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Role of Ca^+-dependent K-channels in the membrane potential and contractility of aorta from spontaneously hypertensive rats

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1 Contractile responses to KCl and membrane potentials were determined in aortic rings from spontaneously hypertensive rats (SHR), normotensive Wistar rats (NWR) and Wistar Kyoto rats (WKY) both in the absence and in the presence of the Ca^{2+}-dependent K-channel blockers, apamin and tetraethylammonium (TEA).

2 Compared to NWR, aortic rings from WKY and SHR were less reactive and their Ca^{2+} uptake after stimulation with K^+ was decreased.

3 Smooth muscle cell membrane potentials were higher in aortae from SHR and WKY than in NWR aortae, whereas SHR had higher K^+ and lower Na^+ intracellular activities than WKY and NWR, suggesting overactivity of the Na^+/K^+ pump in the hypertensive animals.

4 Treatment with apamin caused depolarization of WKY and SHR aortae, and increased their contractile responses to the same level as those of the NWR. Treatment with TEA also caused depolarization of aortae from WKY and SHR, but in the SHR the depolarization induced by TEA was smaller than that produced by apamin and the contractile responses to KCl did not reach the level of those of aortae from NWR.

5 It is concluded that overactivity of Ca^{2+}-dependent K-channels in aortae of WKY and SHR contributes to their higher membrane potentials and lower responsiveness to vasoconstrictor stimuli. In SHR, an overactive Na^+/K^+ pump is also present, and the contribution of apamin-sensitive Ca^{2+}-dependent K-channels to the membrane potential and reactivity appears to be more relevant than that of TEA-sensitive channels.

Keywords: Aorta; Ca^{2+}-dependent K-channels; hypertension; membrane potential; smooth muscle contraction; spontaneously hypertensive rats

Introduction

In order to understand the mechanisms underlying hypertension, several studies on vascular reactivity have been conducted in hypertensive animals. In conductance vessels, such as the aorta, preparations from spontaneously hypertensive rats (SHR) show decreased contractility when compared to those of normotensive Wistar rats (NWR) (Spector *et al.*, 1969; Shibata *et al.*, 1973).

Control of the membrane potential is critical for determining vascular contractility (Harder & Hermsmeyer, 1983; Nelson *et al.*, 1990), and in aortic smooth muscle Ca^{2+}-dependent K-channels seem to make an important contribution to the membrane potential (Sadoshima *et al.*, 1988; Shoemaker & Worrel, 1991) and therefore to Ca^{2+}-influx and vascular contractility. Single channel measurements (Shoemaker & Worrel, 1991; England *et al.*, 1993) showed that Ca^{2+}-dependent K^+ currents are increased in the SHR aorta when compared to those of normotensive controls.

In the present work we have explored the contributions of membrane potential, calcium influx and Ca^{2+}-dependent K-channels to the reported decreased contractility of SHR aortae. Thus, we attempted to characterize further the Ca^{2+}-dependent K-channels involved, by measuring the effects of tetraethylammonium (TEA) and apamin on the membrane potential and contractility of aortae from SHR, from their normotensive Wistar Kyoto controls (WKY) and from NWR. We also measured the intracellular concentrations of sodium and potassium, as well as the K^+-induced Ca^{2+} influx, in aortic smooth muscle cells from these three animal strains. Our results suggest that the low contractility of SHR aortae may be ascribed to hyperpolarization of the smooth muscle cell membrane, which is due, at least in part, to an overactive Na^+/K^+ pump, to stimulated Ca^{2+}-dependent K-channels (particularly those of the apamin-sensitive type) and to decreased voltage-dependent Ca^{2+} influx.

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Methods

Animals

The Okamoto-Aoki strain of female SHR (170–190 g body weight) and their Wistar-Kyoto normotensive controls (WKY, 210–230 g body weight) were derived from an original colony supplied by the National Institutes of Health, Bethesda, MD, U.S.A. Normotensive Wistar rats (NWR, 190–215 g body weight) were from a colony inbred at the Escola Paulista de Medicina. The animals were of similar age (20–30 weeks).

Tension measurements

The animals were decapitated and bled, the thoracic aorta was removed and placed in Krebs-bicarbonate solution of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.18, $MgSO_4$ 1.18, $NaHCO_3$ 25, glucose 10 (pH 7.40). Rings of 1-cm length were cut and everted. A plastic rod was inserted through the lumen and the edge of the blood vessel was pulled with the help of a pair of tweezers, thus inverting the ring. The endothelium was removed by rubbing with a thin plastic rod wrapped in cotton. The presence or absence of a functional endothelium was tested in all preparations by checking whether acetylcholine induced relaxation of the preparations, a response

which is characteristic for vessels with an intact endothelium (Furchgott, 1981). The preparations were mounted in chambers containing 6.0 ml of Krebs-bicarbonate solution under the optimum passive tension of 1 g, previously determined as described by Gleason *et al.* (1985). The temperature was kept at 37 ± 0.5°C and the solution was bubbled with a gas mixture of 95% O_2 and 5% CO_2. After an equilibration period of 2 h the isometric contractile responses were recorded.

Cumulative dose-response curves were obtained by stepwise increase of the concentration of KCl, performed in the presence of 10^{-6} M phentolamine to exclude a possible influence of endogenous catecholamine release. These experiments were done both in the absence and in the presence of TEA (1 mM) or apamin (100 nM) added to the bath 20 or 7 min before the addition of KCl, respectively.

Measurement of cellular Na^+ content

Aortae from SHR, WKY and NWR were mounted on small plates and kept under tension in a bath containing Krebs-bicarbonate solution to which ^{99m}Tc-diethyleneaminepentaacetic acid was added in a final concentration of 2.5 μCi ml^{-1}. The tissues were equilibrated for 20 min in this medium and then transferred to tubes containing non-radioactive solution. The radioactivity in the tissues was counted in a gamma counter (Nucleopan-Siemens), after which they were dried by blotting with filter paper, and the wet weight was determined. The dry weight was also measured after the tissues were kept for 20 h in an oven at 100°C. The tissues were then ashed in beakers containing 0.5 ml of a mixture of equal amounts of HNO_3 (61 g per 100 ml H_2O) and $HClO_4$ (60 g per 100 ml H_2O), then dissolved in 0.01 N HCl, and the electrolytes were analyzed by atomic emission spectroscopy.

The extracellular space was calculated from the ratio of the radioactivities in the tissue and in the experimental solution (McAfee *et al.*, 1979). The intracellular Na^+ concentration (in mmol kg^{-1}) was calculated by subtracting from the total Na^+ content (in mmol kg^{-1}) the product of the extracellular Na^+ concentrtation (in mmol l^{-1}) and the extracellular space (in $l.kg^{-1}$).

Measurements of cell membrane potential (V_m) intracellular potassium activity (a^i_K) and intracellular sodium activity (a^i_{Na})

Microelectrodes were constructed as previously described (Harvey & Kernan, 1984) by pulling double-barrelled capillaries in a horizontal puller (Narishige model PN3). The thicker barrel was siliconized, its tip was filled with either potassium or sodium ion exchanger and the volume was completed respectively with either 0.1 M KCl or 0.5 M NaCl solution. The reference barrels (narrower) were filled with either a 1:3 (v:v) mixture of 1 M NaCl and 1 M sodium acetate solutions (potassium microelectrode) or with 1 M KCl (sodium microelectrode) and were used to measure membrane potential. The resistance of the reference barrels ranged between 150 and 200 × 10^6 Ω and their tip potential was less then 5 mV.

The properties of the potassium electrodes were tested in solutions containing 10, 50, 100 and 150 mM KCl, and in isotonic solutions containing mixtures of KCl and NaCl in different proportions. The resistance of the selective barrel averaged 3.75 ± 0.48 × 10^9 Ω and a potential change of 59.8 ± 0.8 mV ($n = 20$) was found for a decade change in a_K. The selectivity coefficients of the electrodes ($K_{K/Na}$) averaged 0.016 ± 0.007, indicating that the interference from Na^+, and hence the possible error in the a^i_K, was small. Therefore, a^i_K was calculated by means of the following equation:

$$a^i_K = a^e_K \times 10^{(\Delta V s^{-1})}$$

where a^e_K is the extracellular potassium activity, ΔV is the difference between the voltages recorded in the selective barrel and in the reference barrel and S is the slope of the selective electrode's calibration curve.

The sodium electrodes were tested in pure NaCl solutions (5, 10, 35 and 70 mM) and in isotonic solutions prepared with KCl in different proportions. Their resistances averaged 1.2 ± 0.1 × 10^{11} Ω, and a potential change of 53.0 ± 1.5 mV ($n = 40$) was found for a decade change in Na^+ activity. Their selectivity coefficient ($K_{Na/K}$) averaged 0.020 ± 0.004, and consequently, no correction for interferent ion (K^+) was necessary. a^i_{Na} was calculated by means of the following equation:

$$a^i_{Na} = a^e_{Na} \times 10^{(\Delta V s^{-1})}$$

The electrodes were mounted in Ag/AgCl half-cells on a micromanipulator (Leitz) and connected to the two channels of an electrometer (WP Instruments, model FD223). The signals from the reference and differential channels were also recorded on a potentiometric chart recorder (ECB, model RB102).

The preparations were placed in a 2-ml chamber and superfused at a rate of 3 ml min^{-1} with Krebs-bicarbonate solution at 37°C, bubbled with a gas mixture containing 5% CO_2 and 95% O_2. As the microelectrode was inserted into the cell from the intimal side, everted rings were used. The experiments were performed in preparations that had been previously mounted under a tension of 1 g during the 2 h that preceded the impalements. Some measurements of membrane potential were also carried out in rings that had been incubated for 7 min with 100 nM apamin or for 20 min with 1 mM TEA before the impalements.

The criteria for accepting an impalement were: (a) abrupt change in the potential recorded with microelectrodes upon entry and withdrawal from the cell; (b) stable potential (± 3 mV) for at least 1 min after impalement; (c) minimal changes in microelectrode tip potential (<20%) and resistance (<10%) after impalement; (d) reproducible calibration curves of the ion-selective barrel before and after experiments. According to the electrical characteristics (Firth & De Felice, 1971), the average diameter of the electrode tip was estimated to be ca. 0.2 μm.

Cell culture

Primary cultures of smooth muscle cells were obtained from the tunica media of NWR, WKY and SHR thoracic aorta as previously described (Yamamoto *et al.*, 1983; Chamley *et al.*, 1985). The cells were dispersed by incubating the tissue with solution containing 1 mg ml^{-1} collagenase type I, 12 u ml^{-1} elastase type I and 1% bovine serum albumin at 37°C for 2 h in humidified atmosphere of 5% CO_2 and 95% air. The cells were seeded in 35-mm Falcon tissue culture dishes (10^5 cells/dish) and grown in Dulbecco's Modified Eagle Medium containing 10% foetal bovine serum, 100 u ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Every three days the medium was renewed, and the cells were confluent after approximately 10 days, when they were subcultured. The identity and homogeneity of the cells were indicated by positive fluorescence with antibodies against myosin and actin (Yamamoto *et al.*, 1983). The viability of the cells was determined by the trypan blue exclusion method and was found to be greater than 90%. Cultures seeded at the same time were used in each experiment.

Ca^{2+} uptake

The culture medium was removed and the cells were rinsed and incubated for 1 h at 37°C in a physiological salt solution (PSS) of the following composition (in mM): NaCl 137, KCl 2.7, $MgCl_2$ 0.49, $CaCl_2$ 1.36, glucose 5.5 and HEPES 10. After this period of equilibration, 1 ml of the PSS and 2 μCi

of $^{45}Ca^{2+}$ were added to the plates in the absence (controls) or presence of 50 mM KCl. After 120 s, the assay medium was siphoned off and the cells were quickly rinsed twice with 1 ml of ice-cold 0.1 M $MgCl_2$ solution containing 10 mM HEPES and 10 mM $LaCl_3$. The plates were then quickly rinsed by successively dipping in three beakers containing 500 ml of ice-cold 0.1 M $MgCl_2$ (with 10 mM HEPES). Intracellular $^{45}Ca^{2+}$ was extracted with 1 ml of 0.1 N NaOH and the radioactivity was measured by liquid scintillation counting. The Ca^{2+} uptake in the controls was defined as 100% in each strain and after exposure to KCl stimulation the uptake was calculated as a percentage of the controls.

Drugs

Apamin, tetraethylammonium and phentolamine were from Sigma. ^{99m}Tc-diethyleneaminepentaacetic acid was from IPEN (São Paulo, Brazil).

Statistics

Mean values of individual data points from the concentration-response curves were analyzed by one-way analysis of variance followed by the Newman-Keuls test (Snedecor & Cohran, 1976) to compare results in NWR, WKY and SHR aortae, or employing Student's t test for paired data to compare results in treated and untreated aortae from each strain. The measurements of membrane potentials, intracellular K^+ and Na^+ activities and Ca^{2+} uptake were compared by using one-way analysis of variance followed by the Newman-Keuls test. A probability of $P<0.05$ was considered significant.

Results

Mechanical responses to high K^+

Dose-response curves to K^+ were obtained in aortic rings under hyperosmotic conditions in which KCl was simply added to the medium to attain the desired concentration. Figures 1a and 2a show that the contractile responses to K^+ were significantly higher for NWR than for WKY and SHR. Some experiments were also performed under isosmotic conditions, by the replacement of NaCl in the Krebs solution, and similar results were obtained (data not shown).

Incubation with apamin (100 nM) did not affect the responses of aortic rings from NWR, but caused a marked enhancement in the WKY and SHR responses, which reached the same level of the NWR responses (Figure 1b).

When the preparations were incubated with 1 mM TEA (Figure 2b) the responses of the NWR aortae were unaltered, whereas those of the WKY aortae increased significantly, up to the level of the NWR responses. The responses of the SHR aortae were little affected by TEA, with only the lower portion of the dose-response curve being significantly raised.

The TEA and apamin concentrations used in these experiments (1 mM and 100 nM, respectively) did not produce contraction in the aortic preparations from any of the three strains. Preparations which were previously treated with TEA (1 mM) showed contractile responses to apamin concentrations in the range from 200 to 700 nM (data not shown), indicating that 1 mM TEA was not inhibiting apamin-sensitive channels.

Membrane potential

Measurements of the membrane potentials of aortic rings from the three strains showed that the SHR preparations were hyperpolarized in relation to those from the two normotensive controls. The membrane potential of aortic rings from WKY had an intermediate value between those of SHR and NWR (Figure 3).

After incubation with apamin (100 nM), a significant depolarization was observed in aortic rings from WKY and SHR but not in those from NWR. Treatment with TEA (1 mM) also caused a significant depolarization in WKY and SHR. The depolarization induced by TEA in SHR aortae, however, was smaller than that induced by apamin. Some original records of membrane potential both in the absence and in the presence of apamin are shown in Figure 4.

Intracellular K^+ and Na^+

Intracellular K^+ and Na^+ activities were measured by impaling aortic smooth muscle cells from rings that were previously stretched under the same conditions that occurred in the preparations used for the tension measurement experiments (1 g for 2 h).

Table 1 shows that the K^+ activity was higher and the Na^+ activity was lower in aortic rings from SHR than in those from NWR and WKY, which did not differ from each

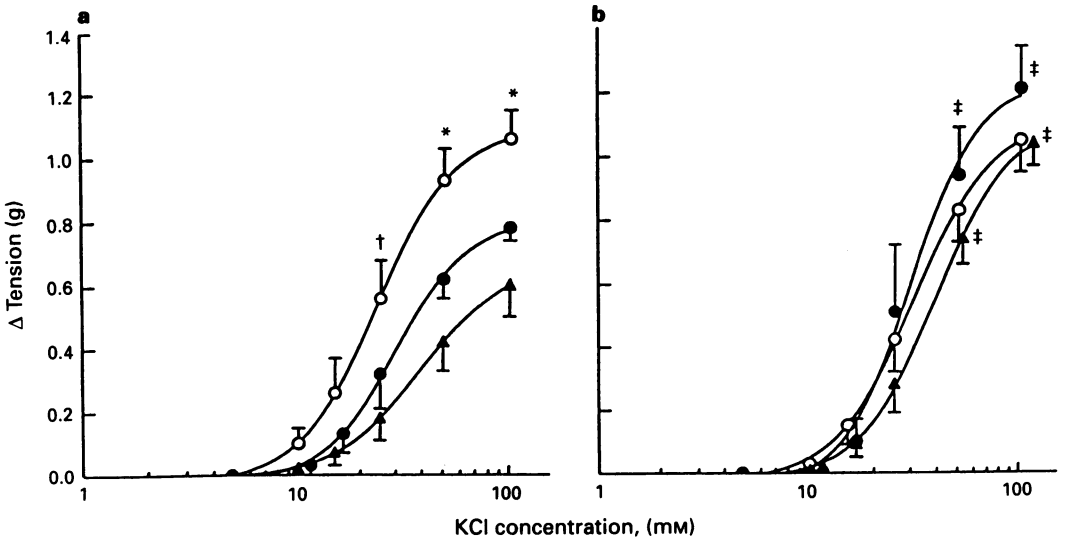

Figure 1 Responses of aortic rings from NWR (○, $n = 8$), WKY (●, $n = 5$) and SHR (▲, $n = 8$) to cumulative increases in KCl concentration in the absence (a) and in the presence (b) of apamin (100 nM). Data are means with s.e.means. *Significantly different from WKY and SHR. †Significantly different from SHR. ‡Significantly different from the respective controls without apamin ($P<0.05$).

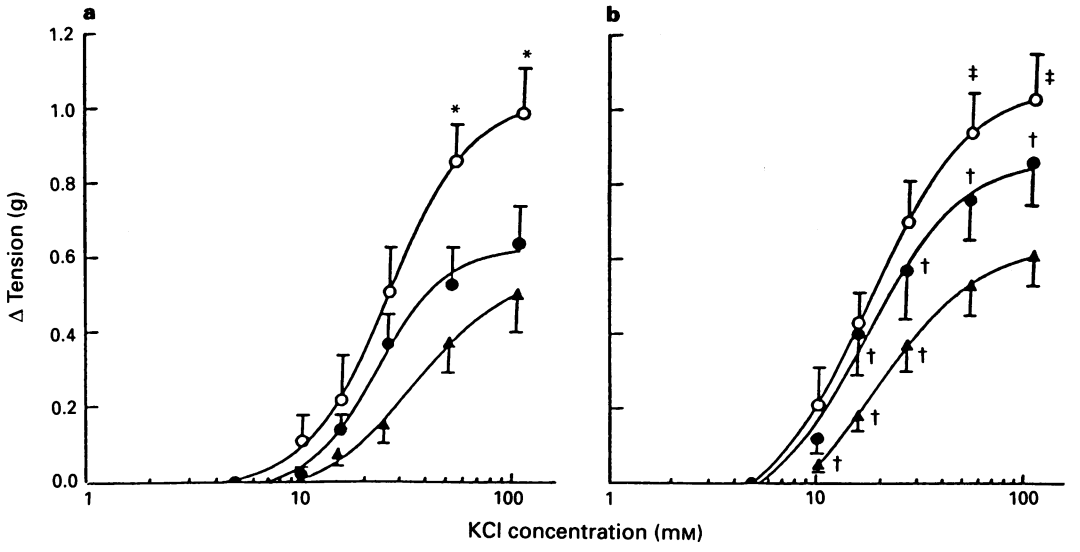

Figure 2 Responses of aortic rings from NWR (○, $n = 5$), WKY (●, $n = 6$) and SHR (▲, $n = 7$) to cumulative increases in KCl concentration in the absence (a) and in the presence (b) of TEA (1 mM). Data are means with s.e.means. *Significantly different from WKY and SHR. †Significantly different from the respective controls without TEA. ‡Significantly different from SHR ($P < 0.05$).

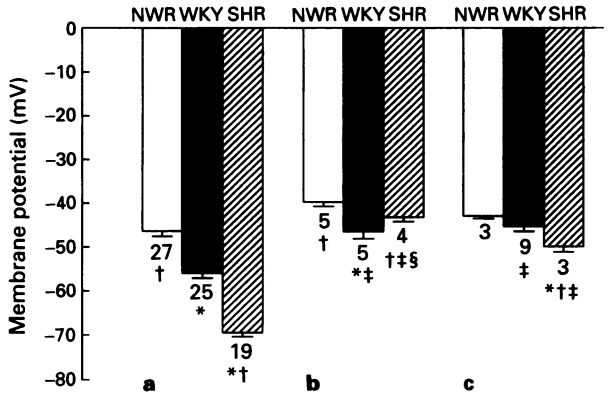

Figure 3 Membrane potentials of aortic rings from NWR, WKY and SHR in the absence (a) and in the presence of 100 nM apamin (b) or TEA 1 mM (c). Values are means and the s.e.means of the number of experiments are given below the columns. *Significantly different from NWR. †Significantly different from WKY. ‡Significantly different from the respective controls without apamin or TEA. §Significantly different from the respective measurements in the presence of TEA ($P < 0.05$).

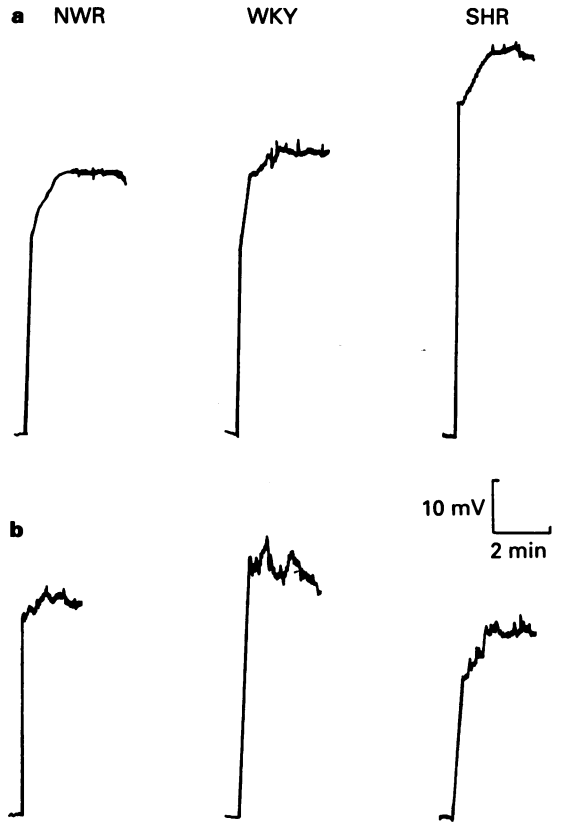

Figure 4 Original records of measurements of membrane potential obtained from aortae of NWR, WKY and SHR in the absence (a) and in the presence of 100 nM apamin (b).

other. These results were corroborated by the measurements of the intracellular Na^+ content performed by chemical analysis, which showed significantly lower values in SHR than in either NWR or WKY (Table 1).

Equilibrium potential for potassium

The values of equilibrium potential for potassium (E_K) were calculated from the values of the intracellular K^+ activity (a^i_K) measured in aortae from NWR, WKY and SHR, by using the Nernst equation. Table 2 shows that, although the E_K values differed from the respective membrane potentials in aortic preparations from WKY and SHR, the difference was significantly more pronounced in preparations from NWR.

Ca^{2+} uptake

The results of the measurements of Ca^{2+} uptake in cultured aortic cells from NWR, WKY and SHR in response to KCl (50 mM) are shown in Figure 5. The stimulation of the cells with KCl for 120 s induced an uptake of Ca^{2+} which was significantly higher in NWR (74.3 ± 7.3% in relation to the control without KCl) than in WKY (29.5 ± 11.5%) and SHR (11.5 ± 1.5%) cells. The basal values of Ca^{2+} uptake in a representative triplicate were 0.52 ± 0.03, 0.65 ± 0.09 and 0.65 ± 0.17 nmol/10^6 cells. 120 s in aortic cells from NWR, WKY and SHR, respectively.

Table 1 Potassium activity, sodium activity and sodium content values for rings of NWR, WKY and SHR aortae

Preparation	*Potassium activity* (mM)	*Sodium activity* (mM)	*Sodium content* (mmol kg^{-1})
NWR	62.1 ± 5.8 (11)	16.4 ± 1.2 (15)	36.3 ± 3.4 (10)
WKY	53.5 ± 5.1 (10)	14.0 ± 0.7 (15)	37.4 ± 4.4 (11)
SHR	79.7 ± 3.7 (9)*	9.0 ± 0.5 (10)*	23.6 ± 4.3 (10)*

Intracellular potassium and sodium activities were measured with potassium and sodium-sensitive microelectrodes, and sodium content was measured by atomic emission spectroscopy. Values are means and the s.e.means of the number of experiments are shown in parentheses. *Significantly different from NWR and WKY ($P<0.05$).

Table 2 Membrane potential (V_m) and equilibrium potential for potassium (E_K) in aortae from NWR, WKY and SHR

Preparation	V_m (mV)	E_K (mV)
NWR	−46.9 ± 1.2 (27)*	−68.9 ± 2.3 (11)
WKY	−56.0 ± 1.1 (15)*	−65.1 ± 2.1 (10)
SHR	−69.5 ± 0.9 (19)*	−76.3 ± 1.2 (9)

Membrane potentials were measured with microelectrodes and the K^+ equilibrium potentials were determined from the intracellular potassium activities by using the Nernst equation. Values are means and s.e.mean of the number of experiments shown in parentheses. *Significantly different from the respective values of E_K ($P<0.05$).

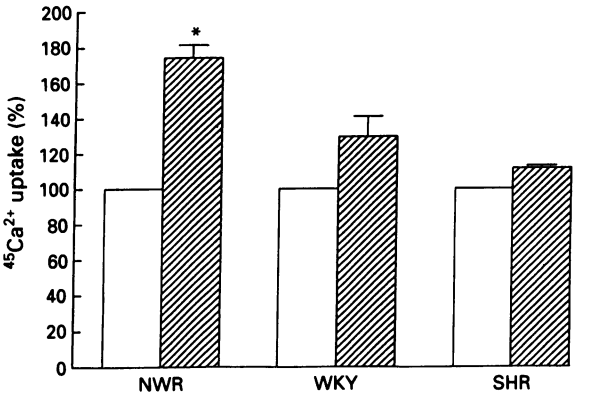

Figure 5 Ca^{2+} uptake in aortic cells of NWR ($n = 3$), WKY ($n = 2$) and SHR ($n = 2$) in response to the stimulation with KCl (50 mM) for 120 s. Ca^{2+} uptake after KCl stimulation was calculated as a percentage of the controls. Each experiment was made in triplicate and the values are means with s.e.means. *Significantly different from WKY and SHR ($P<0.05$).

Discussion

Previous work has shown that preparations from SHR have decreased contractility when compard to those of normotensive Wistar rats (Spector *et al.*, 1969; Shibata *et al.*, 1973). We have observed, however, that WKY aortae are also less reactive than those of NWR to stimulation with potassium (Figures 1a and 2a). Apparently, some properties of smooth muscles from WKY may be closer to those of the SHR than to those of NWR, and therefore may not be pertinent to the hypertensive state. Thus, previous work from our laboratory showed that duodenum preparations from both WKY and SHR develop contractile responses to bradykinin, which are not observed in those from NWR (Feres *et al.*, 1992a).

The lower reactivity of SHR and WKY aortae may be due to the fact that their smooth muscle cell membranes are hyperpolarized in relation to those of NWR. In fact, Figure 3 shows that the membrane potential mesured in aortic smooth muscle cells is larger in WKY than in NWR and larger in SHR than in WKY aortae. This finding differs from that of England *et al.* (1993), who reported comparable membrane potentials in current clamped cells of WKY and SHR. However, these authors recognized that membrane potential values obtained by current clamp methods are somewhat less reliable than those measured with microelectrode methods. Tomobe *et al.* (1992), however, used microelectrode measurements and found SHR aortae to be less polarized than those of WKY. This divergence from our results might be related to the fact that these authors used aortic strips, which were shown to be depolarized in comparison to aortic rings, possibly because of Na^+ leakage into the cells due to lesions made during preparation of the strips (Frediani-Neto *et al.*, 1991).

To obtain further information on the status of our cells, we also measured the intracellular Na^+ and K^+ activities by using ion-selective microelectrodes, a technique not previously employed in aortae from hypertensive rats. Table 1 shows that the intracellular activity of K^+ is higher and that of Na^+ is lower in aortic preparations of SHR than in those of WKY and NWR. The lower intracellular content of Na^+ in SHR aortae was also evidenced through measurements done by chemical analysis. These findings indicate that the sodium pump is more active in aortae from SHR than in those of WKY and NWR, and are in agreement with the previous findings that SHR aortae are more responsive to ouabain and to K^+-free solutions, when compared with WKY (Moreland *et al.*, 1986). Moreover, an overactive sodium pump may explain the hyperpolarized membrane potential that we observed in SHR aortae.

It is known that the contractile responses to potassium are dependent on the influx of calcium from the extracellular medium through voltage-dependent calcium channels (Van Breemen *et al.*, 1972). Since we found that the cellular membranes of aortae from WKY and SHR were more polarized than those of NWR aortae, it is possible that a smaller number of calcium channels is activated during stimulation by potassium, resulting in reduced Ca^{2+} influx and decreased responses of the WKY and SHR aortae in relation to those of NWR. The smaller Ca^{2+} uptake observed in K^+-stimulted aortae from WKY and SHR in relation to those from NWR (Figure 4) supports this idea.

We have also investigated further the hypothesis that Ca^{2+}-dependent K-channels are involved in the differences observed in the behaviour of aortae from SHR, WKY and NWR. Three basic types of Ca^{2+}-dependent K-channels have been reported in muscle cells: (a) a large-conductance (135–250 pS) voltage-dependent channel which is inhibited by concentrations of TEA below 1 mM, described in a variety of non-vascular and vascular smooth muscle cells (Sadoshima *et al.*, 1988; Nelson *et al.*, 1990; Brayden *et al.*, 1992); (b) a weakly voltage-dependent channel with intermediate conductance (55 pS), described in vascular smooth muscle cells (Shoemaker & Worrel, 1991); (c) a small-conductance (10–14 pS) channel which is voltage-independent and is specifically blocked by apamin, described in many tissues (Romey & Lazdunski, 1984; Blatz & Magleby, 1986; Feres *et al.*, 1992b), although not as yet in vascular smooth muscle cells. An increase in the activity of any of these channels would lead to the augmented K^+ permeability described in aortae from hypertensive rats (Jones, 1973; Tamura *et al.*, 1986; Nakamura *et al.*, 1988) and could also result in alterations of vascular membrane potential and contractility.

We have studied the effect of the two Ca^{2+}-dependent K-channel blockers, apamin and TEA, on the contractile responses to K^+ and on the membrane potential of aortae from the three rat strains. In tension measurement experiments (Figure 1), we found that the contractile responses to K^+, which were relatively impaired in SHR and

WKY, were enhanced by treatment with apamin, reaching the level of the responses of the NWR aortae. Treatment with TEA also increased the responses of WKY and SHR aortae, but those of the WKY were significantly more enhanced than those of the SHR (Figure 2). This suggests that either the inhibition by 1 mM TEA was not specific, affecting also apamin-sensitive channels, or that both apamin-sensitive and TEA-sensitive channels may be involved in the lower reactivity of WKY and SHR aortae to K^+. In any case, the impaired SHR responses to K^+ are more dependent than those of WKY on apamin-sensitive channels.

The hypothesis that an increased activity of Ca^{2+}-dependent K-channels may contribute to the hyperpolarized state of smooth muscle membranes of aortae from WKY and SHR was also investigated by studying the effect of apamin and TEA on the membrane potentials. When the aortic rings from NWR were incubated with TEA or apamin, no changes in the membrane potential were observed in relation to the non-treated controls, but preparations from WKY and SHR were significantly depolarized (Figure 3), indicating that the activities of apamin- and TEA-sensitive Ca^{2+}-dependent K^+ channels are enhanced in aortae from both WKY and SHR. However, as the depolarization induced by both Ca^{2+}-dependent K^+ channel blockers was higher in aortae from SHR than in those from WKY, the activity of these channels is probably higher in the SHR than in WKY. In addition, whereas WKY aortae were equally depolarized by TEA and apamin, the depolarization induced by apamin in the SHR was greater than that induced by TEA. These results, together with those of the tension measurements discussed above, suggest that the contribution of the apamin-sensitive channels may be more important in SHR aortae than that of TEA-sensitive Ca^{2+}-dependent K^+ channels to the maintenance of an increased membrane potential and decreased reactivity.

Our results are partly in agreement with recent observations obtained from single channel measurements. Rusch *et al.* (1992) have established a link between K-channel modulation and Ca-channel activation in SHR aorta. Shoemaker & Worrel (1991) described intermediate conductance channels the open probability of which was higher in SHR than in WKY aortae and also higher in WKY than in NWR aortae. England *et al.* (1993) found that high conductance Ca^{2+}-dependent K^+ channels were more active in SHR than in WKY aortic cells. However, these reports do not mention the low-conductance apamin-sensitive channels, the existence of which is suggested by our present results.

The activity of the Ca^{2+}-dependent K-channels of smooth muscles (Singer & Walsh, 1984; Cecchi *et al.*, 1986) and other kinds of cells (French & Wells, 1977; Yellen, 1984) can be reduced by internal Na^+ ions. Thus, besides the intrinsic overactivity of these channels in hypertensive animals reported by Shoemaker & Worrel (1991) and England *et al.* (1993), they may also be activated by a lower intracellular Na^+ concentration due to the increased activity of the Na^+/K^+ pump that we have observed in aortae from SHR. Moreover, it has been demonstrated that the intracellular Ca^{2+} concentration is increased in SHR vascular smooth muscles (Bohr & Webb, 1988), including the aorta (Sada *et al.*, 1990), and this could also result in the increased activity of Ca^{2+}-dependent K-channels.

Thus, our results suggest that the higher membrane potential and the lower contractility observed in WKY and SHR in relation to NWR aortae could rather be attributed to an increased activation of Ca^{2+}-dependent K-channels related both to the rat strain and to hypertension. In SHR and WKY, but not in NWR aortae, the values for E_K are very close to the respective calculated values of membrane potential (Table 2) which could indicate that the permeability for potassium is increased in aortae from these animals. However, in SHR, but not in WKY, an increased activity of the Na^+/K^+ pump and the consequent decrease in the intracellular Na^+ concentration could favour the activation of the Ca^{2+}-dependent K^+ channels, contributing to the more polarized state of the SHR aorta.

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