentially inhibit the low-noise current ($I_{K(V)}$), and the noisy current at high voltages ($I_{K(Ca)}$) was hardly affected by 4-AP (Fig. 7A,B). To quantitate the inhibitory effects of 4-AP, they were compared at various potentials. Mean concentration-response curves at +10 mV, +30 mV, and +50 mV are illustrated in Fig. 7C, and the data are well described by the Eq. 2. The data points were well fit by this equation with a K_d of 2.57, 2.70, and 2.81 mM at +10 mV, +30 mV and +50 mV, respectively. Thus 4-AP exhibited similar blocking sensitivities to the currents recorded at these three different membrane potentials, with that recorded at more positive (> +60 mV) potentials being little sensitive to the drug. The Hill coefficients (N) and 4-AP-insensitive fractions were not significantly different at three test potentials ; 1.32, 1.34, 1.10 for N, and 0.30, 0.39, 0.38 for C at +10 mV, +30 mV and +50 mV, respectively.

'A-like' transient outward K current (I_{to})

As mentioned above, in addition to $I_{K(V)}$ another type of voltage-dependent outward current, transient outward K current (I_{to}) could be activated by depolarizing step pulses in Cd^{2+}-free bath solution. I_{to} was rapidly inactivated following activation, and then superimposed on the $I_{K(V)}$ (Figs. 1Bc and 8A). Since I_{to} was not affected by removal of Ca^{2+} from the external medium and internal high Ca^{2+} buffer, it could be considered as a Ca^{2+}-independent current. Fig. 8 shows the insensitivity of I_{to} to TEA that is the most important characteristics of the current. I_{to} was rarely affected by TEA even at high concentrations up to 30 mM while 10 mM TEA markedly inhibited $I_{K(V)}$ (Fig. 8B,C).

In the presence of 10 mM TEA, the I-V relationship of I_{to} is illustrated in Fig. 9A. The first detectable outward current was activated between −10 mV and 0 mV. The current increased in a nearly linear fashion with increasing voltage steps. Since the I_{to} has a fast activation and inactivation kinetics, deactivating tail currents could not be recorded. The steady-state activation curve, therefore, was estimated from the I-V curve (Fig. 8A) using the following equation:

$$I=g_K(V\text{-}E_{rev}) \qquad \text{(Eq. 3)}$$

assuming that the reversal potential of −77 mV. The continuous line was drawn using a Boltzmann equation (Eq. 1) where $V_{1/2}$ and k_1 are +28.6 mV and −17.8 mV (Fig. 9C, open circles). The steady-state inactivation curve was also obtained in the presence of 10 mM TEA, by using a double pulse protocol (Fig. 9B). The peak current amplitude on returning the membrane potential to a constant test pulse of +60 mV after conditioning prepulses ranging from −80 mV to +40 mV with increment of 10 mV (duration 200 ms) was measured. These currents were normalized and plotted against pre-pulse potentials. The curve shows that the current was completely inactivated at a potential positive to −10 mV (Fig. 9C, filled squares). The continuous line through the data was drawn using a Boltzmann equation where $V_{1/2}$ is −17.13 mV and k_1 is +4.71 mV.

The voltage- and time-dependent behaviours of I_{to} were analyzed (Fig. 10). The time-courses of acti-

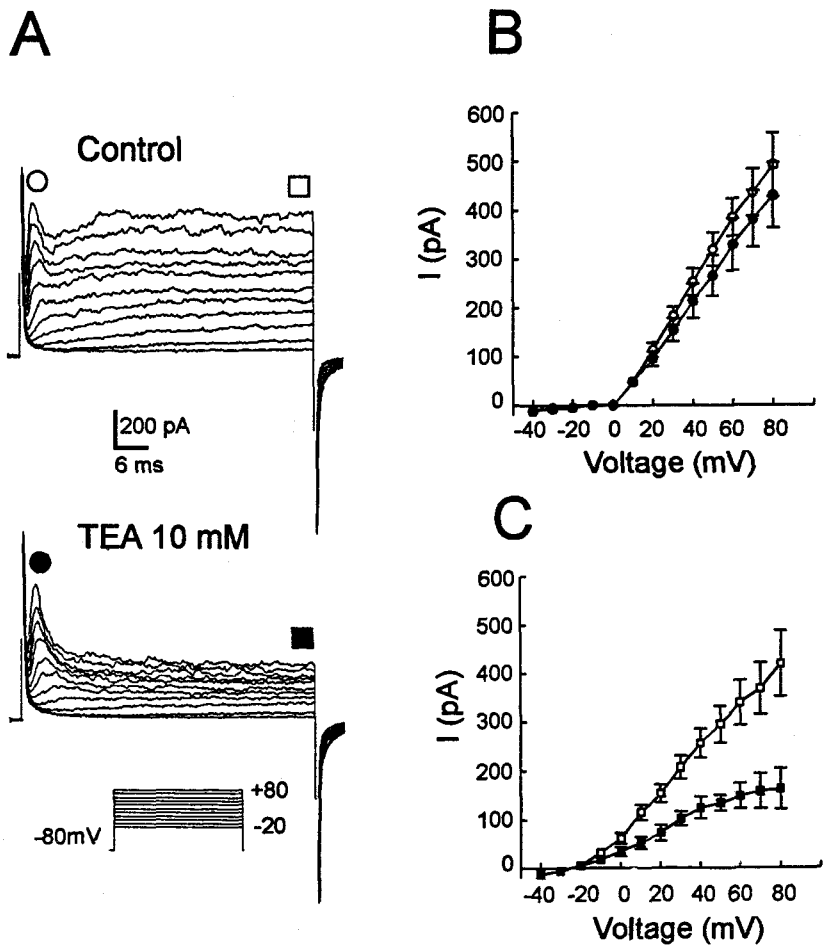

Fig. 8. TEA-insensitive transient outward K current (I_{to}).
A. Recordings showing the effect of TEA (10 mM) on whole-cell K current. Inhibitory effect of TEA was prominent to the later sustained part of the current. Transient outward current was hardly affected by TEA. B. Current-voltage relationship of the transient K current before (○) and after (●) the administration of TEA (10 mM). C. Current-voltage relationship of the sustained part of the current before (□) and after (■) the administration of TEA. TEA reduced the sustained K current ($I_{K(V)}$) about 50 to 70% (n=6).

vation were fitted by n^4 power function. The activation time constants were obtained at the voltage from +20 to +80 mV. Activation time courses of I_{to} were about 10 times faster than that of $I_{K(V)}$ (compare Figs. 4 and 10). The inactivation kinetics of I_{to} was analyzed after treatment of 10 mM TEA. The inactivation time courses were fitted to a single exponential function. The time constants were 4.04± 0.11 ms (n=6) at +50 mV (data not shown). The kinetics of recovery from inactivation was measured using a double-pulse protocol: both pre- and test-pulses were stepped to +80 mV for 45 ms from a holding potential of −60 mV and the interpulse interval between pre- and test-pulse was progressively increased. Recovery was then determined as a function of interpulse interval. Recovery was relatively rapid and well described by a single exponential process. Recovery time constant was 58.62±21.38 ms (n=2) at −60 mV (data not shown).

4-AP dose-dependently inhibited the I_{to}. Since the inhibitory effect of 4-AP was not selective to I_{to}, it is difficult to separate this current from $I_{K(V)}$ only by

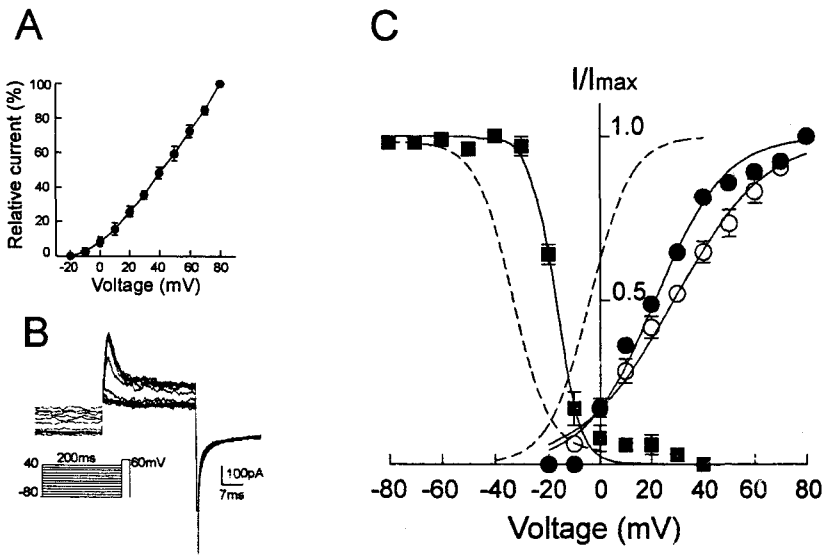

Fig. 9. Steady-state voltage dependence of transient outward K current (I_{to}).
A. I-V curve for TEA (10 mM)-insensitive I_{to}. B. The steady-state inactivation kinetics was obtained by using a conventional double pulse protocol (see *text*). Currents elicited by conditioning prepulses were trimmed. C. The inactivation points (■) were fitted by the Boltzmann equation (Eq. 1), where $V_{1/2}=-17.13$ mV and $k_1=+4.71$ mV (n=4). The activation points (○) were obtained from the I-V curve of TEA-insensitive transient K current (A) by using Eq. 3, where $V_{1/2}=+28.6$ mV and $k_1=-17.8$ mV (n=9) and from Cd^{2+}-sensitive currents (see *text*; ●), where $V_{1/2}=+21.44$ mV and $k_1=-13.4$ mV. Dotted lines that represent the steady-state voltage dependence of $I_{K(V)}$ are shown for comparison.

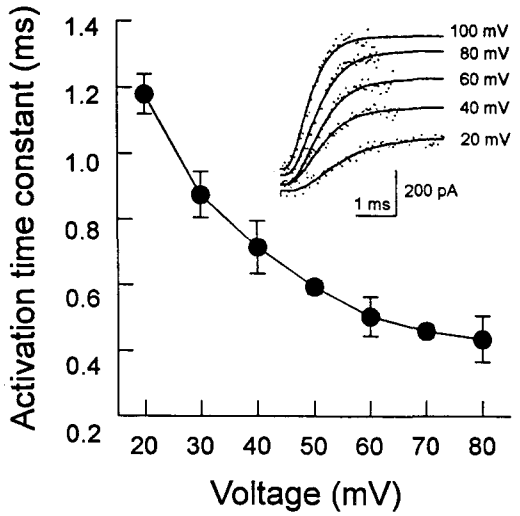

Fig. 10. Activation kinetics of TEA-insensitive transient outward K current (I_{to}).
In the presence of 10 mM TEA, current traces (*dots*) from a representative cell were illustrated in *inset*. The continuous lines in *inset* are the best fit to the following equation : $n=n_\infty(1-\exp(-t/\tau))^4+\text{offset}$. Individual values of activation time-constants are plotted against membrane potentials (n=7).

treatment of 4-AP (Fig. 11; see also Beech & Bolton (1989b)). K_d values were 0.91, 1.19, 1.21, 1.17, 1.15 and 1.24 mM at +30, +40, +50, +60, +70 and +80 mV, respectively (n=2~5). Hill coefficient (N) were 0.65, 0.62, 0.74, 0.72, 0.87 and 0.81 at each potentials.

Amplitude of I_{to} was markedly attenuated by external Cd^{2+} in the presence of external Ca^{2+} (Fig. 12), but Cd^{2+} could not completely block the current at voltage range more positive than +70 mV. Because low concentration of Cd^{2+} (0.5 mM) shifted the I-V relationship to the positive direction without change of maximum conductance (Fig. 12B), effect of Cd^{2+} might not be a direct block of the channel. Cd^{2+}, in contrast, had no appreciable effects on the sustained component of $I_{K(V)}$ (Fig. 12C). Cd^{2+}-sensitive component of the current was calculated by mathematical subtraction from the current recorded in the absence of Cd^{2+} (data not shown). From the I-V curve of the subtracted current, steady-state activation curve could be drawn by using Eq. 3 where $V_{1/2}$ and k_l are +21.4 mV and −13.4 mV, respectively (Fig. 9C, closed cir-

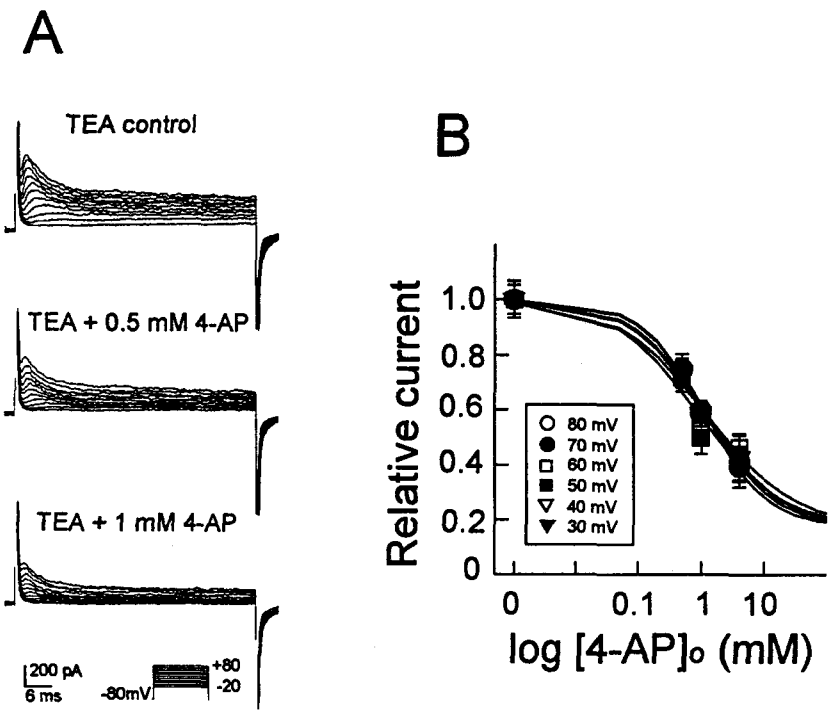

Fig. 11. Effect of 4-AP on transient outward K current (I_{to}).
A. Recordings showing the inhibitory effect of 4-AP on I_{to}. B. Mean concentration-response curves at +30, +40, +50, +60, +70 and +80 mV were illustrated. K_d values are 0.91, 1.19, 1.21, 1.17, 1.15, 1.24 mM, and Hill coefficient (N) are 0.65, 0.62, 0.74, 0.72, 0.87, 0.81 at +30, +40, +50, +60, +70 and +80 mV, respectively (n=2~5).

cles).

DISCUSSION

The results presented in this paper suggest that at least three types of K currents : (a) delayed rectifier K current ($I_{K(V)}$), (b) TEA-insensitive 'A-like' transient outward K current (I_{to}) and (c) large-conductance Ca^{2+}-activated K current ($I_{K(Ca)}$) are present in SMCs of rabbit basilar artery. Interestingly, I_{to} is activated and inactivated at more depolarized potential range than $I_{K(V)}$.

Cell-length and membrane capacitance measured in this experiments were similar to that of the rat basilar (Stockbridge et al, 1992), rabbit basilar (Worley et al, 1991), guinea-pig basilar (West et al, 1992) and cat cerebral (Bonnet et al, 1991) artery. Many investigators reported that the membrane potentials obtained from enzymatically dispersed vascular SMCs were range from −25 to −65 mV and exhibited marked variation between vascular beds and species : −32 mV for rabbit coronary artery (Matsuda et al, 1990), −48 mV for rabbit portal vein (Hume & Leblanc, 1989), −52 mV for canine renal artery (Gebland & Hume, 1992), −36 mV for human cystic artery (Akbarali et al, 1992). Cerebral or basilar arteries penetrated with intracellular microelectrode techniques in vitro have a membarne potentials between −49 and −70 mV : −70 mV for cat middle cerebral arteries (Harder, 1980), -57.4 (before chemical denervation) or −56 mV (after chemical denervation) (Harder, 1981), −49.4 mV and −51.7 mV for canine basilar and middle cerebral arteries, respectively (Fujiwara et al, 1982). In our experiments, membrane potentials of rabbit basilar arterial SMCs were −29±1.2 mV. These values are within the range obtained from enzymatically dispersed vascular SMCs, but somewhat lower than that of the intact tissue strips. Although the discrepancy is not fully understood in this ex-

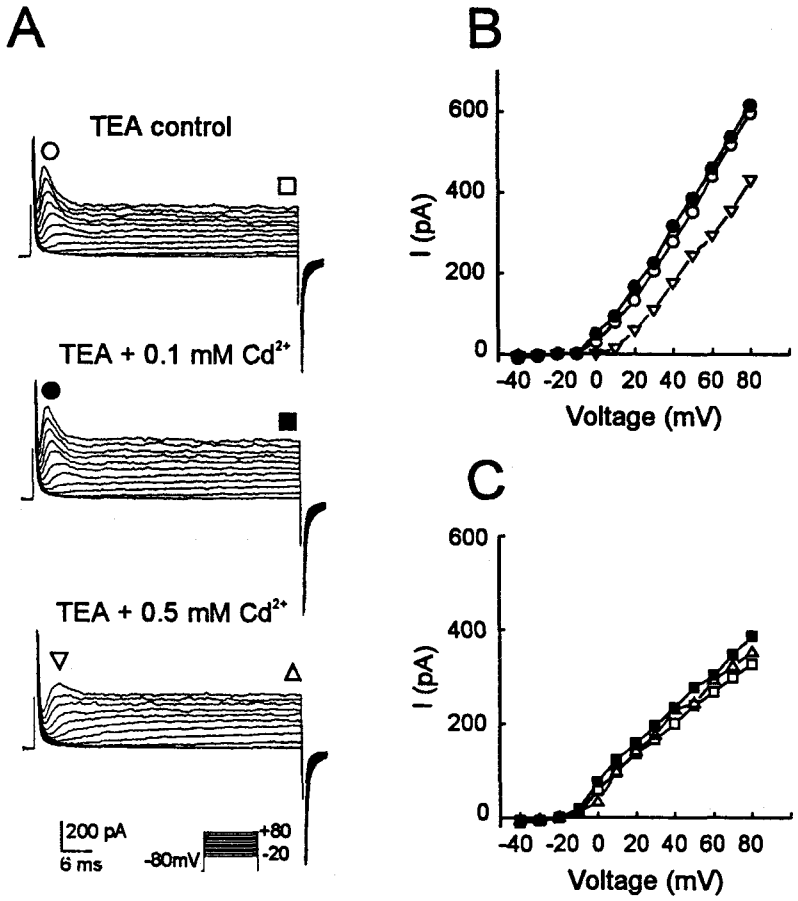

Fig. 12. Parallel shift of the current-voltage relationship of transient outward K current by Cd^{2+}.

A. Recordings showing the selective suppression of transient K current by external Cd^{2+}. Bath solution contained 10 mM TEA. B. Application of Cd^{2+} shifted the I-V relationship of I_{to} to the positive direction (control; ○, 0.1 mM; ●, 0.5 mM; ▽). C. I-V relationship of $I_{K(V)}$ was not changed by Cd^{2+} (control; □, 0.1 mM; ■, 0.5 mM; △).

periment, possible explanations are (1) alteration in resting conductance associated with cell disaggregation (Fleischmann et al, 1993) and (2) high Ca^{2+} buffer in cytosol (10 mM EGTA in this study). If an intracellular Ca^{2+}-activated channels such as BK-channel are active in the resting membrane potential, strong Ca^{2+} buffers in whole-cell patch-clamp mode make the potential to depolarize.

Experiments using internal solutions with high Ca-buffering capacity have demonstrated low-noise $I_{K(V)}$, which are insensitive to intracellular Ca^{2+}, relatively insensitive to TEA, but blocked by 4-AP. Hodgkin and Huxley (1952) first proposed a gating model for $I_{K(V)}$ in squid giant axons. Gating formalisms of this current have proven useful for describing the kinetic behavior of $I_{K(V)}$ in many neuronal and cardiac cells, although the number of gating particles necessary to simulate the observed kinetic behavior has varied tremendously from 1 to 25 (Basler et al, 1990). In the present study, the time course of activation was well fitted by n^2 kinetics over a range of potentials (0 to +50 mV). In other SMCs, it has been reported that activation kinetics of $I_{K(V)}$ can be fitted by n^4 (Beech & Bolton, 1989a; Fleischmann et al, 1993) or n^2 (Volk et al, 1991) processes. In rat basilar artery, the activation kinetics of $I_{K(V)}$ was fitted by a power of 3.6 (Stockbridge et al, 1992). It is known that the $I_{K(V)}$ inactivate to

non-zero current with complex kinetics generally well fitted by a bi-exponential process in various SMCs (rabbit portal vein (Beech & Bolton, 1989a), canine and porcine airway SMCs (Boyle et al, 1992)). These inactivation kinetics is consistent with present experiments. The voltage dependence of activation and inactivation and the availability of the current at each potentials resemble more closely classical $I_{K(V)}$ (Rudy, 1988). The values for the half inactivation potentials are more similar to that observed in the rabbit portal vein ($V_{1/2}$ = −30.2 mV) (Beech & Bolton, 1989a) than rabbit coronary artery ($V_{1/2}$= −24.2 mV) (Volk et al, 1991). Both 4-AP and TEA reduced the $I_{K(V)}$ dose-dependently and the effect of 4-AP was more potent than that of the TEA. Neither 4-AP nor TEA, however, completely inhibited the current even at high concentration (10 mM). These pharmacological characteristics are similar to the 4-AP-sensitive current identified in rabbit portal vein (Beech & Bolton, 1989a), coronary artery (Volk et al, 1991) and cat cerebral arteries (Bonnet et al, 1991).

The properties of I_{to}, in the present study, including rapid activation and inactivation, 4-AP sensitivity and TEA-insensitivity, and steady-state activation and inactivation kinetics suggested that it falls into the category of 'A-like' K current (Rudy, 1988). The rapidly activating and inactivating transient K currents have also been observed recently in other SMCs (Beech & Bolton, 1989b; Lang, 1989; Imaizuni et al, 1990). These have tended to activate and inactivate in a more negative potential range than the I_{to} described in this report, and have therefore been categorized as 'true' A-currents. For these 'true' A-currents, its negative voltage range of inactivation as well as their selective blockade by 4-AP have facilitated the separation of A-currents from other voltage-gated K currents such as $I_{K(V)}$. In the present study on rabbit basilar artery, however, both the relatively positive voltage range over which I_{to} inactivated, and the apparent non-selective action of 4-AP on I_{to} and $I_{K(V)}$, made a separation based on these approaches alone less convincing. In rabbit basilar SMCs, the voltage range of inactivation for I_{to} was significantly more positive than that for $I_{K(V)}$. In addition, I_{to} recovered more quickly from inactivation than did $I_{K(V)}$. Cd^{2+} could exert significant effects on the amplitude and availability of I_{to} but not $I_{K(V)}$. These data suggest strongly that the transient and sustained components of the TEA-insensitive current represent current flowing through at least two populations of K channels. In cultured rat dorsal root ganglion cells, A-current was significantly depressed by various divalent cations (Cd^{2+}, Mn^{2+}, Co^{2+}, Ca^{2+}), and this depressant effects resulted from a depolarizing shift of the steady-state activation and inactivation curves (Mayer & Sugiyama, 1988). In contrast to its effect on A-current, divalent cations did not cause any appreciable change for $I_{K(V)}$. They suggested that this modulatory effect of divalent cations on the gating of A-current appears to reflect binding to a specific saturable site, either the A-channel protein itself or phospholipids electrically close to the gating apparatus. Most of the transient outward currents which have recently been described in SMCs are sensitive to extracellular Ca^{2+} and Cd^{2+}. Increasing extracellular Ca^{2+} concentration greatly reduced the amplitude of A-current in rabbit portal vein (Beech & Bolton, 1989b) and human mesenteric arterial cells (Smirnov & Aaronson, 1992). Suppression of the transient outward K current by submillimolar concentrations of Cd^{2+} was also reported in other SMCs (guinea-pig ureter (Imaizuni et al, 1990), rabbit pulmonary artery (Clapp & Gurney, 1991)). A unifying hypothesis to explain the suppressive effect of divalent cations described in their reports is analogous to that described by Mayer and Sugiyama (1988).

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