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# Charybdotoxin-sensitive $K^+$ channels regulate the myogenic tone in the resting state of arteries from spontaneously hypertensive rats

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**1** To determine the possible role of $Ca^{2+}$-activated $K^+$ ($K_{Ca}$) channels in the regulation of resting tone of arteries from spontaneously hypertensive rats (SHR), the effects of agents which interact with these channels on tension and $^{86}Rb$ efflux were compared in endothelium-denuded strips of carotid, femoral and mesenteric arteries from SHR and normotensive Wistar-Kyoto rats (WKY).

**2** Strips of carotid, femoral and mesenteric arteries from SHR exhibited a myogenic tone; that is, the resting tone decreased when either the Krebs solution was changed to a $0\text{-}Ca^{2+}$ solution or $10^{-7}$ M nifedipine was added.

**3** The addition of charybdotoxin (ChTX, $10^{-9}$–$10^{-7}$ M), a blocker of large conductance $K_{Ca}$ channels, to the resting strips of these arteries produced a concentration-dependent contraction, which was significantly greater in SHR than in WKY. Relatively low concentrations of tetraethylammonium (0.05–5 mM) produced a concentration-dependent contraction which was similar to the ChTX-induced contraction in these strips.

**4** The ChTX-induced contractions in SHR were greatly attenuated by $10^{-7}$ M nifedipine and by $3 \times 10^{-6}$ M cromakalim, a $K^+$ channel opener. Cromakalim alone abolished the myogenic tone in SHR.

**5** The addition of apamin (a blocker of small conductance $K_{Ca}$ channels, up to $10^{-6}$ M), or of glibenclamide (a blocker of ATP-sensitive $K^+$ channels, up to $5 \times 10^{-6}$ M ), to the resting strips failed to produce a contraction.

**6** In resting strips of carotid, femoral and mesenteric arteries preloaded with $^{86}Rb$, the basal $^{86}Rb$ efflux rate constants were significantly greater in SHR than in WKY. The addition of $10^{-7}$ M nifedipine to the resting strips decreased the basal $^{86}Rb$ efflux rate constants only in SHR.

**7** The cellular $Ca^{2+}$ uptake in the resting state of carotid and femoral arteries from SHR was significantly increased when compared to WKY, and this increase in SHR was significantly reduced by $10^{-7}$ M nifedipine.

**8** These results suggest that the ChTX-sensitive $K_{Ca}$ channels were highly activated to regulate the myogenic tone in the resting state of carotid, femoral and mesenteric arteries from SHR. The increased $K_{Ca}$ channel functions in SHR arteries appeared to be secondary to the increased $Ca^{2+}$ influx via L-type voltage-dependent $Ca^{2+}$ channels in the resting state of these arteries.

**Keywords:** $Ca^{2+}$-activated $K^+$ channels; charybdotoxin; $^{86}Rb$ efflux; voltage-dependent $Ca^{2+}$ channels; $^{45}Ca$ influx; $Ca^{2+}$ uptake; spontaneously hypertensive rats (SHR); arterial smooth muscle

## Introduction

The total peripheral resistance is increased in patients with hypertension and in various models of experimental hypertension. The increased resistance may be caused by increased arterial contractility. It has been demonstrated that the cellular $Ca^{2+}$ uptake and/or content are increased in various tissues and cells isolated from patients and experimental animals with essential hypertension. Particularly, an abnormality in the $Ca^{2+}$ handling of arterial smooth muscle has been demonstrated in spontaneously hypertensive rats (SHR) (for review, see Postnov & Orlov, 1985; Lau & Eby, 1985; Bohr & Webb, 1988). Since the myofilaments in arterial smooth muscle are activated by a rise in the cellular concentration of free $Ca^{2+}$, the alteration of the cellular $Ca^{2+}$ uptake and/or content may conceivably provide a logical interpretation of some of the abnormal functions associated with hypertension, including an increased incidence of myogenic tone, hyperreactivity to vasoconstrictors and decreased relaxation to vasodilators.

Arteries isolated from SHR exhibit a spontaneous active tone (Noon *et al.*, 1978; Fitzpatrick & Szentivanyi, 1980; Winquist & Bohr, 1983; Asano *et al.*, 1986). The spontaneous active tone is independent of regional innervation and circulating hormones and has been termed myogenic. The myogenic tone is abolished by the removal of external $Ca^{2+}$ (Noon *et al.*, 1978; Fitzpatrick & Szentivanyi, 1980; Winquist & Bohr, 1983) and by calcium channel blockers (Asano *et al.*, 1986), suggesting that the myogenic tone depends on $Ca^{2+}$ influx via voltage-dependent $Ca^{2+}$ channels. The ability of arteries to produce myogenic tone and to maintain it in the resting state also suggests the ability to produce membrane depolarization and subsequent $Ca^{2+}$ influx. To counteract this ability, a negative feedback mechanism may exist that is activated by membrane depolarization and/or cellular $Ca^{2+}$. $Ca^{2+}$-activated $K^+$ ($K_{Ca}$) channels are an ideal candidate for such a mechanism because they are activated by both cellular $Ca^{2+}$ and membrane depolarization (for review, see Cook, 1988; Castle *et al.*, 1989). $K_{Ca}$ channels are subdivided into at least three types; large, intermediate and small conductance $K_{Ca}$ channels, according to the difference in single channel conductance. Differences in the mechanisms of activation of these channels may reflect their functional roles, with some channels maintaining resting tone and others terminating or limiting contraction induced by vasoconstrictors. Pharmacologically, charybdotoxin (ChTX) and apamin are

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relatively selective and potent blockers of large and small conductance $K_{Ca}$ channels, respectively (Cook, 1988; Castle *et al.*, 1989). ChTX-sensitive large conductance $K_{Ca}$ channels have been identified in single cells from vascular smooth muscles (Sugg *et al.*, 1990; Pavenstadt *et al.*, 1991; Brayden & Nelson, 1992). Although small conductance $K_{Ca}$ channels have been found in vascular smooth muscles, it has not been determined whether the channels are sensitive to apamin (Inoue *et al.*, 1985; Benham *et al.*, 1986). In the present study, we examined the possible role of $K_{Ca}$ channels in the regulation of myogenic tone in the resting state of arterial smooth muscle from SHR, by using standard tension-recording methods and $^{86}Rb$ efflux (used to monitor $K^+$ permeability) experiments.

## Methods

Male SHR, 13 weeks of age, and age-matched male normotensive Wistar-Kyoto rats (WKY) were bred in our laboratory. Systolic blood pressures at this age, measured by the tail-cuff plethysmography, were significantly ($P<0.001$) higher in SHR ($199 \pm 3$ mmHg, $n = 20$) than in WKY ($138 \pm 2$ mmHg, $n = 20$). Body weights were not significantly different between SHR ($250 \pm 7$ g, $n = 20$) and WKY ($268 \pm 7$ g, $n = 20$).

### *Preparation of arterial strips and measurements of mechanical activity*

The rats were stunned by a blow to the head and then exsanguinated. The common carotid artery (1.0–1.2 mm outside diameter), the femoral artery (0.7–0.9 mm o.d.) and the main branch of the superior mesenteric artery (0.7–0.9 mm o.d.) were excised and placed in Krebs solution of the following composition (in mM): NaCl 115.0, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25.0, $KH_2PO_4$ 1.2 and dextrose 10.0. Helical strips (0.8 mm × 8 mm) of these arteries were prepared as described previously (Asano *et al.*, 1988). The endothelium of the strip was removed by gently rubbing the endothelial surface with cotton pellets.

Strips were mounted vertically in water-jacketed muscle baths containing 10 ml Krebs solution (37°C). The Krebs solutions were aerated with 95% $O_2$ and 5% $CO_2$. The isometric tension was recorded with a force-displacement transducer (TB-612T, Nihon Kohden Kogyo Co., Tokyo, Japan). Strips were stretched passively to optimal length by imposing resting tension (carotid, 0.4 g; femoral, 0.6 g; mesenteric, 0.5 g) and a 90 min equilibration period preceded each experiment. The optimal resting tension was determined by a length-passive tension study (Asano *et al.*, 1988). All experiments were conducted in phenoxybenzamine-treated strips to eliminate possible α-adrenoceptor responses to noradrenaline that may be released from adrenergic nerve endings by depolarizing stimuli (Asano *et al.*, 1988).

After determination of the contractile responses of the strips to Krebs solution containing 65.9 mM KCl ($K^+$) ($K^+$ substitution for $Na^+$), the contractile responses to ChTX, apamin, tetraethylammonium (TEA; a relatively nonselective blocker of $K^+$ channels) or glibenclamide (a blocker of ATP-sensitive $K^+$ channels) were determined.

In some experiments, the effect of $0\text{-}Ca^{2+}$ solution, nifedipine (a blocker of L-type voltage-dependent $Ca^{2+}$ channels) or cromakalim (a $K^+$ channel opener) on the resting tone was examined. The $0\text{-}Ca^{2+}$ solution was prepared by omission of $Ca^{2+}$ from the Krebs solution and by addition of 0.1 mM EGTA.

### *Measurement of $^{86}Rb$ efflux from arterial strips*

$^{86}Rb$ efflux was measured simultaneously with tension changes as described previously (Masuzawa *et al.*, 1990; 1991). Briefly, after a 90 min equilibration, strips were incubated for an additional 3 h in the Krebs solution to which 14–20 μCi $ml^{-1}$ $^{86}Rb$ had been added. Each strip was then dipped (15 s) into non-radioactive Krebs solution and transferred to a superfusion chamber. A resting tension of 1.2 g (carotid), 1.8 g (femoral) or 1.5 g (mesenteric) was applied because larger strips were used. The superfusate was sampled by use of a collection period of 2 min and counted for radioactivity in an Aloka autowell gamma counter. On completion of the efflux, each strip was solubilized. The rate constant of $^{86}Rb$ efflux was then calculated as described previously (Masuzawa *et al.*, 1990; 1991).

### *Measurement of cellular $Ca^{2+}$ uptake*

Cellular $Ca^{2+}$ uptake was measured from the basal $^{45}Ca$ influx as described previously (Asano & Hidaka, 1985). Briefly, isolated arteries were equilibrated in a Tris-buffered solution of the following composition (in mM): NaCl 154.0, KCl 5.4, $CaCl_2$ 2.5, dextrose 11.0 and Tris 6.0 (pH 7.4). The Tris-buffered solutions were aerated with 100% $O_2$. Arteries were then transferred to the Tris-buffered solution to which 1 μCi $ml^{-1}$ $^{45}Ca$ had been added. After a 5 min incubation time, the arteries were placed in test tubes containing the 80.8 mM $La^{3+}$-substituted solution at 0.5°C for 60 min. Each artery was then placed in a glass scintillation vial containing 0.2 ml NCS tissue solubilizer (Amersham International, Buckinghamshire). Solubilized tissues were mixed with 5 ml Amersham ACS II scintillant and counted for radioactivity in an Aloka liquid scintillation counter. Values for cellular $Ca^{2+}$ uptake (expressed as nmol $g^{-1}$ tissue wet weight) were then calculated as described previously (Asano & Hidaka, 1985).

### *Statistical analysis*

Unless specified, results shown in the text, tables and figures are expressed as means ± s.e.mean ($n$ = number of preparations). Student's $t$ test for paired or unpaired data, or variance analysis was used to determine the significance of differences between means, and a $P$ value of $<0.05$ was taken as significant.

### *Drugs and isotopes*

The drugs used were ChTX (Peptide Institute Inc., Minoh, Japan), TEA chloride (Sigma Chemical Co., St. Louis, MO, U.S.A.), apamin (Sigma), glibenclamide (Sigma), nifedipine (Bayer Yakuhin Ltd., Osaka, Japan), papaverine hydrochloride (Wako Pure Chemical Industries, Osaka, Japan), cromakalim (Beecham Pharmaceuticals Research Division, Harlow, Essex, UK) and phenoxybenzamine hydrochloride (Nakarai Chemicals, Kyoto, Japan). $^{86}RbCl$ (specific activity initially 1.5–2.9 mCi $mg^{-1}$) and $^{45}CaCl_2$ (specific activity initially 16.7–28.5 mCi $mg^{-1}$) were obtained from Amersham International (Buckinghamshire).

Nifedipine (1 mM) and phenoxybenzamine (1 mM) were dissolved in 50% ethanol with further dilution in distilled water before use. Cromakalim (10 mM) was dissolved in 60% ethanol with further dilution in distilled water before use. Glibenclamide (1 mM) was dissolved in 50% ethanol with further dilution in the same solvent before use. Aqueous stock solutions were prepared for other drugs. Concentrations of drugs are expressed as final molar concentrations.

## Results

### *Myogenic tone in the resting state of spontaneously hypertensive rat arteries*

Carotid arteries isolated from SHR exhibited a myogenic tone; typically small magnitude oscillations superimposed on a tonic contraction (Figures 1 and 2). Such oscillation was

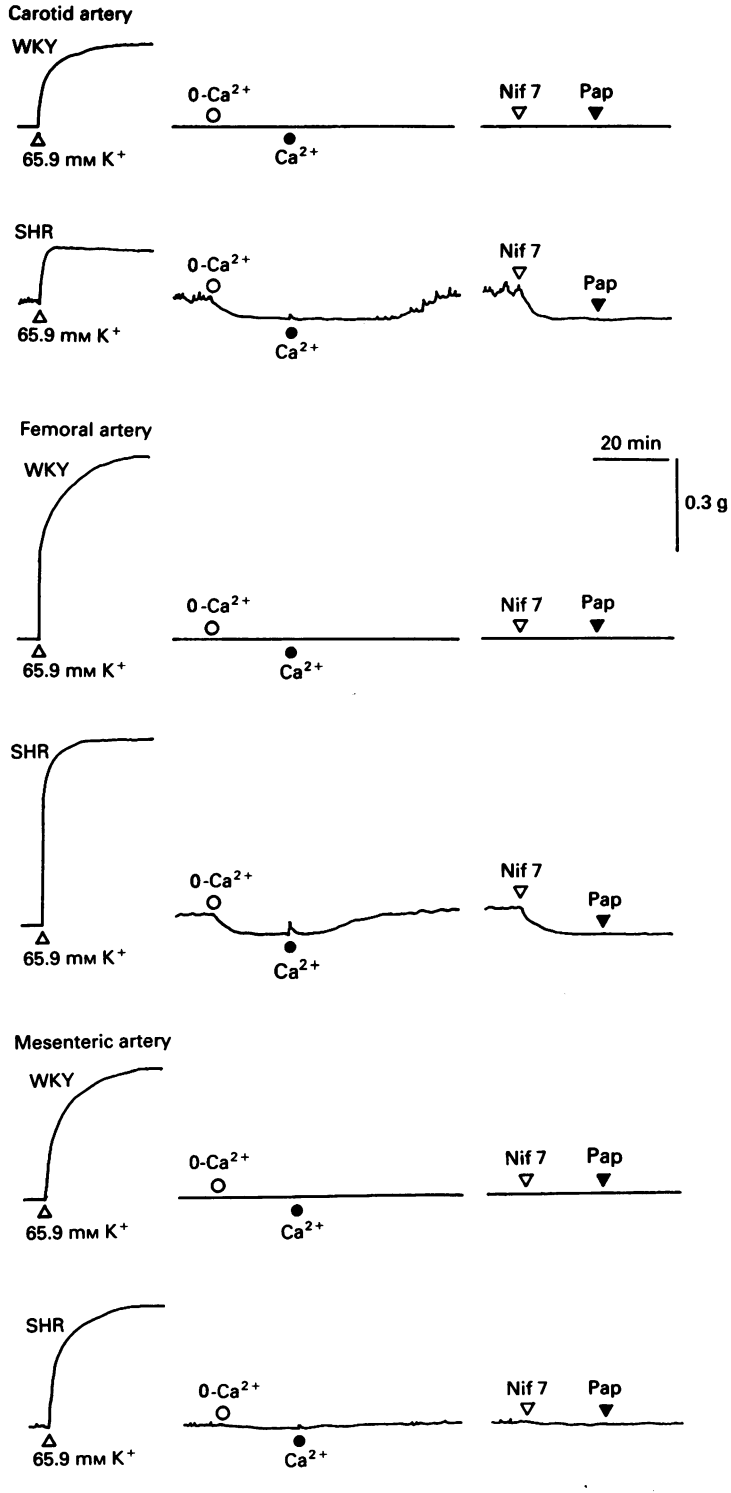


**Figure 1** Typical recordings of the effects of $0-Ca^{2+}$ solution ($0-Ca^{2+}$ ) and nifedipine (Nif) on resting strips of carotid, femoral and mesenteric arteries from 13 week old WKY and SHR. After the 90 min equilibration, strips were maximally activated by repeated application of 65.9 mM $K^+$ until the responses were reproducible. Following washout with a Krebs solution, the solution was replaced with a $0-Ca^{2+}$ solution for 20 min, and then 2.5 mM $Ca^{2+}$ ($Ca^{2+}$) was added to the $0-Ca^{2+}$ solution. The strips were then washed with a Krebs solution for 40 min, and were exposed to $10^{-7}$ M Nif. At the end of each experiment, $10^{-4}$ M papaverine (Pap) was added to identify the position of the maximum relaxation. Note the existence of an oscillatory contraction in carotid and mesenteric arteries from SHR and of a tonic contraction in femoral arteries from SHR in the resting state.

occasionally observed in mesenteric arteries from SHR (13 out of 35 preparations), but was rare in femoral arteries from SHR (9 out of 51 preparations) in which a tonic contraction was usually observed (Figures 1 and 2). These arteries relaxed from the resting tone when placed in a $0-Ca^{2+}$ solution (Figure 1). The relaxation in these arteries was $28.9 \pm 3.1\%$ (carotid, $n = 14$), $9.8 \pm 1.8\%$ (femoral, $n = 22$) and $2.7 \pm 0.6\%$ (mesenteric, $n = 12$), respectively, of the 65.9 mM $K^+$-induced maximum contraction. After the 20 min exposure to the $0-Ca^{2+}$ solution, the addition of 2.5 mM $Ca^{2+}$ restored the myogenic tone completely. The addition of $10^{-7}$ M nifedipine to the resting strips of these arteries placed in a Krebs solution also caused a relaxation in SHR which was comparable to the relaxation induced by $Ca^{2+}$ removal

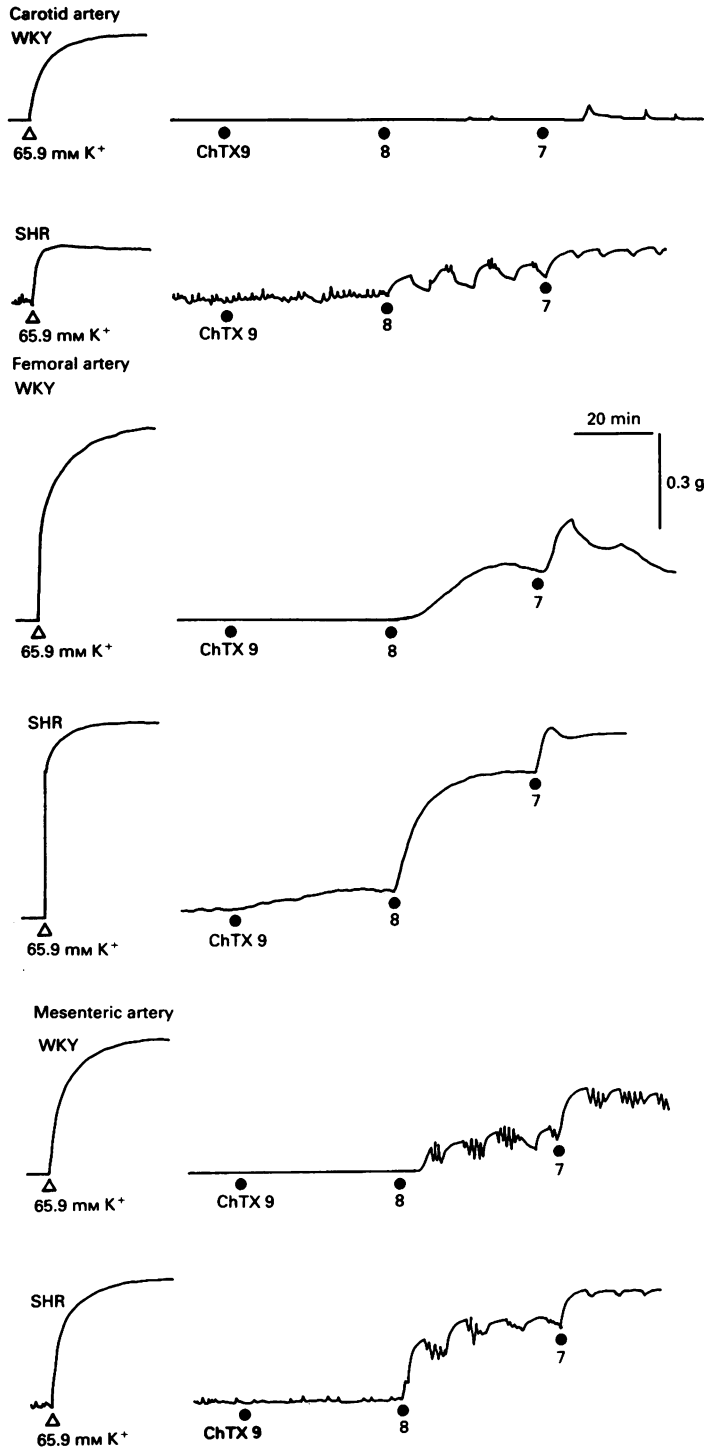


**Figure 2** Typical recordings of the contractile effect of charybdotoxin (ChTX) on resting strips of carotid, femoral and mesenteric arteries from 13 week old WKY and SHR. After the application of 65.9 mM $K^+$, and washout with a Krebs solution as in Figure 1, the concentration-response curve for ChTX was constructed in a cumulative fashion. Concentrations of ChTX are expressed as the negative log of the molar concentration.

(Figure 1). No further relaxation was obtained when $10^{-4}$ M papaverine was added to these arteries.

Carotid, femoral and mesenteric arteries isolated from WKY did not respond to $Ca^{2+}$ removal or nifedipine (Figure 1).

### *Charybdotoxin and tetraethylammonium-induced contraction*

The addition of ChTX to the resting strips of SHR arteries caused a concentration-dependent oscillatory contraction in carotid and mesenteric arteries and a tonic contraction in femoral arteries (Figure 2). On the other hand, the ChTX-induced contractions in WKY arteries were relatively weak and transient (Figure 2). Concentration-response curves for the contractile effect of ChTX are compared in arteries from SHR and WKY in Figure 3. In the three arteries used, the ChTX-induced contractions were significantly greater in SHR than in WKY. The difference in the ChTX-induced contraction between SHR and WKY was in order of carotid> femoral> mesenteric. The peak contractions induced by $10^{-7}$ M ChTX in arteries from SHR were comparable to the

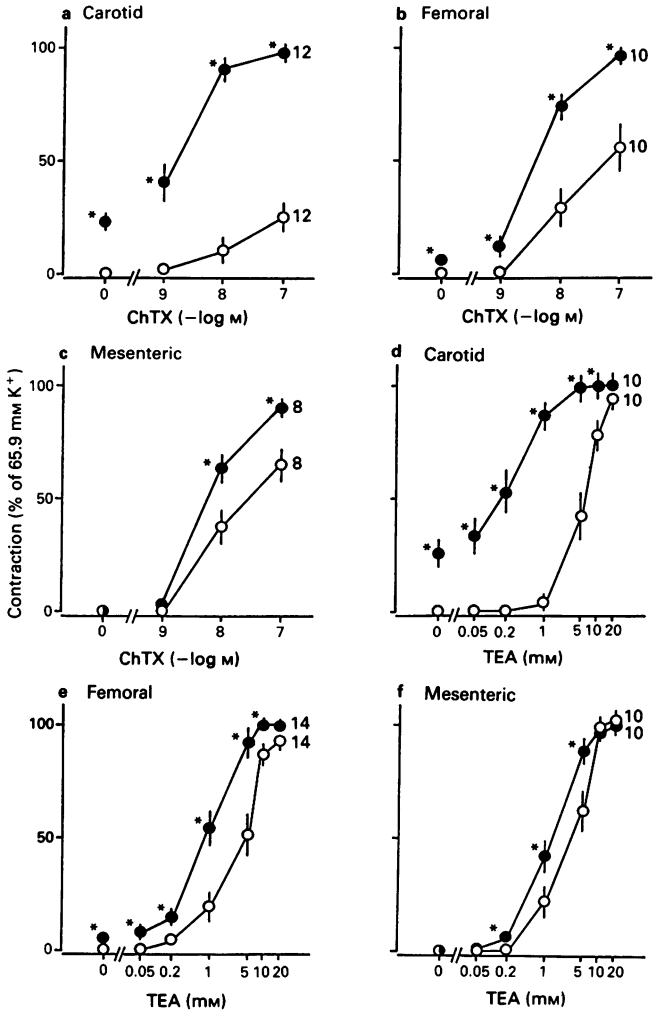


**Figure 3** Concentration-response curves for the contractile effects of charybdotoxin (ChTX; a,b,c) and tetraethylammonium (TEA; d,e,f) on resting strips of carotid (a,d), femoral (b,e) and mesenteric (c,f) arteries from WKY (○) and SHR (●). Experimental conditions were the same as in Figure 2. The peak contractions induced by each concentration of ChTX and TEA are expressed as % of the maximum contraction induced by 65.9 mM $K^+$. At zero on the abscissa scale, the oscillatory contraction or the tonic contraction before the addition of ChTX or TEA is expressed as % of the maximum contraction induced by 65.9 mM $K^+$. Data points are means of the number of preparations indicated by each curve, and s.e.mean is shown by vertical bars. *Significantly different from WKY ($P<0.05$).

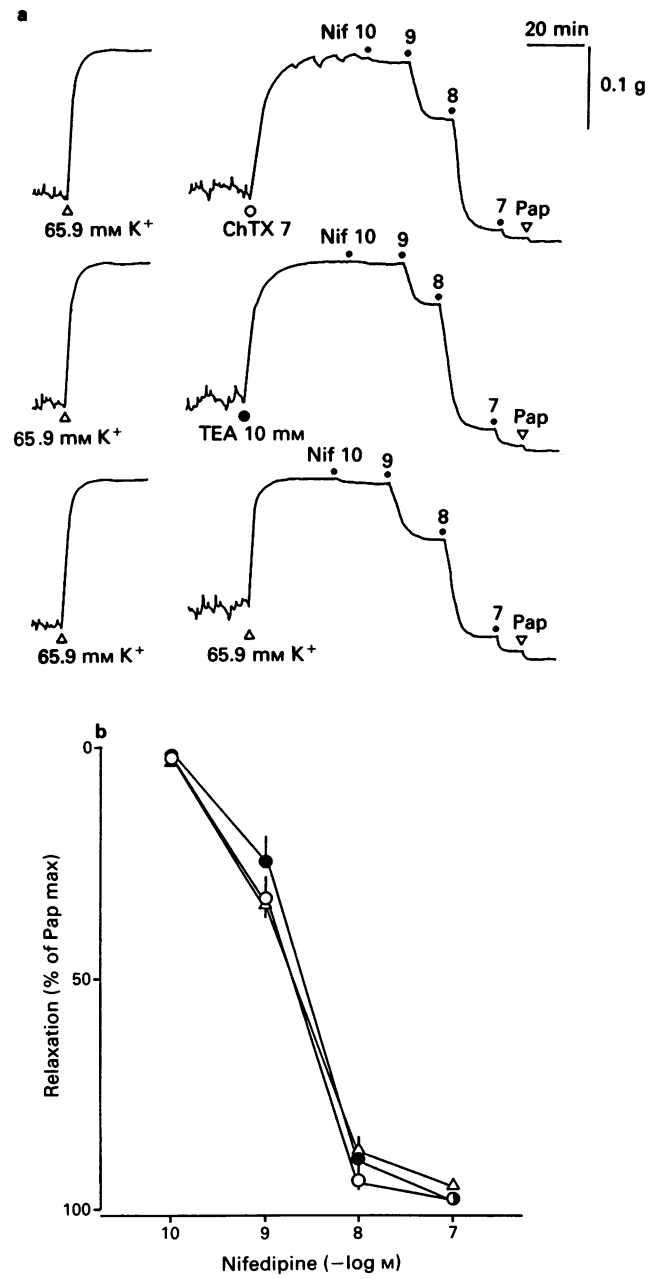


**Figure 4** The relaxant effect of nifedipine (Nif) on (13 weeks old) SHR carotid arteries contracted with charybdotoxin (ChTX), tetraethylammonium (TEA) and 65.9 mM $K^+$. (a) Typical recordings. After the application of 65.9 mM $K^+$, and washout with a Krebs solution as in Figure 1, the strips were contracted with $10^{-7}$ M ChTX, 10 mM TEA or 65.9 mM $K^+$. Nif was added in a cumulative fashion after the contraction had reached a plateau. At the end of each experiment, $10^{-4}$ M papaverine (Pap) was added to identify the position of the maximum relaxation. Concentrations of Nif are expressed as the negative log of the molar concentration. (b) Concentration-response curves for the relaxant effect of Nif on $10^{-7}$ M ChTX (○), 10 mM TEA (●) and 65.9 mM $K^+$ (△)-contracted strips. Relaxations induced by each concentration of Nif are expressed as % of the maximum relaxation induced by Pap. Data points are means of the number of preparations (ChTX, TEA and $K^+$, $n=5$) and s.e.mean is shown by vertical bars.

maximum contractions induced by 65.9 mM $K^+$.

The addition of TEA to the resting strips caused a concentration-dependent contraction in carotid, femoral and mesenteric arteries from SHR and WKY (Figure 3). Although the maximum contraction induced by 20 mM TEA in each artery was not significantly different between SHR and WKY, the threshold concentration for initiating contraction and the $EC_{50}$ value for TEA in each artery were significantly lower in SHR than in WKY. It is noteworthy that the contractions induced by relatively low concentrations (below 5 mM) of TEA in these arteries were similar to the ChTX-induced contractions. The difference in the TEA-induced contraction between SHR and WKY was in the order of carotid > femoral > mesenteric.

The addition of apamin (up to $10^{-6}$ M, $n=3-5$), or of glibenclamide (up to $5\times10^{-6}$ M, $n=3-5$), to the resting strips of the three arteries failed to cause a contraction in either SHR or WKY (data not shown).

### *Mechanism of charybdotoxin-induced contraction*

After the contraction of SHR carotid arteries induced by $10^{-7}$ M ChTX had reached a plateau, the addition of nifedipine produced a concentration-dependent relaxation (Figure 4a). Nifedipine also relaxed the arteries contracted by

either 10 mM TEA or 65.9 mM $K^+$ (Figure 4a). Concentration-response curves for the relaxant effect of nifedipine were similar among the ChTX-, TEA- and $K^+$-contracted arteries (Figure 4b). The contractile responses of SHR carotid arteries to ChTX were greatly attenuated by $10^{-7}$ M nifedipine or by $3 \times 10^{-6}$ M cromakalim (Figure 5). Nifedipine or cromakalim alone abolished the myogenic tone in SHR carotid arteries (Figure 5a). In the presence of either drug, ChTX produced only a transient contraction which was similar to that of WKY carotid arteries shown in Figures 2 and 3.

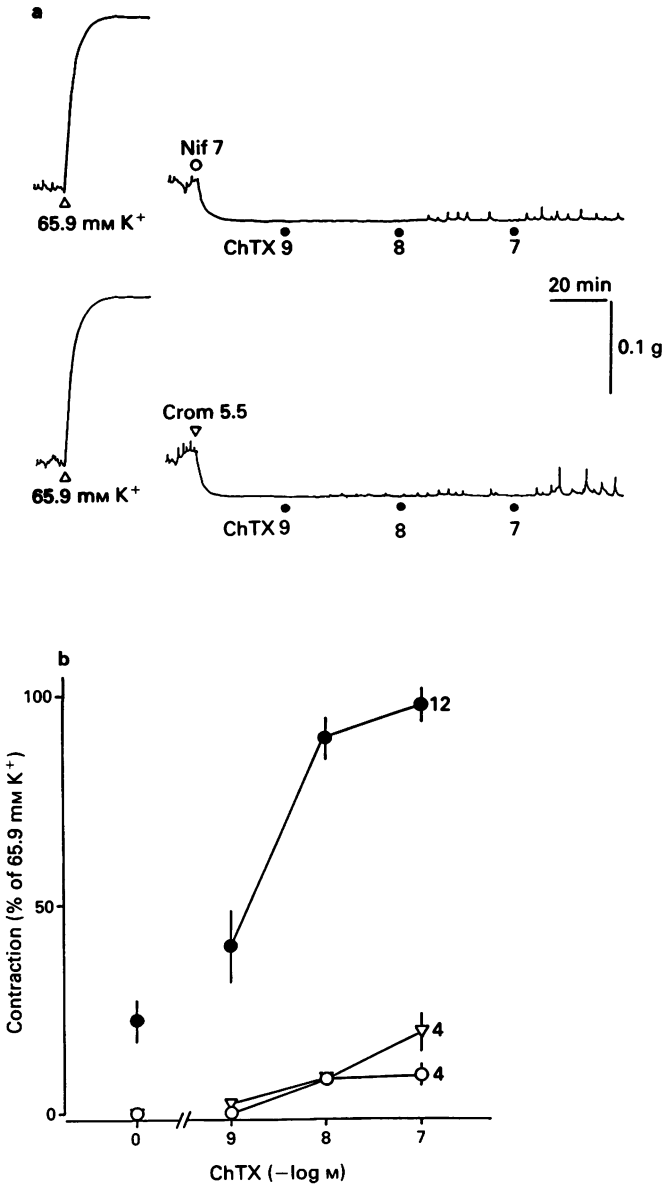


**Figure 5** Effects of nifedipine (Nif) and cromakalim (Crom) on the charybdotoxin (ChTX)-induced contraction in (13 weeks old) SHR carotid arteries. (a) Typical recordings. Nif ($10^{-7}$ M) or Crom ($3 \times 10^{-6}$ M) was added 30 min before the determination of the ChTX-induced contraction. The ChTX-induced contraction was determined as in Figure 2. Concentrations of Nif, Crom and ChTX are expressed as the negative log of the molar concentration. (b) Concentration-response curves for the ChTX-induced contraction in the absence (control, ●) and presence of either $10^{-7}$ M Nif (○) or $3 \times 10^{-6}$ M Crom (▽). The peak contractions induced by each concentration of ChTX are expressed as % of the maximum contraction induced by 65.9 mM $K^+$. At zero on the abscissa scale, the oscillatory contraction before the addition of ChTX is expressed as % of the maximum contraction induced by 65.9 mM $K^+$. Data points are means of the number of preparations indicated by each curve, and s.e.mean is shown by vertical bars.

### *$^{86}$Rb efflux from resting strips*

The basal $^{86}$Rb efflux rate constants in resting strips of carotid, femoral or mesenteric arteries were significantly greater in SHR than in WKY (Figure 6, Table 1). The addition of $10^{-7}$ M nifedipine to the resting strips of carotid, femoral and mesenteric arteries from SHR significantly decreased the basal $^{86}$Rb efflux rate constants (Figure 6, Table 1). Nifedipine also produced a relaxation in carotid and femoral arteries from SHR (Figure 6). However, $10^{-7}$ M nifedipine did not have an effect on $^{86}$Rb efflux and tension in the three arteries from WKY (Figure 6, Table 1). After exposure to $10^{-7}$ M nifedipine, the $^{86}$Rb efflux rate constants in carotid, femoral or mesenteric arteries from SHR were still significantly greater than those from WKY (Table 1).

### *Cellular $Ca^{2+}$ uptake*

The cellular $Ca^{2+}$ uptake in the resting state of carotid and femoral arteries from SHR was significantly increased when compared to WKY (Table 2). In carotid arteries, $10^{-7}$ M nifedipine abolished the increase in the cellular $Ca^{2+}$ uptake in SHR, but had no effect on WKY. The cellular $Ca^{2+}$ uptake in the presence of nifedipine became similar in the carotid arteries from SHR and WKY (Table 2). In the femoral arteries, although nifedipine reduced the cellular $Ca^{2+}$ uptake only in SHR, the cellular $Ca^{2+}$ uptake in the presence of nifedipine was still significantly increased in SHR when compared to WKY (Table 2).

## Discussion

The present study clearly demonstrated that the ChTX-sensitive $K_{Ca}$ channels were highly activated in the resting state of carotid, femoral and mesenteric arteries from SHR when compared to WKY. In SHR arteries, the primary defect responsible for the increased activation of ChTX-sensitive $K_{Ca}$ channels could be an increase in the cellular $Ca^{2+}$ uptake. Such $K_{Ca}$ channel activation may act as a negative feedback mechanism to regulate the level of myogenic tone in the resting state of SHR arteries. This conclusion arises from the following observations: (1) the addition of ChTX to the resting strips from SHR produced a potent contraction which was comparable to the maximum contraction induced by high $K^+$-depolarization; (2) a similar contraction was obtained in SHR when relatively low concentrations (below 5 mM) of TEA were added to the resting strips; (3) arterial contractions induced by ChTX and TEA were weak in WKY; (4) the $^{86}$Rb efflux from resting strips was significantly increased in SHR; (5) the cellular $Ca^{2+}$ uptake in resting arteries was significantly increased in SHR; and (6) nifedipine reduced the $^{86}$Rb efflux and the cellular $Ca^{2+}$ uptake only in SHR.

We initially compared the effect of ChTX on carotid, femoral and mesenteric arteries from SHR and WKY, because SHR arteries have been demonstrated to exhibit a myogenic tone in the resting state (Noon *et al.*, 1978; Fitzpatrick & Szentivanyi, 1980; Winquist & Bohr, 1983; Asano *et al.*, 1986). ChTX was used to determine if $K_{Ca}$ channels play a role in regulating resting tone in isolated arteries. The assumption was that if ChTX-sensitive $K_{Ca}$ channels regulate the resting tone, the blockade of the $K_{Ca}$ channels will contract arterial smooth muscle by causing membrane depolarization and the subsequent $Ca^{2+}$ influx via voltage-dependent $Ca^{2+}$ channels (Brayden & Nelson, 1992). If the depolarization and $Ca^{2+}$ influx activate $K_{Ca}$ channels, then a hyperpolarization of the membrane or inhibition of $Ca^{2+}$ influx should diminish the arterial contraction induced by $K_{Ca}$ channel blockers. This is the probable mechanism of ChTX-induced contractions demonstrated in the present study, because either the hyperpolarization of the membrane through the activation of $K^+$ channels by cromakalim or the

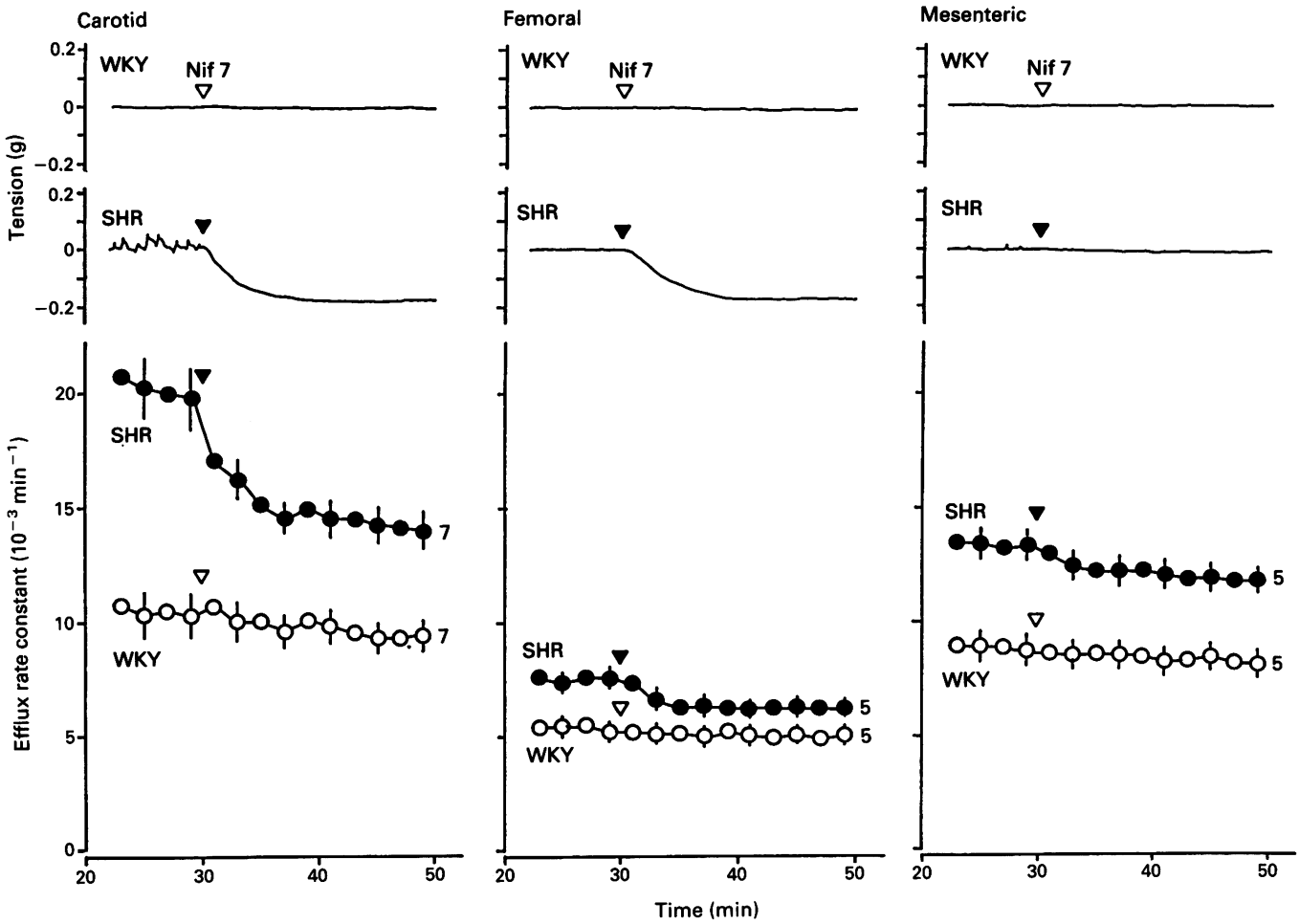


**Figure 6** The effect of nifedipine (Nif) on tension (two top panels) and $^{86}$Rb efflux (bottom panels) in resting strips of carotid, femoral and mesenteric arteries from WKY and SHR. The strips were incubated with $^{86}$Rb for 3 h in Krebs solution and then were washed out for the first 30 min with Krebs solution, followed by a 20 min washout with Krebs solution containing $10^{-7}$ M Nif. Ordinate scale: change in tension expressed as g or $^{86}$Rb efflux rate constant expressed as $10^{-3}$ min$^{-1}$. Abscissa scale: time (min) after start of the efflux period. The rate constants are means of the number of preparations indicated by each curve, and s.e.mean (representative values) is shown by vertical bars.

**Table 1** The effect of $10^{-7}$ M nifedipine on $^{86}$Rb efflux from resting strips of carotid, femoral and mesenteric arteries from WKY and SHR

| | | | $^{86}$Rb efflux rate constant ($10^{-3}$ min$^{-1}$)[a] | |
|---|---|---|---|---|
| *Artery* | *Rat* | n | *Before Nif*[b] | *After Nif*[c] |
| Carotid | WKY | 7 | 10.2 ± 1.0 | 9.9 ± 0.7 |
| | SHR | 7 | 19.7 ± 1.4* | 14.5 ± 0.8*† |
| Femoral | WKY | 5 | 5.2 ± 0.4 | 5.1 ± 0.3 |
| | SHR | 5 | 7.2 ± 0.4* | 6.2 ± 0.4*† |
| Mesenteric | WKY | 5 | 8.8 ± 0.7 | 8.5 ± 0.6 |
| | SHR | 5 | 13.4 ± 0.7* | 12.0 ± 0.6*† |

[a] The experimental conditions were the same as in Figure 6.
[b] $^{86}$Rb efflux rate constants before the exposure to $10^{-7}$ M nifedipine (Nif) were measured between the 26th and 30th min of efflux.
[c] $^{86}$Rb efflux rate constants after the exposure to $10^{-7}$ M nifedipine (Nif) were measured between the 40th and 44th min of efflux.
Data are expressed as means ± s.e.mean, and *n* indicates the number of preparations used.
*Significantly different from WKY ($P<0.05$).
†Significantly different from 'Before Nif' ($P<0.05$).

inhibition of $Ca^{2+}$ influx by nifedipine almost abolished the ChTX-induced contraction. The present study clearly demonstrates the increased function of ChTX-sensitive $K_{Ca}$ channels in SHR arteries. The ChTX-induced contractions can be explained pharmacologically by a blocking action of ChTX on large conductance $K_{Ca}$ channels. A similar explanation can be given for the TEA-induced contractions. In the present study, TEA, at concentrations below 5 mM, caused a contraction which was similar to the ChTX-induced contraction in each artery. At low concentrations, TEA preferentially blocks large conductance $K_{Ca}$ channels, although at high concentrations it blocks other types of $K^+$ channels (Cook, 1988; Nelson *et al.*, 1990; Brayden & Nelson, 1992). Furthermore, the large conductance $K_{Ca}$ channel is the only known $K^+$ channel in arterial smooth muscle that is blocked by either ChTX or low concentrations of TEA (Nelson *et al.*, 1990).

The $^{86}$Rb efflux experiments also demonstrated the increased $K^+$ permeability in carotid, femoral and mesenteric arteries from SHR as compared to WKY. An increased $K^+$ permeability in SHR arteries was initially reported by Jones (1973; 1974a,b). The finding that nifedipine reduced the basal $^{86}$Rb efflux rate constant only in SHR arteries clearly indicates that extracellular $Ca^{2+}$ enters the cell via L-type voltage-dependent $Ca^{2+}$ channels in the resting state of SHR arteries, activates $K_{Ca}$ channels, and thus contributes to the increased $K^+$ permeability. Similar results were reported for the resting state of the aorta from aldosterone-salt hypertensive rats in which the effects of calcium channel blockers on basal tension and the elevated $^{42}$K efflux were evaluated (Smith & Jones, 1990). Judging from the effect of nifedipine

**Table 2** The effect of $10^{-7}$ M nifedipine on the cellular $Ca^{2+}$ uptake in the resting state of carotid and femoral arteries from WKY and SHR

| *Condition* | *Rat* | *Cellular $Ca^{2+}$ uptake* (nmol $g^{-1}$ wet tissue)[a] *Carotid* | *Femoral* |
|---|---|---|---|
| Control | WKY | 60.1 ± 1.6 (18) | 82.1 ± 2.4 (15) |
| | SHR | 71.2 ± 1.7* (18) | 103.1 ± 3.4* (15) |
| Nifedipine $10^{-7}$ M[b] | WKY | 58.6 ± 1.3 (18) | 84.3 ± 2.8 (15) |
| | SHR | 61.4 ± 2.0† (18) | 94.1 ± 2.0*† (15) |

[a]Isolated carotid and femoral arteries were incubated with $^{45}Ca$ for 5 min in Tris-buffered solution (5.4 mM $K^+$) before a 60 min washout in 80.8 mM $La^{3+}$-substituted solution.
[b]Nifedipine was added 60 min before the application of and also during the $^{45}Ca$ incubation period.
Data are expressed as means ± s.e.mean, and numbers in parentheses indicate the number of preparations used.
*Significantly different from WKY ($P<0.05$).
†Significantly different from 'Control' ($P<0.05$).

on $^{86}Rb$ efflux shown in Figure 6, the contribution of the $Ca^{2+}$ influx to the increased $K^+$ permeability was greater in carotid and femoral arteries than in mesenteric arteries.

High activation of $K_{Ca}$ channels in the resting state of SHR arteries may reflect the increased cellular $Ca^{2+}$ uptake in these arteries, because the $K_{Ca}$ channels are activated by both cellular $Ca^{2+}$ and membrane depolarization (Cook, 1988; Castle *et al.*, 1989). The increased cellular $Ca^{2+}$ uptake or content in the resting state has been demonstrated in aortae from SHR (van Breemen *et al.*, 1986; Jelicks & Gupta, 1990; Sada *et al.*, 1990). The present study also demonstrated the increased cellular $Ca^{2+}$ uptake in the resting state of carotid and femoral arteries from SHR. The mechanisms responsible for the increased cellular $Ca^{2+}$ uptake in SHR was different between carotid and femoral arteries. In SHR carotid arteries, the increase in cellular $Ca^{2+}$ uptake was abolished by nifedipine, suggesting that the increased $Ca^{2+}$ influx through the opening of L-type voltage-dependent $Ca^{2+}$ channels contributed to the increased cellular $Ca^{2+}$ uptake. However, additional factors are also contributing to the increased cellular $Ca^{2+}$ uptake in SHR femoral arteries, because the increase in the cellular $Ca^{2+}$ uptake was not completely abolished by nifedipine. The opening of voltage-dependent $Ca^{2+}$ channels in arterial smooth muscle normally requires membrane depolarization (Bean *et al.*, 1986). Therefore, a likely explanation for the increased $Ca^{2+}$ influx in resting strips of SHR appears to be that SHR arteries are more depolarized in the resting state than WKY arteries (Asano *et al.*, 1986). Although no measurements of the resting membrane potential are available in the present study, membrane depolarization in SHR arteries has been demonstrated in the mesenteric artery (Fujii *et al.*, 1992), the tail artery (Cheung, 1984), and the aorta (Tomobe *et al.*, 1991). The level of the resting membrane potential in arterial smooth muscle largely determines the resting tone and the sensitivity to vasoconstrictors, since the distribution and conductance of ions across the plasma membrane are influenced by the resting membrane potential. However, we do not exclude the possibility that the voltage-dependent $Ca^{2+}$ channels in resting strips of SHR exhibited a different dependence on membrane potential when compared to WKY. Verification that the SHR arteries are depolarized or that the voltage-dependent $Ca^{2+}$ channels in these arteries behave differently at a normal resting membrane potential will require detailed electrophysiological studies on each artery.

The increased cellular $Ca^{2+}$ uptake produces the myogenic tone. The assumption was that if the membrane depolarization and the subsequent $Ca^{2+}$ influx produce the myogenic tone, then a hyperpolarization of the membrane or inhibition of $Ca^{2+}$ influx should reduce the myogenic tone. This is the probable mechanism of the appearance of myogenic tone, because either the hyperpolarization of the membrane through the activation of $K^+$ channels by cromakalim or the inhibition of $Ca^{2+}$ influx by nifedipine almost abolished the myogenic tone. Therefore, the resting tone of SHR arteries is determined by the net balance of two opposing phenomena; a contraction due to the increased cellular $Ca^{2+}$ uptake (probably through the activation of myosin light chain kinase), and a relaxation due to the activation of $K_{Ca}$ channels. The net balance of the two phenomena resulted in the oscillatory and/or tonic contraction that we observe in resting strips of SHR. The finding of myogenic tone in resting strips of SHR clearly indicates that the contraction due to the increased cellular $Ca^{2+}$ uptake exceeded the relaxation due to the activation of $K_{Ca}$ channels. Our results were consistent with a previous report of enhanced TEA-induced contractions in carotid arteries of stroke-prone SHR compared with WKY (Thompson *et al.*, 1987). These authors concluded that an alteration of voltage-dependent $Ca^{2+}$ channels in these arteries was a likely explanation for the differential effect of TEA.

In the resting state of WKY arteries, $K_{Ca}$ channels must be very infrequently open, because the function of $K_{Ca}$ channels in arterial smooth muscle is to open when cellular $Ca^{2+}$ rises and so exert a repolarizing action on the membrane potential. This may be the case in carotid arteries. However, the $K_{Ca}$ channels were moderately activated in the resting state of femoral and mesenteric arteries from WKY, because ChTX produced moderate contractions in these arteries. The $K_{Ca}$ channels in these two arteries probably were involved in the repolarization of the membrane which resulted in the disappearance of the myogenic tone in these arteries. The moderate activation of $K_{Ca}$ channels in femoral and mesenteric arteries suggests that the basal $Ca^{2+}$ contents in these arteries are higher than the carotid arteries. The difference in the basal $Ca^{2+}$ content among the WKY arteries may result in the variation of the $K_{Ca}$ channel function in these arteries, and hence the regional difference in the ChTX- or TEA-induced contraction between SHR and WKY. The difference in the basal $Ca^{2+}$ content among the WKY arteries did not depend on the $Ca^{2+}$ influx via L-type voltage-dependent $Ca^{2+}$ channels. These observations suggest a direct link between the increased $Ca^{2+}$ influx via L-type voltage-dependent $Ca^{2+}$ channels and the activation of $K_{Ca}$ channels in SHR arteries.

Small conductance $K_{Ca}$ channels might be activated in the resting state of SHR arteries by the increased cellular $Ca^{2+}$ uptake. However, this is unlikely, because apamin had no effect on the myogenic tone. Although small conductance $K_{Ca}$ channels have been found in some vascular smooth muscles, it has not been determined whether the channels are sensitive to apamin (Inoue *et al.*, 1985; Benham *et al.*, 1986). Thus, it is likely that apamin-sensitive $K_{Ca}$ channels do not exist in the arteries used in the present study. The myogenic tone was not affected by glibenclamide but was abolished by cromakalim, suggesting that the cromakalim-activated $K^+$ channels are present but do not appear to be activated in association with the myogenic tone in SHR arteries.

In conclusion, the present study clearly demonstrated that the ChTX-sensitive $K_{Ca}$ channels were highly activated in the

resting state of SHR arteries. We propose that the activation of ChTX-sensitive $K_{Ca}$ channels, caused by the increase in the cellular $Ca^{2+}$ uptake, is an important negative feedback mechanism that regulates the resting tone in SHR arteries. This regulatory mechanism is likely to influence the resting tone in many arteries, including carotid, femoral and mesenteric arteries. In these arteries, the primary defect responsible for the increased $K^+$ permeability could be an increase in the transmembrane $Ca^{2+}$ influx via L-type voltage-dependent $Ca^{2+}$ channels.

## References


ASANO, M., AOKI, K. & MATSUDA, T. (1986). Contractile effects of Bay k 8644, a dihydropyridine calcium agonist, on isolated femoral arteries from spontaneously hypertensive rats. *J. Pharmacol. Exp. Ther.*, **239**, 198–205.

ASANO, M. & HIDAKA, H. (1985). Pharmacological properties of N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7), a calmodulin antagonist in arterial strips from rats and rabbits. *J. Pharmacol. Exp. Ther.*, **234**, 476–484.

ASANO, M., MASUZAWA, K. & MATSUDA, T. (1988). Evidence for reduced β-adrenoceptor coupling to adenylate cyclase in femoral arteries from spontaneously hypertensive rats. *Br. J. Pharmacol.*, **94**, 73–86.

BEAN, B.P., STUREK, M., PUGA, A. & HERMSMEYER, K. (1986). Calcium channels in muscle cells isolated from rat mesenteric arteries: modulation by dihydropyridine drugs. *Circ. Res.*, **59**, 229–235.

BENHAM, C.D., BOLTON, T.B., LANG, R.J. & TAKEWAKI, T. (1986). Calcium-activated potassium channels in single smooth muscle cells of rabbit jejunum and guinea-pig mesenteric artery. *J. Physiol.*, **371**, 45–67.

BOHR, D.F. & WEBB, R.C. (1988). Vascular smooth muscle membrane in hypertension. *Annu. Rev. Pharmacol. Toxicol.*, **28**, 389–409.

BRAYDEN, J.E. & NELSON, M.T. (1992). Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science*, **256**, 532–535.

CASTLE, N.A., HAYLETT, D.G. & JENKINSON, D.H. (1989). Toxins in the characterization of potassium channels. *Trends Neurosci.*, **12**, 59–65.

CHEUNG, D.W. (1984). Membrane potential of vascular smooth muscle and hypertension in spontaneously hypertensive rats. *Can. J. Physiol. Pharmacol.*, **62**, 957–960.

COOK, N.S. (1988). The pharmacology of potassium channels and their therapeutic potential. *Trends Pharmacol. Sci.*, **9**, 21–28.

FITZPATRICK, D.F. & SZENTIVANYI, A. (1980). The relationship between increased myogenic tone and hyporesponsiveness in vascular smooth muscle of spontaneously hypertensive rats. *Clin. Exp. Hypertens.*, **2**, 1023–1037.

FUJII, K., TOMINAGA, M., OHMORI, S., KOBAYASHI, K., KOGA, T., TAKATA, Y. & FUJISHIMA, M. (1992). Decreased endothelium-dependent hyperpolarization to acetylcholine in smooth muscle of the mesenteric artery of spontaneously hypertensive rats. *Circ. Res.*, **70**, 660–669.

INOUE, R., KITAMURA, K. & KURIYAMA, H. (1985). Two Ca-dependent K-channels classified by the application of tetraethylammonium distribute to smooth muscle membranes of the rabbit portal vein. *Pflügers Arch.*, **405**, 173–179.

JELICKS, L.A. & GUPTA, R.K. (1990). NMR measurement of cytosolic free calcium, free magnesium, and intracellular sodium in the aorta of the normal and spontaneously hypertensive rat. *J. Biol. Chem.*, **265**, 1394–1400.

JONES, A.W. (1973). Altered ion transport in vascular smooth muscle from spontaneously hypertensive rats. Influences of aldosterone, norepinephrine, and angiotensin. *Circ. Res.*, **33**, 563–572.

JONES, A.W. (1974a). Reactivity of ion fluxes in rat aorta during hypertension and circulatory control. *Fed. Proc.*, **33**, 133–137.

JONES, A.W. (1974b). Altered ion transport in large and small arteries from spontaneously hypertensive rats and the influence of calcium. *Circ. Res.*, **34 & 35** (Suppl. I). I-117–I-122.

LAU, K. & EBY, B. (1985). The role of calcium in genetic hypertension. *Hypertension*, **7**, 657–667.

MASUZAWA, K., ASANO, M., MATSUDA, T., IMAIZUMI, Y. & WATANABE, M. (1990). Comparison of effects of cromakalim and pinacidil on mechanical activity and $^{86}Rb$ efflux in dog coronary arteries. *J. Pharmacol. Exp. Ther.*, **253**, 586–593.

MASUZAWA, K., MATSUDA, T. & ASANO, M. (1991). The diverse effects of cromakalim on tension and $^{86}Rb$ efflux in canine arterial smooth muscle. *Br. J. Pharmacol.*, **103**, 1033–1040.

NELSON, M.T., PATLAK, J.B., WORLEY, J.F. & STANDEN, N.B. (1990). Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am. J. Physiol.*, **259**, C3–C18.

NOON, J.P., RICE, P.J. & BALDESSARINI, R.J. (1978). Calcium leakage as a cause of the high resting tension in vascular smooth muscle from the spontaneously hypertensive rats. *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 1605–1607.

PAVENSTADT, H., LINDEMAN, S., LINDEMAN, V., SPATH, M., KUNZELMANN, K. & GREGER, R. (1991). Potassium conductance of smooth muscle cells from rabbit aorta in primary culture. *Pflügers Arch.*, **419**, 57–68.

POSTNOV, Y.V. & ORLOV, S.N. (1985). Ion transport across plasma membrane in primary hypertension. *Physiol. Rev.*, **65**, 904–945.

SADA, T., KOIKE, H., IKEDA, M., SATO, K., OZAKI, H. & KARAKI, H. (1990). Cytosolic free calcium of aorta in hypertensive rats. Chronic inhibition of angiotensin converting enzyme. *Hypertension*, **16**, 245–251.

SMITH, J.M. & JONES, A.W. (1990). Calcium antagonists inhibit elevated potassium efflux from aorta of aldosterone-salt hypertensive rats. *Hypertension*, **15**, 78–83.

SUGG, E.E., GARCIA, M.L., REUBEN, J.P., PATCHETT, A.A. & KACZOROWSKI, G.J. (1990). Synthesis and structural characterization of charybdotoxin, a peptidyl inhibitor of the high conductance $Ca^{2+}$-activated $K^+$ channel. *J. Biol. Chem.*, **265**, 18745–18748.

THOMPSON, L.P., BRUNER, C.A., LAMB, F.S., KING, C.M. & WEBB, R.C. (1987). Calcium influx and vascular reactivity in systemic hypertension. *Am. J. Cardiol.*, **59**, 29A–34A.

TOMOBE, Y., ISHIKAWA, T., YANAGISAWA, M., KIMURA, S., MASAKI, T. & GOTO, K. (1991). Mechanisms of altered sensitivity to endothelin-1 between aortic smooth muscles of spontaneously hypertensive and Wistar-Kyoto rats. *J. Pharmacol. Exp. Ther.*, **257**, 555–561.

VAN BREEMEN, C., CAUVIN, C., JOHNS, A., LEIJTEN, P. & YAMAMOTO, H. (1986). $Ca^{2+}$ regulation of vascular smooth muscle. *Fed. Proc.*, **45**, 2746–2751.

WINQUIST, R.J. & BOHR, D.F. (1983). Structural and functional changes in cerebral arteries from spontaneously hypertensive rats. *Hypertension*, **5**, 292–297.


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