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MODULATION OF K^+ AND Ca^{2+} CHANNELS BY HISTAMINE H_1-RECEPTOR STIMULATION IN RABBIT CORONARY ARTERY CELLS

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SUMMARY

1. The modulation of whole-cell K^+ and Ca^{2+} currents by stimulation of histamine H_1-receptors in freshly isolated single smooth muscle cells from the rabbit coronary artery was characterized using the patch-clamp technique at 35 °C. Single-channel K^+ currents were also analysed using the cell-attached patch configuration.

2. The histamine H_1-receptor agonist, 2-(2-aminoethyl)pyridine (AEP) (0·1 mM), increased the amplitude of voltage-activated inward Ba^{2+} currents, recorded using the perforated-patch recording technique, which could be completely blocked by the dihydropyridine antagonist, nicardipine (1 μM).

3. Whole-cell outward K^+ currents in rabbit coronary artery cells could be classified into at least two components: (a) a slowly inactivating, 4-aminopyridine (4-AP)-sensitive low-noise current, and (b) a non-inactivating, tetraethylammonium (TEA)-sensitive high-noise current.

4. AEP (0·1 mM) caused changes in whole-cell outward K^+ currents which depended upon membrane voltage. Specifically: (a) AEP enhanced the amplitude of outward currents at voltages between −30 and 0 mV, and (b) AEP decreased the outward currents at more positive potentials.

5. The removal of extracellular Ca^{2+} caused little inhibition of the effects of AEP on K^+ currents, whereas the depletion of intracellular Ca^{2+} stores by pretreatment with ryanodine and caffeine prevented the effects of AEP on K^+ channels. Moreover, acute exposure to ryanodine (10 μM) or thapsigargin (1 μM), a Ca^{2+}-ATPase inhibitor, caused voltage-dependent changes in the outward currents similar to those observed with AEP. These results suggest that the voltage-dependent effects of AEP on K^+ currents are mainly mediated by release of Ca^{2+} from intracellular stores.

6. The dual stimulatory and inhibitory effect of AEP on whole-cell K^+ currents was shown to be due to a differential effect on two distinct types of K^+ channels. The stimulatory effect observed over the voltage range −30 to 0 mV was prevented by pretreatment of cells with low concentrations of TEA (1 mM), whereas the inhibitory effect observed at positive potentials was prevented by pretreatment of cells with 4-AP (3 mM).

7. Single-channel recordings revealed two types of unitary K^+ currents with conductances of 225 and 70 pS in the cell-attached configuration with symmetrical

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K^+ solutions (150 mM K^+ in pipette–150 mM K^+ in bath). Bath application of AEP (0·1 mM) caused a marked increase in the open probability of the large conductance channels.

8. In ring segments of rabbit coronary artery (endothelium denuded), AEP (0·1 mM) caused a sustained contraction. This contraction was significantly enhanced in the presence of charybdotoxin (100 nM).

9. These results suggest that the histamine H_1-receptor agonist AEP significantly increases large conductance Ca^{2+}-activated K^+ channel activity in the physiological range of the membrane potential in rabbit coronary artery cells by increasing the intracellular Ca^{2+} concentration via releasing Ca^{2+} from intracellular stores. This action of AEP on K^+ channels would tend to limit the degree of contraction generated with AEP.

INTRODUCTION

The regulation of vascular tone is dependent on the binding of hormones and neurotransmitters to their specific receptors. Ion channels appear to play an essential role in this regulation. Activation of voltage-dependent Ca^{2+} channels causes an increase in Ca^{2+} influx, which induces cell contraction. Vasoconstrictors, such as noradrenaline (Benham & Tsien, 1988; Nelson, Standen, Brayden & Worley, 1988) and angiotensin II (Bkaily *et al.* 1988), are shown to enhance the activity of voltage-dependent Ca^{2+} channels. In contrast, opening of K^+ channels leads to cellular hyperpolarization which, in turn, may deactivate voltage-dependent Ca^{2+} channels and cause relaxation of the muscle. A number of studies have reported the characteristics of K^+ channels in vascular smooth muscle cells and suggested the contribution of K^+ channels to vascular tone (for review see Kajioka, Nakashima, Kitamura & Kuriyama, 1991).

Histamine is one of the most potent vasoconstrictors of the human coronary artery, the effect of which is mediated by histamine H_1-receptors (Toda, 1983), and is regarded as one candidate for the genesis of coronary vasospasm. In previous studies of the rabbit coronary artery it has been shown that the histamine H_1-receptor agonist, 2-(2-aminoethyl)pyridine (AEP), enhances $^{45}Ca^{2+}$ influx and increases $^{45}Ca^{2+}$ efflux into Ca^{2+}-free solution. The enhanced $^{45}Ca^{2+}$ efflux was attributed to the release of Ca^{2+} from intracellular stores (Khoyi, Bowen & Keef, 1991). Thus, AEP apparently increases $[Ca^{2+}]_i$ via two distinct pathways leading ultimately to contraction of the muscle. However, a rise in $[Ca^{2+}]_i$ would also be predicted to modify the activity of Ca^{2+}-sensitive K^+ channels. The goal of the present study was to characterize the properties of K^+ channels in rabbit coronary artery cells and to investigate the extent to which histamine H_1-receptor activation modifies the properties of K^+ and Ca^{2+} currents in these cells. A preliminary report of these findings has appeared in abstract form (Ishikawa, Eckman, Hume & Keef, 1992).

METHODS

Cell isolation

Single vascular smooth muscle cells were isolated from rabbit coronary artery using a dispersal procedure with collagenase. Male albino rabbits (1·5–2·0 kg) were anaesthetized with an intravenous injection of pentobarbitone (30 mg/kg). The hearts were removed and the coronary arteries were

dissected and cleared of adhering tissue and fat in a Krebs solution (mM: 118·5 NaCl, 4·2 KCl, 1·8 $CaCl_2$, 1·2 $MgCl_2$, 23·8 $NaHCO_3$, 1·2 KH_2PO_4, 11 glucose, aerated with 95% O_2–5% CO_2). The vessels were cut into small segments (about 2 mm in length) and placed in a Ca^{2+}-free Hanks' solution (mM: 125 NaCl, 5·36 KCl, 15·5 $NaHCO_3$, 0·336 Na_2HPO_4, 0·44 KH_2PO_4, 10 glucose, 2·9 sucrose, aerated with 95% O_2–5% CO_2) for 30 min at room temperature. The segments were then placed in the Ca^{2+}-free Hanks' solution containing 1 mg/ml collagenase (cls2, Worthington Biochemical Co., Freehold, NJ, USA), 2 mg/ml bovine serum albumin (BSA), 2 mg/ml trypsin inhibitor (type II-S, Sigma Chemical Co., St Louis, MO, USA), 0·1 mg/ml ATP-Na_2, 0·1 mg/ml protease (type XIV, Sigma) and incubated for about 30 min with gentle agitation at 35 °C. After completion of the digestion, single cells were dispersed by gentle agitation in the Ca^{2+}-free Hanks' solution. The cells obtained were stored in Hanks' solution (Ca^{2+}, 0·1 mM) containing 1 mg/ml BSA and 0·5 mg/ml trypsin inhibitor at 4 °C.

Electrophysiological studies

The dispersed cells were placed in a small chamber (300 μl) on the stage of an inverted microscope. The bath solution was superfused through the chamber by gravity at a rate of about 1 ml/min. Recordings of whole-cell and single-channel currents were performed using conventional patch-clamp techniques (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). The voltage-clamp protocols were computer driven using pCLAMP software (Axon Instruments, Foster City, CA, USA) and delivered through the headstage of a patch-clamp amplifier (Axopatch 1-D, Axon Instruments).

Experiments were carried out on relaxed spindle-shaped cells. To measure whole-cell outward currents, standard tight-seal whole-cell recordings using suction electrodes were used. After the patch membrane was disrupted, about 5 min was allowed to dialyse the cell. Most of the experiments were performed within 15 min of gaining access, during which time macroscopic currents remained stable. Longer protocol usually resulted in significant 'run-down' of macroscopic current. In some experiments intracellular Ca^{2+} stores of the cells were depleted by treatment with ryanodine prior to patch-clamping the cells. In this way current 'run-down' was minimized. The ryanodine treatment consisted of the following steps: (1) 10 min superfusion with Ca^{2+}-free physiological salt solution (PSS) containing 10 μM ryanodine (solution R), (2) 3 min superfusion with solution R plus 5 mM caffeine, (3) 30 min superfusion with solution R. Access to cells was then gained and currents were recorded in PSS containing 10 μM ryanodine. For measurement of whole-cell inward currents, the perforated-patch technique was used to reduce 'run-down' of L-type Ca^{2+} channel currents. In this experiment 240 μg/ml of amphotericin B was added to the pipette solutions (Rae, Cooper, Gates & Watsky, 1991) and 10–20 min was required in order to achieve an access resistance of 10–20 MΩ. The junction potential between pipette solution and intracellular solution was not compensated in the perforated whole-cell configuration. The whole-cell currents recorded were low-pass filtered at 5 kHz (−3 dB) and leak subtraction was not performed. The bath temperature was maintained at 35 °C using a temperature controller (TC-1, NBD, Stony Brook, NY, USA).

For single-channel measurements in the cell-attached configuration, membrane potential was held at potentials ranging between −30 and +60 mV for 1 min. Single-channel recordings were low-pass filtered at 2 kHz (−3 dB). The experiments were performed at room temperature.

Solutions

The following solutions were used for recording whole-cell outward currents. PSS in the bath had the following composition (mM): 130 NaCl, 10 $NaHCO_3$, 4·2 KCl, 1·2 KH_2PO_4, 0·5 $MgCl_2$, 1·0 $CaCl_2$, 5·5 glucose, and 10 Hepes and was titrated to pH 7·35 with NaOH. High-K^+ solution in the pipette had the following composition (mM): 120 potassium gluconate, 20 KCl, 5 ATP-K_2, 2 phosphocreatinine, 0·5 $MgCl_2$, 0·1 EGTA, and 10 Hepes and was titrated to pH 7·2 with KOH. In some whole-cell current-measuring experiments, cytochalasin B (30 μM) was included in the pipette solution to prevent cell contractions (Dresel & Knickle, 1987; Donaldson & Hill, 1985). For Ca^{2+}-free solutions the $CaCl_2$ in PSS was replaced by an equimolar amount of $MgCl_2$.

In recording whole-cell inward currents using the perforated-patch technique, the bath solution was of the following composition (mM): 70 NaCl, 10 CsCl, 0·5 $MgCl_2$, 10 $BaCl_2$, 50 TEA-Cl, 5·5 glucose, and 10 Hepes and was titrated to pH 7·35 with NaOH, High-Cs^+ pipette solution contained (mM): 120 caesium aspartate, 22·6 CsCl, 1·2 $MgCl_2$, 1·0 EGTA, 5·5 glucose, and 5·0 Hepes and was titrated to pH 7·2 with CsOH.

In the cell-attached configuration the composition of both pipette and bath solutions was the following (mM): 140 potassium gluconate, 10 KCl, 0·5 $MgCl_2$, 0·1 EGTA, 5·5 glucose, and 10 Hepes (pH to 7·2 with KOH).

Contraction studies

The left ventricular coronary artery was dissected and cleared of adhering tissue and fat in the Krebs solution. The endothelium was removed by gentle scrubbing of the lumen with a stainless-steel rod. The successful removal of the endothelium was judged by a lack of relaxant response to application of acetylcholine (ACh, 1 μM). The preparation was mounted vertically in a tissue bath containing Krebs solution maintained at 37 °C and aerated with 95% O_2–5% CO_2. The upper triangular wire was attached to a strain gauge (Gould, Oxnard, CA, USA) and the lower to a stable mount. A resting force of 0·5 g was applied to 3 mm long segments of vessel. After equilibration for 1 h, the isometric contraction was measured.

In preliminary studies, the threshold concentration of AEP for eliciting contraction was 3 μM and the maximal response was attained at 0·3 mM. AEP has previously been shown to be a specific agonist of H_1-receptors in other tissues (Durant, Ganellin & Parsons, 1975; Donaldson & Hill, 1985). This conclusion was confirmed in the rabbit coronary artery by determining that AEP (0·3 mM)-induced contraction was completely blocked by the H_1-receptor antagonist chlorpheniramine (10 μM) and that no relaxation was elicited with AEP in the presence of chlorpheniramine in tissues precontracted with ACh (10 μM). For the present studies we used AEP at a concentration of 0·1 mM, which caused vasocontraction corresponding to 80–90% of its maximal effect.

Drugs

2-(2-Aminoethyl)pyridine (AEP) (Aldrich Chemical Co., Milwaukee, WI, USA); charybdotoxin (CTX) (Peninsular Laboratories, Belmont, CA, USA); amphotericin B, chlorpheniramine maleate, 4-aminopyridine (4-AP), tetraethylammonium chloride (TEA), and thapsigargin (Sigma), and ryanodine (AgriSystems International, Wind Gap, PA, USA) were used.

Statistics

Statistical analyses were performed with the one-way analysis of variance (ANOVA) followed by the Bonferroni method or Student's t test for paired values. Changes were considered significant at $P < 0.05$. Data are expressed as means ± S.E.M.

RESULTS

Effects of AEP on whole-cell Ca^{2+} currents

Previous studies in the rabbit coronary artery have shown that AEP increases the influx of $^{45}Ca^{2+}$ into cells and that the contractile response to AEP is significantly reduced in Ca^{2+}-free solution or in the presence of nifedipine (Khoyi *et al.* 1991). These data suggest that AEP either directly and/or indirectly enhances the entry of Ca^{2+} through L-type Ca^{2+} channels. In the present study experiments were undertaken to characterize Ca^{2+} currents in isolated rabbit coronary artery cells and determine whether these currents are significantly modified by H_1-receptor stimulation.

Inward currents were obtained using amphotericin B-perforated whole-cell configuration (Rae *et al.* 1991). The voltage-dependent Ca^{2+} currents measured with this technique did not exhibit the 'run-down' phenomenon for up to 1 h. Under these conditions, an inward current was observed following 200-ms depolarizing voltage steps from a holding potential of −60 mV, with a peak inward current response of 125 ± 7 pA ($n = 5$) at a membrane potential of 0 mV (Fig. 1) using 10 mM Ba^{2+} as the charge carrier. This inward current was identified as a current through L-type Ca^{2+}

channels since it was blocked with the dihydropyridine Ca^{2+} channel blocker, nicardipine (1 μM, data not shown, $n = 3$). As illustrated in Fig. 1, AEP (0·1 mM) increased the amplitude of inward currents, and in the continued presence of AEP, 1 μM nicardipine completely blocked all inward current (Fig. 1*A*), suggesting that

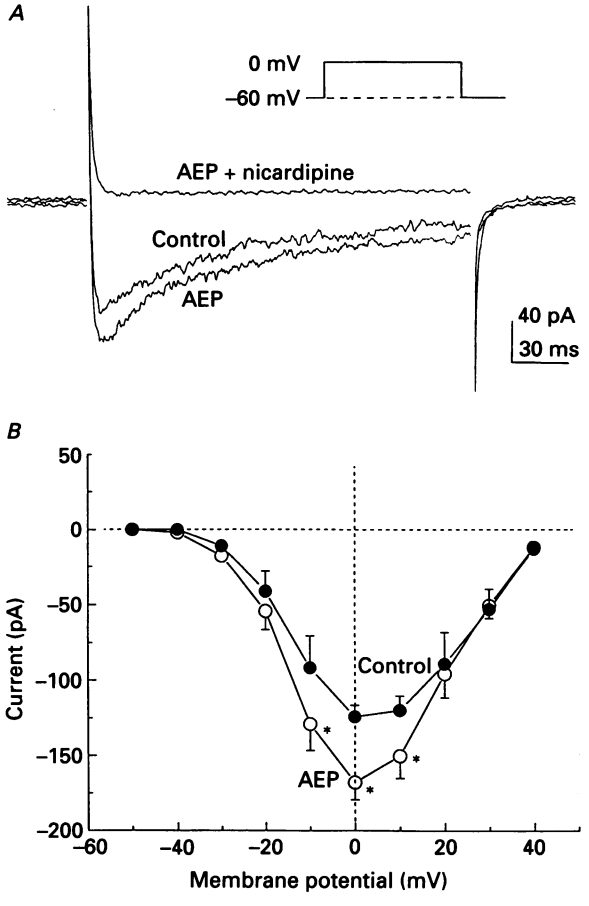

Fig. 1. Effect of 2-(2-aminoethyl)pyridine (AEP) on depolarization-activated Ca^{2+} currents in the perforated whole-cell configuration. *A*, current response to a voltage step to 0 mV (200 ms duration) from a holding potential of −60 mV was recorded under the control condition, in the presence of AEP (0·1 mM), or in the presence of AEP (0·1 mM) and nicardipine (1 μM). *B*, the current–voltage relationship for the peak inward current under control conditions and in the presence of AEP (0·1 mM) was obtained with voltage steps from a holding potential of −60 mV to potentials of −50 to +40 mV applied in 10 mV increments. Shown are mean values ± S.E.M. from five cells. * Significant difference from the current before exposure to AEP at each voltage (Student's *t* test for paired values). Ba^{2+} (10 mM) was used as a charge carrier through the Ca^{2+} channel.

the actions of AEP were mediated by activation of L-type Ca^{2+} channels. Although AEP enhanced the amplitude of Ba^{2+} currents it did not shift the current–voltage relationship along the voltage axis (Fig. 1*B*).

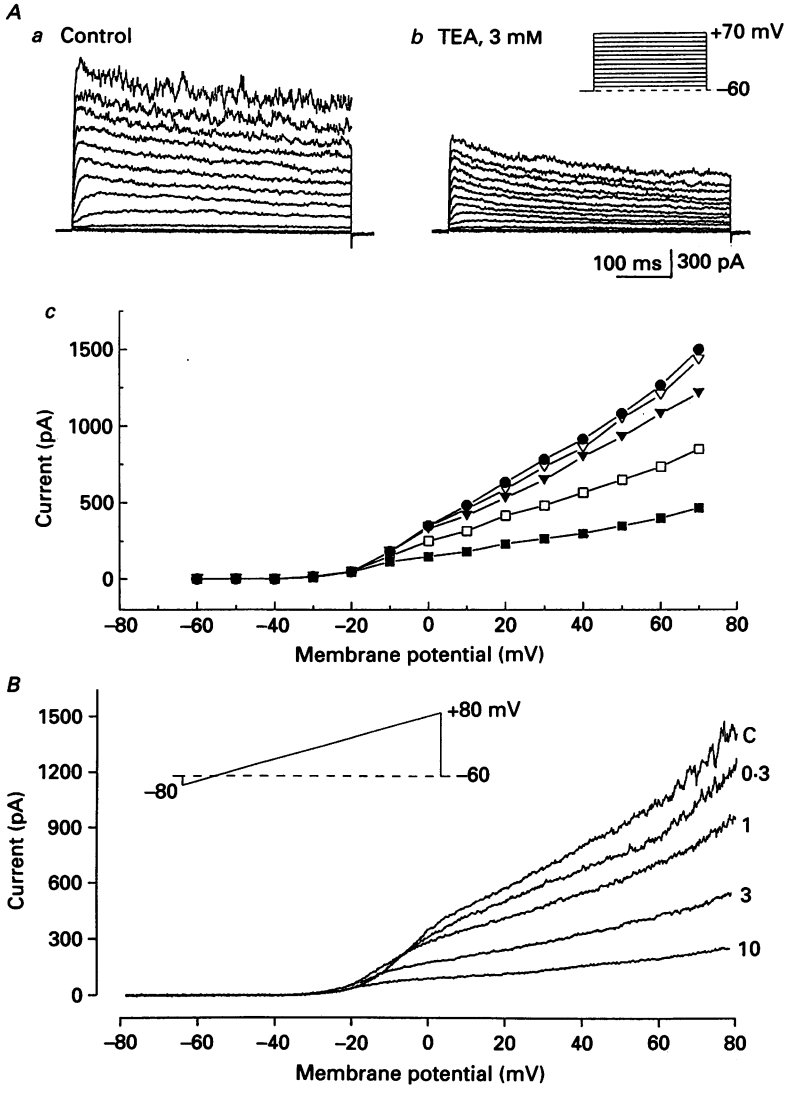

Fig. 2. Effect of tetraethylammonium (TEA) on whole-cell outward currents. *A*, each family of currents was obtained with voltage steps from −50 to +70 mV for 500 ms at a holding potential of −60 mV before (*a*) and after (*b*) exposure to TEA, 3 mM. *c*, peak outward currents were plotted against test potentials before (●) and after exposure to various concentrations of TEA (▽, 0·3; ▼, 1; □, 3; ■, 10 mM). *B*, current–voltage relationship obtained with ramp pulses from −80 to +80 mV for 900 ms at a holding potential of −60 mV before (C) and after exposure to various concentrations of TEA (0·3, 1, 3, and 10 mM). Each trace in *B*, shows the average of five currents. All traces in *A* and *B* were obtained from the same cell.

Characterization of whole-cell K^+ currents

In cells dialysed with high-K^+ pipette solution and bathed in PSS, outward currents were elicited with 500-ms depolarizing voltage steps applied from a holding potential of −60 mV in the standard whole-cell configuration (Figs 2*A* and 3*A*). Detectable outward currents were observed at potentials positive to approximately

−30 mV. With steps to more positive potentials the currents activated faster and exhibited a small degree of inactivation. Steps to very positive potentials (i.e. > +40 mV) produced large noisy currents. Studies of other smooth muscles have shown that the outward currents induced by depolarization are usually preceded by

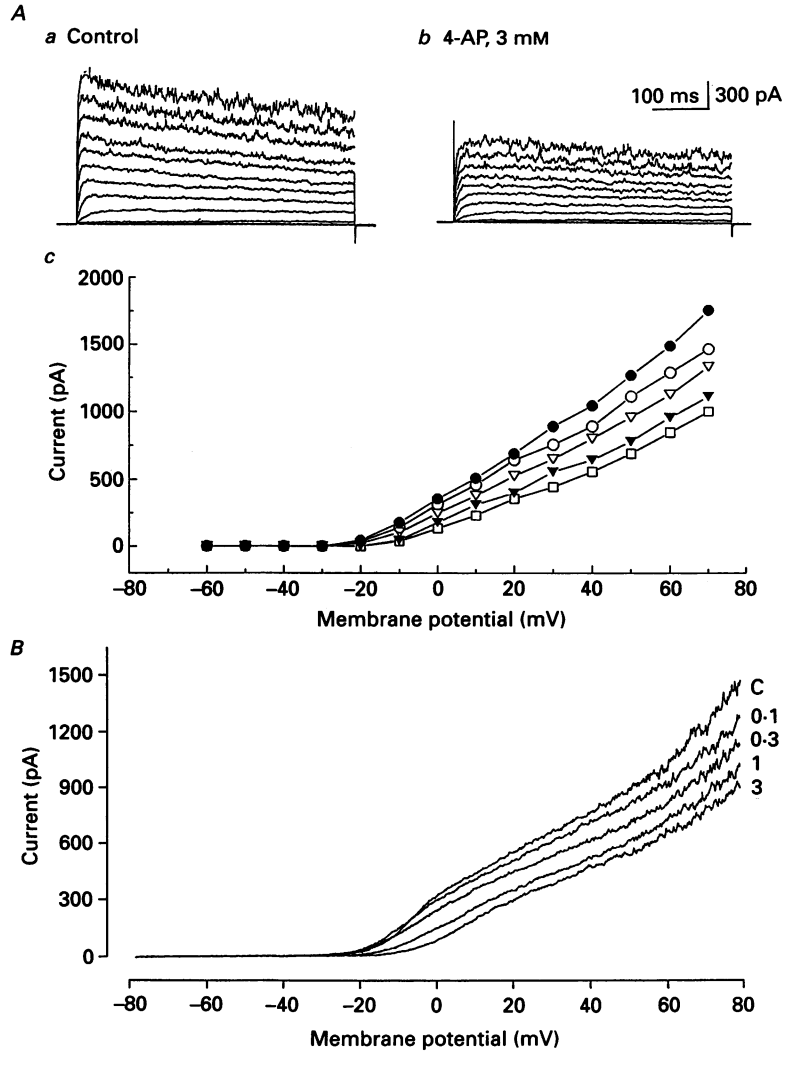

Fig. 3. Effect of 4-aminopyridine (4-AP) on whole-cell outward currents. *A*, each family of currents was obtained with voltage steps as in Fig. 2 before (*a*) and after (*b*) exposure to 4-AP, 3 mM. *c*, peak outward currents were plotted against test potentials before (●) and after exposure to various concentrations of 4-AP (○, 0·1; ▽, 0·3; ▼, 1; □, 3 mM). *B*, current–voltage relationship obtained with ramp pulses as in Fig. 2 before (C) and after exposure to various concentrations of 4-AP (0·1, 0·3, 1 and 3 mM). Each trace in *B*, shows the average of five currents. All traces in *A* and *B* were obtained from the same cell.

a small transient net inward current. However, in the present preparations net inward current was not observed, but may have been obscured by the larger outward currents.

The current–voltage relationship of outward currents was also investigated using ramp depolarizing pulses in the whole-cell configuration. A ramp of voltage from −80 to +80 mV was applied over a 900-ms period of time from a holding potential of −60 mV (Figs 2*B* and 3*B*). Under control conditions, the first detectable outward

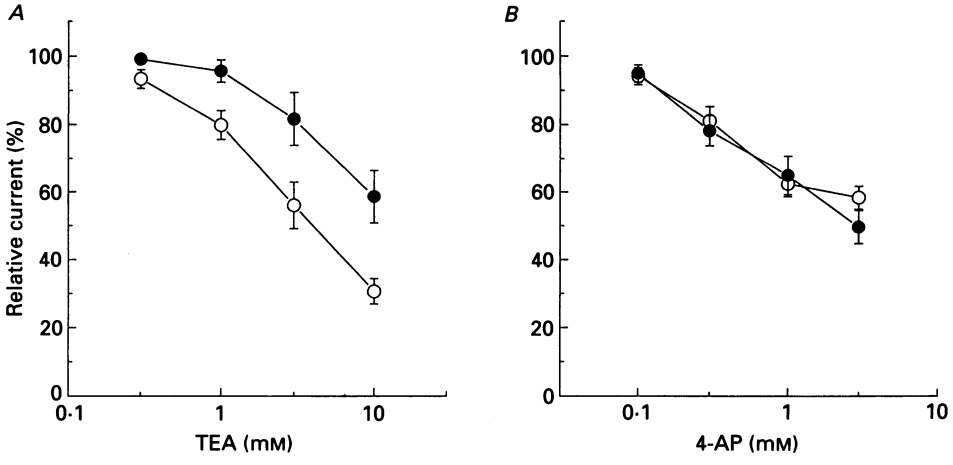

Fig. 4. Differences between effects of TEA and 4-AP on whole-cell outward currents. Concentration–response relationship of effects of TEA (*A*) and 4-AP (*B*) on the outward currents is shown (means ± S.E.M., $n = 6$). Peak currents elicited by voltage steps to 0 (●) and +60 mV (○) from a holding potential of −60 mV were measured in the presence of various concentrations of TEA or 4-AP. The amplitude of currents was normalized to the peak current in the absence of the drugs.

current was observed when the voltage ramp reached approximately −30 mV. For voltages positive to this the magnitude of outward current increased and at very positive potentials (i.e. > +40 mV) the current became noisy. Although the amplitude of currents elicited by ramp pulses was slightly smaller than that of peak currents elicited by step pulses, there was no remarkable difference in current–voltage relationship between them. The outward currents were not observed in Cs^+-dialysed cells with 10 mM TEA (data not shown, $n = 6$), suggesting that the outward currents were due to K^+ channels.

Effects of 4-AP and TEA on outward currents. It has been shown that macroscopic outward currents in smooth muscle cells can be divided into two components using two well-known K^+ channel blockers, TEA and 4-AP (Beech & Bolton, 1989; Gelband & Hume, 1992). Therefore, we investigated the effects of these K^+ channel blockers on the outward current in rabbit coronary artery cells. Bath application of TEA or 4-AP reduced the amplitude of the outward currents in a concentration-dependent manner (Figs 2 and 3). However, some differences were observed regarding the effects of TEA and 4-AP. Low concentrations of TEA (0·3–1 mM) selectively inhibited outward currents at membrane potentials positive to 0 mV, whereas higher concentrations of TEA (3–10 mM) reduced outward currents at all membrane potentials (Fig. 2*A c*). A similar inhibitory effect of TEA was observed in experiments in which currents were elicited with ramp pulses (Fig. 2*B*). TEA also reduced current

noise which was observed at very positive potentials (Fig. 2*A b* and *B*). In contrast to TEA, the effects of 4-AP were similar at all concentrations, i.e. 4-AP (0·1–3 mM) reduced outward currents at all potentials but did not reduce current noise (Fig. 3). Increasing the concentrations of 4-AP to 10 mM did not produce further inhibition

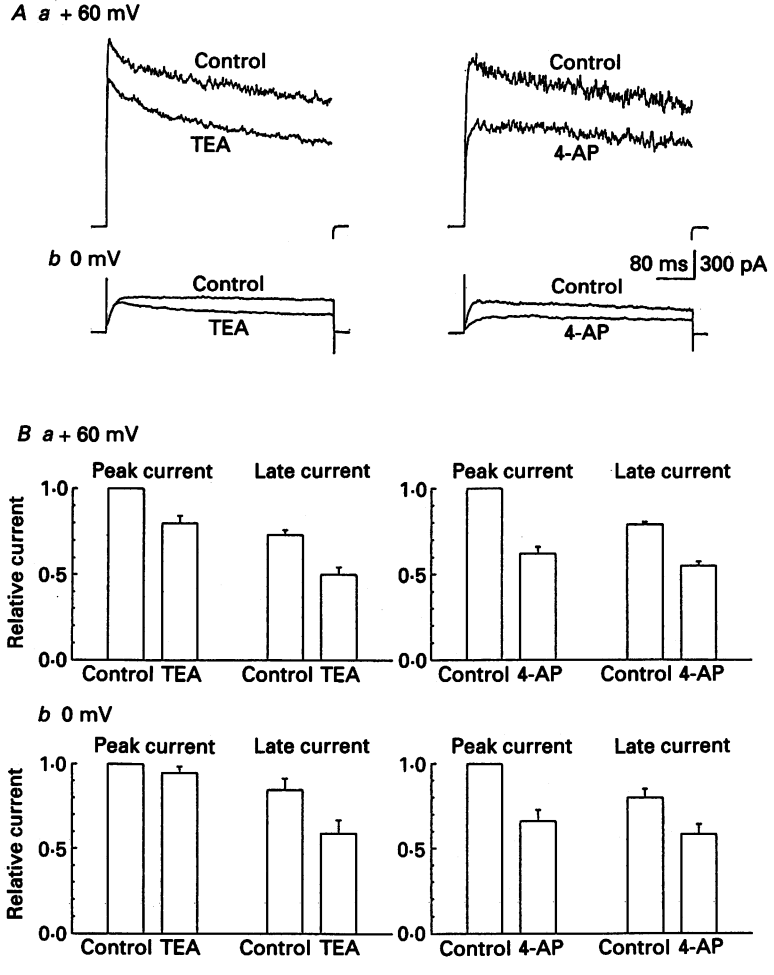

Fig. 5. Differences between effects of TEA and 4-AP on whole-cell outward currents. *A*, typical traces of outward currents evoked by voltage steps to +60 mV (*a*) and 0 mV (*b*) from a holding potential of −60 mV before (control) and after exposure to TEA (1 mM; left panel) and 4-AP (1 mM; right panel). *B*, mean current (mean ± S.E.M., $n = 6$) at the peak (peak current) and the end of step pulses (late current) evoked by voltage steps to +60 mV (*a*) and 0 mV (*b*) from a holding potential of −60 mV were plotted for control and after exposure to TEA (1 mM; left panel) and 4-AP (1 mM; right panel). The amplitude of currents was normalized to the peak current in control.

(data not shown, $n = 4$). These characteristics of the blockers were clearly shown in their concentration–response relationship on the outward currents evoked by voltage steps to 0 and +60 mV. As shown in Fig. 4, there was a clear difference in the sensitivity to TEA at these two membrane potentials. Thus, the current obtained

with a step pulse to +60 mV was more sensitive to TEA than that at 0 mV. In contrast, the concentration–response relationship for 4-AP was the same at 0 and +60 mV.

TEA and 4-AP also showed different inhibitory effects on peak outward current and outward current at the end of step pulses (late current) evoked by voltage

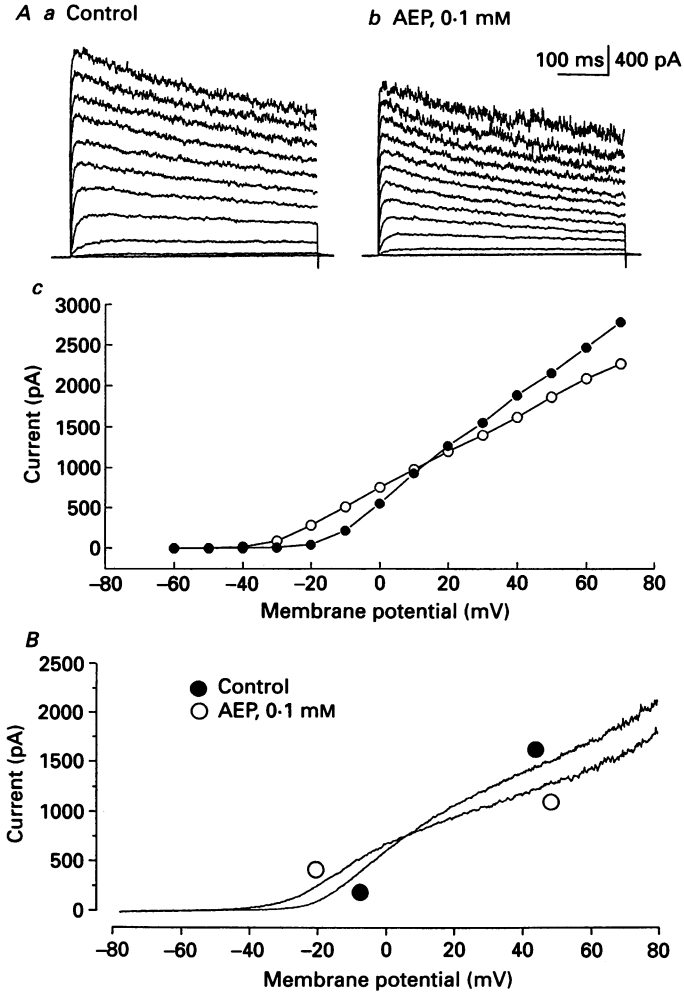

Fig. 6. Effect of AEP on whole-cell outward currents. *A*, outward currents were elicited by voltage steps as in Fig. 2 and recorded before (*a*) and after (*b*) exposure to AEP (0·1 mM). Panel *c* shows the current–voltage relationship for the peak outward current before (●) and after (○) exposure to AEP (0·1 mM). *B*, current–voltage relationship was also observed with the ramp pulses as in Fig. 2 before (●) and after (○) exposure to AEP (0·1 mM). Each trace shows the average of five currents. All traces in *A* and *B* were obtained from the same cell.

steps (Fig. 5). The peak current at 0 mV was only slightly reduced with 1 mM TEA whereas the late current was reduced by approximately 30%. The minimal effect of TEA on the peak current may be due to slow activation of TEA-sensitive outward

currents when membrane potential is stepped to 0 mV. When voltage was stepped to +60 mV, TEA significantly depressed both the peak and late currents. In contrast, 4-AP (1 mM) produced a significantly greater percentage reduction of the peak current than the late current at both 0 and +60 mV. This finding suggests that the outward currents inhibited by 4-AP show greater inactivation than those inhibited by TEA. The experiments with TEA and 4-AP suggest that the outward currents in rabbit coronary artery cells can be separated into two components, i.e. (1) an inactivating, 4-AP-sensitive, low-noise current and (2) a non-inactivating low concentration of TEA-sensitive, high-noise current.

Effects of AEP on whole-cell K^+ currents

The effects of AEP (0·1 mM) on whole-cell outward currents were measured using both step voltage and ramp voltage commands. Outward currents elicited by step depolarizations to voltages between approximately −30 and 0 mV were increased by AEP (Fig. 6*Ac*). However, AEP decreased the outward currents at more positive potentials. Almost the same voltage-dependent response to AEP was observed when the ramp pulses were applied (Figs 6*B* and 7*A*). Therefore, the following experiments analysing this voltage-dependent response were performed with the ramp protocol since we could continuously monitor the changes of current–voltage relationship for outward currents by agents. The voltage-dependent effects of AEP were maintained for at least 5 min and the currents were almost recovered within 10 min after wash-out. These effects of AEP were inhibited by the histamine H_1-receptor antagonist, chlorpheniramine (10 μM) (data not shown, $n = 3$).

Role of Ca^{2+} release and Ca^{2+} influx in modulating K^+ channels

Previous studies have shown that AEP enhances both Ca^{2+} influx and the release of Ca^{2+} from intracellular stores in the rabbit coronary artery (Khoyi *et al.* 1991). In addition, our experiments measuring whole-cell inward currents indicate that AEP can enhance the activity of voltage-dependent L-type Ca^{2+} channels. The effects of AEP on K^+ channels may therefore be mediated by an increase in $[Ca^{2+}]_i$, arising from enhanced Ca^{2+} release, enhanced Ca^{2+} influx, or both. To explore these possibilities we examined whether exposure to Ca^{2+}-free solutions or depletion of intracellular Ca^{2+} stores by ryanodine (Ito, Takakura, Sato & Sutko, 1986) would alter the effect of AEP on macroscopic K^+ currents.

First, the effects of AEP were investigated 10 min after incubating cells in the Ca^{2+}-free PSS. As shown in Fig. 7*B*, AEP (0·1 mM) caused significant changes in outward currents which were very similar to the voltage-dependent effects obtained in regular Ca^{2+}-containing PSS (Fig. 7*A*). Next, series of experiments were undertaken in which Ca^{2+} influx was left intact while the release of Ca^{2+} was blocked by pretreatment of cells with ryanodine (10 μM) and caffeine (5 mM). Under these circumstances, the voltage-dependent effects of AEP on outward current were absent (Fig. 7*C*). Since ryanodine is not believed to exert a direct effect on L-type Ca^{2+} channels (Balke & Wier, 1991) these results suggest that the contribution of Ca^{2+} influx to the voltage-dependent effects of AEP is relatively minor compared to the contribution of Ca^{2+} release.

The experiments thus far suggest that an increase in $[Ca^{2+}]_i$ induced mainly by Ca^{2+}

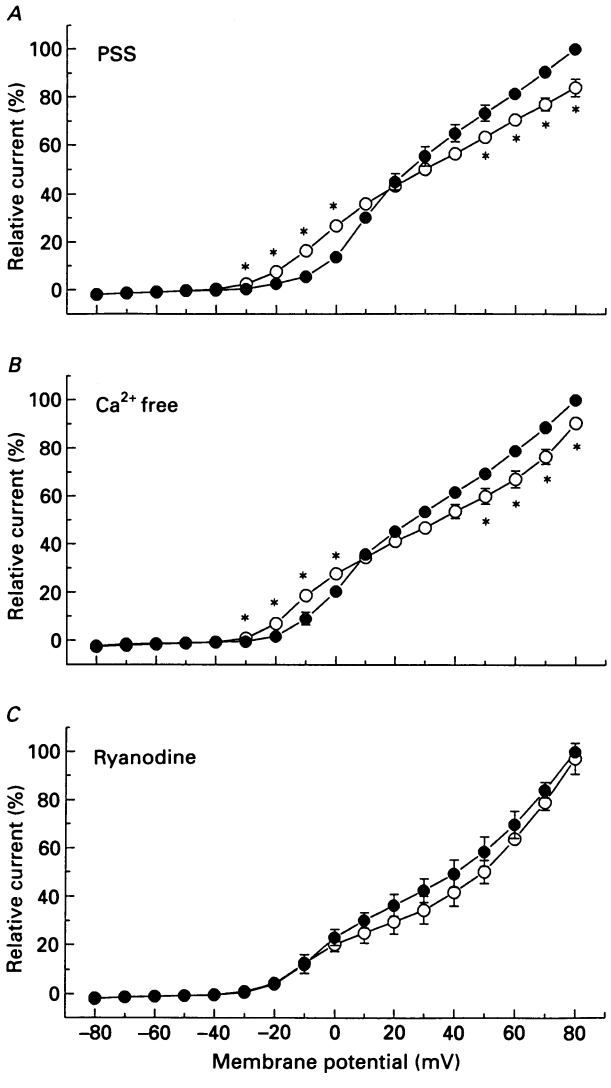

Fig. 7. Dependence of the AEP-induced changes in whole-cell outward currents on extracellular Ca^{2+} and intracellular stored Ca^{2+}. Graphs show mean values (mean ± S.E.M., $n = 4$–6) of the currents obtained using the ramp pulses as in Fig. 2 before (●) and after (○) exposure to AEP (0·1 mM). AEP significantly increased the amplitude of currents between −30 and 0 mV and diminished current amplitude at positive potentials in the PSS (*A*). Similar results were obtained even in the Ca^{2+}-free PSS (*B*). The depletion of intracellular Ca^{2+} stores by pretreatment with ryanodine (see Methods) abolished the voltage-dependent effects of AEP (*C*). The amplitude of currents was measured in 10 mV increments and normalized to the current elicited at +80 mV before exposure to AEP. There was no significant difference in the currents at any potentials between controls (before exposure to AEP) under the three conditions. *Significant difference from the current before exposure to AEP at each voltage (ANOVA followed by the Bonferroni method).

release from intracellular stores could be related to the voltage-dependent effect of AEP on outward currents. Further experiments were performed to clarify this possibility by using other procedures to increase $[Ca^{2+}]_i$. Ryanodine has been shown to increase $[Ca^{2+}]_i$ in the process of depleting intracellular Ca^{2+} stores (Desilets,

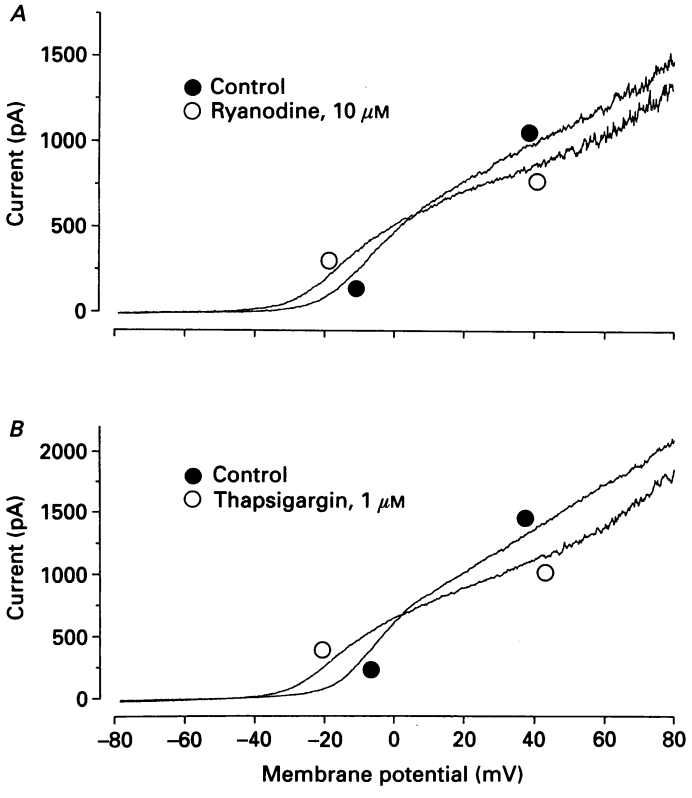

Fig. 8. Effect of ryanodine and thapsigargin on whole-cell outward currents. The outward currents were elicited by the ramp pulses as in Fig. 2 and were recorded before (control) and 10 min after exposure to 10 μM ryanodine (*A*) or 5 min after exposure to 1 μM thapsigargin (*B*). Each trace shows the average of five currents.

Driska & Baumgarten, 1989; Wagner-Mann, Hu & Sturek, 1992). After gaining access to a cell and recording control outward currents, the cell was superfused with ryanodine (10 μM) in PSS for 10 min. At this time ryanodine produced voltage-dependent changes in outward currents which were very similar to those induced by AEP, i.e. ryanodine increased the amplitude of currents evoked by the ramp protocol for potentials between −30 and 0 mV and diminished current at more positive potentials (Fig. 8*A*). A similar voltage-dependent change in outward currents was also produced by thapsigargin (1 μM; Fig. 8*B*). Thapsigargin is a selective inhibitor of Ca^{2+}-ATPase in the sarcoplasmic reticulum and has been shown to increase $[Ca^{2+}]_i$ (Thastrup, 1990). These observations support the hypothesis that an increase in $[Ca^{2+}]_i$ can be responsible for both stimulation of outward currents at negative potentials and inhibition of currents at positive potentials.

All of these results lead to the conclusion that the effects of AEP on macroscopic K^+ currents are mediated primarily by release of Ca^{2+} from intracellular stores, and that stimulation of Ca^{2+} entry through voltage-dependent Ca^{2+} channels plays little role in AEP-induced changes in K^+ currents.

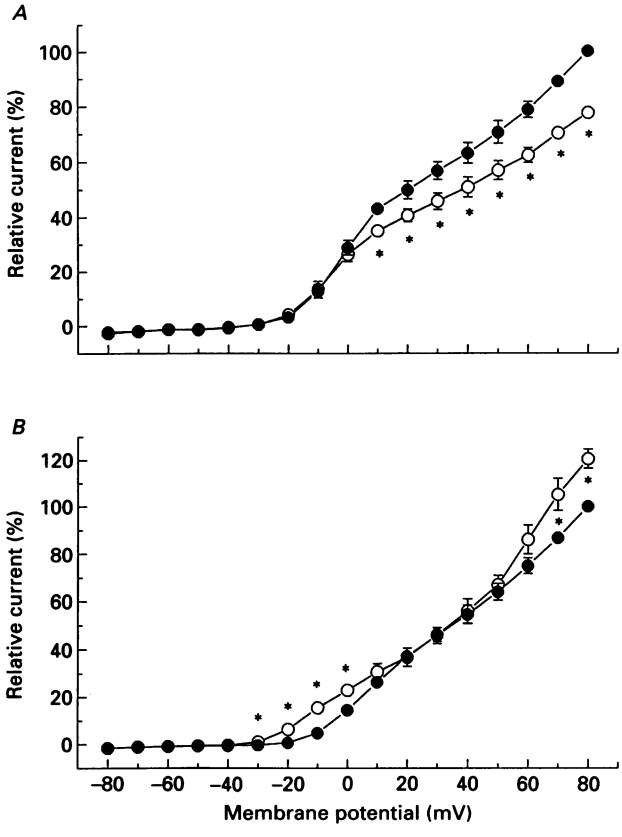

Fig. 9. Effects of TEA and 4-AP on the AEP-induced changes of whole-cell outward currents. Graphs show mean values (mean ± S.E.M., $n = 4$) of the currents obtained using the ramp pulses as in Fig. 2 before (●) and after (○) exposure to AEP (0·1 mM) in the presence of 1 mM TEA (*A*) or 3 mM 4-AP (*B*). The amplitude of currents was measured in 10 mV increments and normalized to the current elicited at +80 mV before exposure to AEP (790 ± 221 (*A*) and 1339 ± 37 (*B*) pA). * Significant difference from the current before exposure to AEP at each voltage (Student's *t* test for paired values).

Identification of the K^+ channels sensitive to AEP

AEP-induced changes in K^+ currents exhibited an unusual dependence upon membrane potential in that currents activated at negative potentials (−30 to 0 mV) were increased, whereas currents activated at positive membrane potentials (> +30 mV) were depressed compared to control currents. Since a similar dual effect on K^+ currents was observed during acute exposure to ryanodine or thapsigargin, it is possible that both the stimulation and inhibition of K^+ currents is due to an increase in release of Ca^{2+} from intracellular stores. What is not clear is whether both

the stimulation and inhibition of K^+ currents is due to the effects of Ca^{2+} release on one population of K^+ channels at two different ranges of membrane potential, or whether the effects of Ca^{2+} release are due to different effects on two separate populations of K^+ channels with distinct voltage activation ranges.

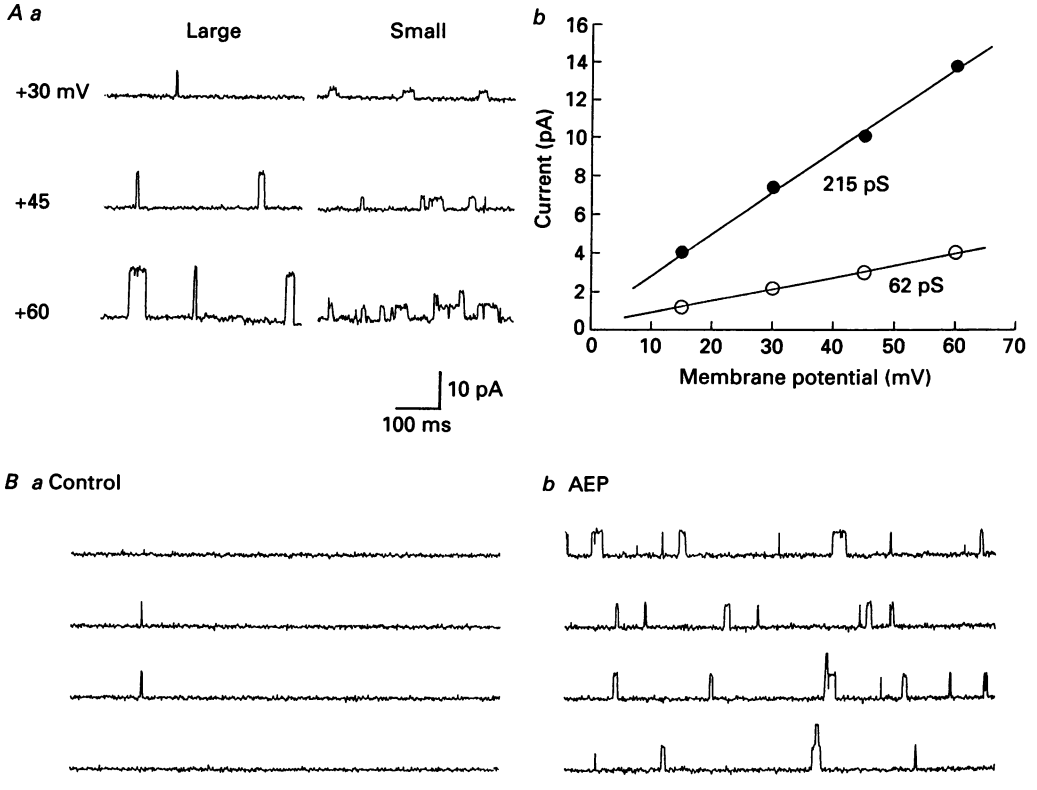

Fig. 10. Single-channel current recordings of K^+ channels in coronary artery cells using cell-attached configuration. *A*, using cell-attached patch configuration, two different amplitudes of unitary currents were recorded with symmetrical K^+ solutions (150 mM K^+ in pipette–150 mM K^+ out). Large conductance currents (left) and small conductance ones (right) were recorded from different patches (*A a*). The current–voltage relations of two kinds of unitary currents are shown in *A b*. *B*, effect of AEP on the large conductance unitary K^+ current in coronary artery cells using cell-attached configuration. Control large conductance channel activity at +30 mV is shown in *B a*. Bath application of AEP (0·1 mM) caused a marked increase in the open probability of these channels, as shown in *B b*.

To determine if two distinct channels might be involved, we examined the influence of the K^+ channel antagonists, TEA and 4-AP, on the voltage-dependent effects of AEP. In the presence of TEA (1 mM), AEP (0·1 mM) did not modify outward current between −30 and 0 mV whereas at positive potentials a significant reduction in current was observed with the ramp protocol (Fig. 9*A*). Thus block of TEA-sensitive K^+ currents prevented the negative voltage shift in K^+ current activation previously observed in response to AEP, suggesting that AEP-induced

Ca^{2+} release altered the voltage dependency of TEA-sensitive K^+ currents in a manner consistent with expected effects of $[Ca^{2+}]_i$ on maxi-K^+ channels (for review see McManus, 1991). The observed K^+ current depression by AEP in the presence of TEA, suggests that an increase in $[Ca^{2+}]_i$ also inhibits a population of TEA-insensitive K^+ channels. This interpretation received additional support from experiments in which we examined the effects of 4-AP on the K^+ current response to AEP. In the presence of 4-AP (3 mM), AEP produced a negative shift in the voltage-dependent activation of K^+ currents and increased K^+ current amplitudes at positive potentials (Fig. 9*B*). These observations are consistent with AEP initiating an increase in $[Ca^{2+}]_i$ which simultaneously stimulates TEA-sensitive K^+ channels and inhibits 4-AP-sensitive K^+ channels.

To provide further evidence for the involvement of maxi-K^+ channels in the actions of AEP, we investigated the effect of AEP on unitary currents in cell-attached patches. As illustrated in Fig 10*A*, unitary K^+ currents were observed in the cell-attached configuration with symmetrical K^+ solutions (150 mM K^+ in pipette–150 mM K^+ in bath). The patch was held at various potentials between -30 and $+60$ mV for 1 min. Two populations of unitary currents were observed at positive membrane potentials although the open probabilities of both were very low under our recording condition. In only two of nineteen patches were both large and small conductance channels clearly present. Eight patches exhibited only the large unitary currents, the mean slope conductance of which was 225 ± 10 pS. Nine patches showed only small unitary currents with a mean slope conductance of 70 ± 1 pS. Although the smaller conductance channels were not further characterized in this study, they may present a 4-AP sensitive, intermediate conductance channel, as previously reported in canine renal artery cells (Gelband & Hume, 1992). In patches containing the large conductance channel, we examined the effect of AEP. Figure 10*B* shows a typical recording of maxi-K^+ channels when the patch membrane was clamped at $+30$ mV. Under control conditions, the open probability of maxi-K^+ channels was very low ($P_o = 0{\cdot}0028$). Bath application of AEP (0·1 mM) caused an increase in the open probability of these channels ($P_o = 0{\cdot}044$). Similar results were obtained from three more experiments. In one of the four patch experiments addition of AEP resulted in the appearance of channel openings a previously quiescent patch held at -30 mV.

Effects of charybdotoxin on AEP-induced contraction in tissue preparations

A key finding from our electrophysiological studies is that AEP causes activation of maxi-K^+ channels at physiological potentials (near the resting membrane potential) in rabbit coronary artery smooth muscle cells. Therefore, this effect of AEP should hyperpolarize the membrane potential, which in turn would be predicted to lead to muscle relaxation in intact tissue. However, AEP causes potent contractions of intact rabbit coronary artery (Khoyi *et al.* 1991). Under these circumstances the action of AEP on maxi-K^+ channels may act as a negative feedback mechanism which limits the degree of contraction elicited by AEP. To examine this possibility, the effects of charybdotoxin, CTX (a blocker of maxi-K^+ channels, Miller, Moczydlowski, Latorre & Phillips, 1985) were investigated on AEP-

induced contractions in intact ring segments of endothelium-denuded rabbit coronary artery. AEP (0·1 mM) alone induced a sustained contraction in this vessel. In the presence of CTX (100 nM) the contractile response to AEP was significantly enhanced (Fig. 11*A* and *B*). CTX itself did not cause any changes in the vessel tonus.

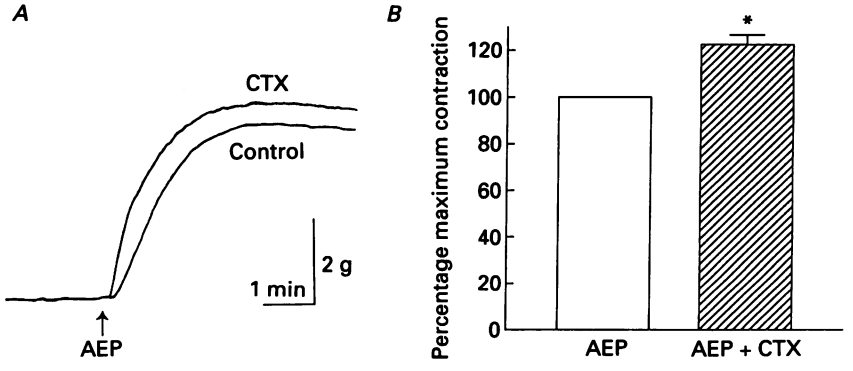

Fig. 11. Effect of charybdotoxin on the AEP-induced contraction in endothelium-denuded rabbit coronary artery. Superimposed contractile records obtained from one preparation are shown in *A*. AEP (0·1 mM) induced a sustained contraction in this vessel. This contractile response was enhanced in the presence of charybdotoxin (CTX) (100 nM). CTX itself did not cause any changes in the vessel tonus. The results of statistical analyses are shown in *B*. The augmenting effect of CTX on the AEP-induced contraction was significant (Student's *t* test for paired values; $n = 4$).

This result suggests that AEP causes an activation of maxi-K^+ channels as well as contractions in the intact tissue and that the effect of AEP on maxi-K^+ channels limits the degree of AEP-induced contraction.

DISCUSSION

In this study we investigated the effect of the histamine H_1-receptor agonist, AEP, on K^+ and Ca^{2+} channels. The data presented above showed that AEP produced voltage-dependent changes in outward currents, i.e. AEP increased the amplitude of currents for potentials between −30 and 0 mV and diminished currents at more positive potentials. A nicardipine-sensitive inward current was also recorded in these cells, which was significantly increased in the presence of AEP. Despite this ability of AEP to enhance Ca^{2+} entry through voltage-dependent Ca^{2+} channels, AEP's ability to enhance the activity of maxi-K^+ channels in the physiological range of membrane potential is due primarily to an increase in the release of Ca^{2+} from intracellular stores.

The outward currents in the rabbit coronary artery cells were divided into at least two components with the use of two different well-known K^+ channel blockers, i.e. TEA and 4-AP. These two blockers had very different voltage-dependent effects on outward currents. Low concentrations of TEA reduced currents at positive voltages to a greater extent than at negative voltages, whereas 4-AP-sensitive currents were observed at negative membrane potentials, and were relatively constant at positive

membrane potentials. Using voltage step depolarizations, we also observed that the currents blocked by TEA and 4-AP had different time dependencies. The TEA-sensitive component was non-inactivating and noisy which is similar to properties previously reported for maxi-K^+ channels (for review see Latorre, Oberhauser, Labarca & Alvarez, 1989; McManus, 1991). The 4-AP-sensitive component was inactivating and exhibited less noise, characteristics similar to the properties of delayed rectifier K^+ currents reported by others (Okabe, Kitamura & Kuriyama, 1987; Beech & Bolton, 1989; Volk, Matsuda & Shibata, 1991; Gelband & Hume, 1992). When the TEA concentration was raised to 3 mM or above, both components of current appeared to be reduced. This observation agrees with the non-selective effects of higher concentrations of TEA on K^+ channels reported by others (Okabe *et al.* 1987; Langton, Nelson, Huang & Standen, 1991).

In the present study inward currents were recorded using the amphotericin B-perforated patch technique (Rae *et al.* 1991). With this technique, the currents through L-type Ca^{2+} channels were recorded without 'run-down' for over 1 h. The existence of two types of voltage-dependent Ca^{2+} currents has been shown in a variety of vascular smooth muscle cells (for review see Bean, 1989). However, our results showed that all voltage-dependent Ca^{2+} currents in rabbit coronary artery cells were abolished in the presence of the Ca^{2+} channel blocker dihydropyridine, suggesting that there is only L-type Ca^{2+} current in these cells. This result is in agreement with other studies of rabbit coronary artery cells (Matsuda, Volk & Shibata, 1990) and rabbit mesenteric artery cells (Nelson & Worley, 1989). Our results also showed that AEP through H_1-receptor activation augmented Ca^{2+} currents in rabbit coronary artery cells. The effect of H_1-receptor stimulation on L-type Ca^{2+} channels which we report here differs somewhat from previous studies of rabbit saphenous artery cells in which histamine H_3-receptor activation has been reported to augment voltage-dependent Ca^{2+} currents (Oike, Kitamura & Kuriyama, 1992). The effects of H_3-receptor activation in coronary arterial cells have yet to be examined.

AEP also produced voltage-dependent effects on outward currents which included an enhancement of current between -30 and 0 mV and inhibition of current at positive potentials. Since some outward currents are known to be Ca^{2+} sensitive (for review see Latorre *et al.* 1989; McManus, 1991), we investigated the contribution of Ca^{2+} to the effects of AEP on outward currents. The voltage-dependent effects of AEP were unaffected by removal of extracellular Ca^{2+}. In contrast, when Ca^{2+} release was abolished by long exposure to ryanodine, AEP no longer produced either significant enhancement or suppression of outward currents. This absence of effect of AEP was observed regardless of whether Ca^{2+} was included in the solution bathing the cells. These results lead to the conclusion that the voltage-dependent stimulatory and inhibitory effects of AEP are mediated primarily by release of Ca^{2+} from intracellular stores.

Ca^{2+} release from the intracellular stores has been reported to enhance the activity of spontaneous transient outward currents (i.e. STOCs) in several vascular smooth muscle preparations (Benham & Bolton, 1986; Ganitkevich & Isenberg, 1990). STOCs are most probably due to the local simultaneous opening of maxi-K^+ channels in response to a sudden discharge of Ca^{2+} from intracellular stores (Benham &

Bolton, 1986). In ileal smooth muscle cells, ryanodine has been shown to cause transient enhancement of single Ca^{2+}-activated K^+ currents in the cell-attached patch configuration (Sakai, Terada, Kitamura & Kuriyama, 1988). It has also been shown that the whole-cell outward current evoked by a voltage step is augmented by the application of caffeine in porcine coronary artery cells (Sturek, Stehno-Bittel & Obye, 1991). The enhancement of outward currents which we observed over the range of potentials between -30 and 0 mV is similar to these findings. The present experiment also showed that the stimulatory effect of AEP at negative potentials was blocked by a low concentration of TEA. Finally, single-channel recordings demonstrated that AEP increased the open probability of maxi-K^+ channels. All of these results support the conclusion that AEP-induced enhancement of outward currents at negative potentials is due to the activation of maxi-K^+ channels by an increase in $[Ca^{2+}]_i$. This is in good agreement with the report in which an increase in $[Ca^{2+}]_i$ produced a leftward shift in the voltage–open probability relation curve of maxi-K^+ channels in inside-out patches from rabbit mesenteric artery cells (Benham, Bolton, Lang & Takewaki, 1986). However, the decrease in outward K^+ current which we observed with AEP at positive potentials has not been widely reported previously.

The mechanism underlying the decline of outward currents induced by AEP at positive potentials seems also to be related to the rise in $[Ca^{2+}]_i$ since this inhibitory effect was blocked by depleting intracellular Ca^{2+} stores with ryanodine and, furthermore, was mimicked by thapsigargin or short exposure to ryanodine. The experiments using K^+ channel blockers showed that AEP diminished the outward current evoked at positive potentials in the presence of TEA whereas AEP enhanced current in the presence of 4-AP. This observation suggests that the inhibitory effects of AEP on K^+ current are related to an indirect ability of Ca^{2+} to inhibit the 4-AP-sensitive current. The exact mechanism of this inhibition is unknown at this time but there is sufficient evidence that divalent cations can inhibit K^+ current in other preparations (Matsuda, Saigusa & Irisawa, 1987; Mazzanti & DeFelice, 1990). Further studies are required to elucidate this point.

Previous studies of the rabbit coronary artery have shown that the AEP-induced contraction is sensitive to nifedipine and associated with a rise in Ca^{2+} influx into the tissue (Khoyi *et al.* 1991). The present study suggests that direct enhancement of L-type Ca^{2+} current by AEP may be partly responsible for the nifedipine-sensitive component of this contraction. In addition to effects on Ca^{2+} entry, AEP also apparently enhances Ca^{2+} release from intracellular stores (Khoyi *et al.* 1991). Direct evidence for histamine-induced increase in $[Ca^{2+}]_i$ has been demonstrated by use of the fura-2 fluorometric method in porcine coronary arteries (Hirano, Kanaide, Abe & Nakamura, 1991). Ca^{2+} release is probably due to formation of inositol 1,4,5-trisphosphate via H_1-receptor stimulation (Donaldson & Hill, 1985). It is of interest that the activation of maxi-K^+ channels by AEP was maintained for at least 5 min although this effect was mediated by Ca^{2+} release from intracellular stores. Many vasoconstrictors including histamine (Hirano *et al.* 1991) are known to cause a release of Ca^{2+} from intracellular stores, leading to a transient increase in $[Ca^{2+}]_i$. This discrepancy may be explained by the concept of 'free Ca^{2+} in the subsarcolemmal region (Ca^{2+}_{is})' (Sturek *et al.* 1991). Sturek *et al.* (1991) have shown that ryanodine or

a low concentration of caffeine increases the maxi-K^+ currents without detectable changes in $[Ca^{2+}]_i$ averaged over the bulk myoplasm in bovine coronary artery cells. Thus, it is possible that AEP caused sustained increases in Ca^{2+}_{is} which induced sustained activation of maxi-K^+ channels even if the $[Ca^{2+}]_i$, i.e. mean cytosolic Ca^{2+} concentration, returned to the basal level.

The present study suggests that Ca^{2+} release may be importantly linked not only to contraction but also to changes in the activity of two separate K^+ channels. In the voltage range at which contraction occurs with AEP (i.e. around −30 mV, Keef & Ross, 1987) we observed enhancement of K^+ current in the presence of AEP. This enhanced K^+ channel activity may tend to limit the degree of membrane depolarization and consequently the extent to which L-type Ca^{2+} channels are activated. This in turn may limit contraction. In support of this hypothesis we observed that contraction with AEP in the intact tissue was enhanced in the presence of the blocker of maxi-K^+ channels, charybdotoxin (Miller *et al.* 1985). A similar role of these K^+ channels in blood vessels has been proposed by others (Trieschmann & Isenberg, 1989; Brayden & Nelson, 1992).

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