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AN INCREASED CALCIUM SENSITIVITY OF MESENTERIC RESISTANCE VESSELS IN YOUNG AND ADULT SPONTANEOUSLY HYPERTENSIVE RATS

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1 We have measured the calcium sensitivity in response to noradrenaline stimulation and potassium depolarization of isolated segments of 100 to 200 μm mesenteric resistance vessels from spontaneously hypertensive (SHR) and control Wistar-Kyoto (WKY) rats. The rats were either young (4 wk) or adult (4 months), that is of ages before or after the SHRs had developed elevated blood pressure. Experiments were performed under conditions in which the effect of noradrenaline uptake by, and the potassium-induced noradrenaline release from, the nerve terminals in the vessel walls was eliminated.

2 The response of the SHR and WKY vessels to noradrenaline under conditions where only the extracellular calcium appeared to have been removed was similar. When subsequently stimulated maximally by noradrenaline, the calcium-sensitivity of the SHR vessels ($Ca\text{-}ED_{50} \simeq 0.1$ mM) was greater than that of the WKY vessels ($Ca\text{-}ED_{50} \simeq 0.2$ mM). When depolarized by potassium, all vessels were less sensitive to calcium and there was little difference in the calcium sensitivity of SHR and WKY vessels in either age group ($Ca\text{-}ED_{50} \simeq 0.8$ mM).

3 The results suggest that whereas the potassium (potential)-dependent calcium permeability of the SHR smooth muscle cell membrane is normal, the noradrenaline-induced calcium permeability is abnormally high. The presence of this abnormality in the vessels from the young SHRs suggests that it may be a factor involved in the aetiology of hypertension in the SHR.

Introduction

Noradrenaline, generally considered to be the principle transmitter of the sympathetic vascular control mechanism, activates vascular smooth muscle by causing an increase of the intracellular free calcium concentration. It is thought (see Bolton, 1979) to do this both by releasing calcium from intracellular stores and by increasing the calcium permeability of the plasma membrane to allow the influx of extracellular calcium. Therefore the sensitivity of the vasculature to noradrenaline is in part dependent on the size of the intracellular stores and on the degree of the calcium permeability induced by noradrenaline.

In an attempt to determine whether differences in the calcium handling properties of the vasculature could be involved in the aetiology of genetic hypertension, investigators have performed perfusion experiments on vascular beds isolated from the spontaneously hypertensive rat (SHR) developed by Okamoto & Aoki (1963). These experiments indicate that the vasculature of the SHR has both a greater noradrenaline sensitivity (Finch & Haeusler, 1974; Lais & Brody, 1978) and a greater calcium sensitivity in response to noradrenaline stimulation (Folkow, Hallbäck, Jones & Sutter, 1977). Perfusion experiments however have the disadvantage that the effects of structural factors are difficult to distinguish, nor is it easy to identify which parts of the vascular bed are responding. Furthermore it is becoming increasingly clear that the noradrenaline sensitivity of the vasculature is greatly influenced by the presence of the nerve terminals in the vessel wall (Vanhoutte, 1978). Therefore it is desirable to investigate the behaviour of isolated vessels, in particular those small enough to contribute to the peripheral resistance. Until recently this has not been possible, and experiments have been confined to aorta and conduit vessels, and the results have been conflicting (Bohr, 1974). However, using the method described by Mulvany & Halpern (1976), we (Mulvany, Aalkjaer & Christensen, 1980a) and our colleagues (Whall, Myers & Halpern, 1980) have demonstrated that small (150 to 200 μm) mesenteric resistance vessels from adult SHRs have, if the effects of the nerve terminals are eliminated, a greater noradrenaline sensitivity than corresponding control Wistar-Kyoto (WKY) vessels. Moreover the increased sensitivity is already present in vessels taken from young (6 wk) SHRs, that is before they have developed elevated blood pressure (Mulvany *et al.*, 1980a).

The purpose of the present investigation was to examine whether these differences in noradrenaline

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sensitivity could be explained by differences in the calcium handling properties of the vessels. The small size of the vessels has so far prevented direct measurement of these parameters using conventional radioisotope techniques. As an alternative we have therefore used the isometric wall tension response of the vessels as an index of the intracellular concentration of free calcium. The amount of intracellular calcium released by noradrenaline has been assessed by examining the noradrenaline response under conditions which indicated that the extracellular space was depleted of calcium. The calcium permeability of the membrane was assessed by determining the calcium sensitivity of the vessels under conditions in which the vessels were either maximally activated by noradrenaline or nearly fully depolarized by exposure to a high potassium solution. The experiments were done under conditions where the effects of the nerve terminals were eliminated by the use of cocaine to block neuronal noradrenaline uptake (de la Lande, Frewin & Watterson, 1967) and phentolamine to inhibit the effect of noradrenaline released from the nerve terminals by potassium (Lorenz & Vanhoutte, 1975). The results suggest that there is little difference either in the amount of calcium released from the intracellular stores or in the calcium permeability induced by depolarization. However, the increase in calcium permeability caused by noradrenaline appears to be greater in the SHR vessels both before (at age 4 wk) and after (at age 4 months) the onset of elevated blood pressure.

Some of the results have already been presented in preliminary form (Mulvany, Christensen & Nyborg, 1980b).

Methods

Animal characteristics

Young (4 wk) or adult (4 months) spontaneously hypertensive (SHR) or control Wistar-Kyoto (WKY) rats were anaesthetized with ketamine (1 mg/g, Ketalar, Parke-Davis). Mean blood pressure measurements were taken by direct cannulation of the abdominal aorta. The heart was then removed and the ventricular weights obtained. These measurements are shown in Table 1, together with details concerning the age and weight of the rats.

Dissection and myograph

Segments of mesenteric resistance vessels were taken from these rats (one vessel per rat) and mounted on a myograph. The myograph was essentially similar to that described earlier (Mulvany & Halpern, 1976), but was designed so that two segments could be mounted in the same chamber. Experiments were therefore carried out with one vessel from a SHR and one from a corresponding WKY. Segments were about 1.5 mm in length and had an internal diameter of about 150 μm. The segments were second generation vessels of the superior mesenteric artery (Mulvany *et al.*, 1980a). The vessels were threaded onto two wires which were attached to a force transducer (Kistler Morse, DSC6) and a micrometer, respectively. Thus the myograph permitted direct measurement of vessel isometric wall tension while the internal circumference was controlled. The myograph was mounted on the stage of a microscope so that vessel dimensions could be measured. Solutions were changed by draining the chamber and refilling with the new solution.

Solutions

Vessels were dissected, mounted and held relaxed in a physiological salt solution (PSS) of the following composition (mM): NaCl 119, $NaHCO_3$ 14.9, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $CaCl_2$ 2.5, ethylenediaminetetraacetic acid (EDTA) 0.026 and glucose, 5.5. K-PSS was the same as PSS, but there was an equimolar exchange of NaCl for KCl. x-Ca-PSS was the same as PSS except that the $CaCl_2$ concentration was x. Ca-free-PSS was the same as PSS except that calcium was omitted and 0.1 mM ethylene glycol bis (β-aminoethylether)-*N*-*N*′-tetraacetic acid (EGTA) was included. EGTA-PSS was the same as Ca-free PSS except that the EGTA concentration was 5 mM. NA-K-PSS was used to activate vessels maximally

Table 1 Characteristics of spontaneously hypertensive (SHR) and control Wistar-Kyoto (WKY) rats used in these experiments

		Young	*Adult*
Age (day)	SHR	30 ± 1	117 ± 6
	WKY	30 ± 1	117 ± 6
Body weight(g)	SHR	64 ± 3*	277 ± 6*
	WKY	74 ± 2	308 ± 9
Heart weight (mg)	SHR	299 ± 11	917 ± 20*
	WKY	270 ± 9	813 ± 12
$\frac{\text{Heart wt.}}{\text{Body wt.}}$ (mg/g)	SHR	4.6 ± 0.1*	3.3 ± 0.1*
	WKY	3.7 ± 0.1	2.6 ± 0.1
Mean blood pressure (mmHg)	SHR	52 ± 3	166 ± 11*
	WKY	53 ± 3	106 ± 5
Pulse (min^{-1})	SHR	197 ± 22	274 ± 18*
	WKY	206 ± 13	211 ± 12

Values given as mean ± s.e.; 10 rats in young groups, 9 rats in adult groups. SHR and WKY values have been compared at each age at *t* test: $*P < 0.05$.

(Mulvany & Warshaw, 1979) and consisted of K-PSS to which 10 μM noradrenaline was added.

All solutions were kept at 37°C, pH 7.4 and bubbled with 5% CO_2 in O_2. Drugs used were (−)-noradrenaline hydrochloride (Sigma), cocaine chloride (May and Baker) and phentolamine (Regitine, CIBA).

Normalization

After mounting, vessels were set to internal circumference $L_0 = 0.9\ L_{100}$ where L_{100} is an estimate of the internal circumference the vessel would have had when relaxed *in situ* under a transmural pressure of 100 mmHg. L_0 is approximately the circumference at which maximum active wall tension is developed (Mulvany & Warshaw, 1979). L_{100} was obtained as described earlier by determining the point on the resting wall tension-internal circumference characteristic which corresponded to 100 mmHg (Mulvany & Halpern, 1977).

Morphology

With vessels set to internal circumference L_0, we measured the thickness of the media within the vessel walls. The microscope was focussed on the wall at the point where the vessel wrapped around the wires. Operating at 500 power with normal bright field illumination, the media layer was clearly defined. Its thickness was measured at four positions with an ocular micrometer.

Pharmacology

The dose-response characteristics of the vessels to noradrenaline and potassium were determined by exposing the vessels to geometrically increasing concentrations of noradrenaline (or potassium) with 2 min at each concentration. The response for each concentration was measured from the wall tension at the end of each 2 min period. Where vessels produced rhythmic activity (see Results) the response was measured from the mean wall tension for the last 20 s of each period. The method of determining the dose-response characteristics of the vessels to Ca was essentially similar: further details are given in Results (see Figure 3). For all dose-response experiments we determined the concentrations required to give half maximal responses (ED_{50}). This was done by linear interpolation of plots of the characteristics with concentrations on a logarithmic scale. Where indicated, ED_{50}s are expressed in terms of their pD_2 values where $pD_2 = \log_{10}(ED_{50}(M))$.

Mechanical characteristics

Experiments were initiated and terminated by activating vessels three times with NA-K-PSS. Moreover throughout experiments following each dose-response determination, the vessel was activated with NA-K-PSS to check its mechanical condition. If the response to NA-K-PSS fell by more than 20% the experiment was terminated.

The mechanical responses have been expressed in three ways (Mulvany & Halpern, 1977): (a) as active wall tension, ΔT, that is the increase in measured force divided by twice the segment length; (b) as active media stress, that is active force per unit media area, calculated as active wall tension divided by media thickness; (c) as effective active pressure, ΔP, that is an estimate of the pressure against which the vessel would have been able to contract *in vivo* at the given level of activation. This is calculated using Laplace's equation from

$$\Delta P = \Delta T/(L/2\pi),$$

where L is the internal circumference.

Statistics

The results have been analyzed as follows. In Table 1 and in the text we have used the two-tailed Student's *t* test to compare SHR parameters with their age-matched WKY controls, differences being considered significant for $P < 0.05$. In Tables 2, 3, 4, where we wished to compare both differences between strains and the effects of age, we have used analysis of variance (Sokal & Rolf, 1979). There the given values of P_{strain} and P_{age} give the probability that the differences seen between, respectively, the strains and the ages of the rats concerned could have arisen by chance. Also shown is $P_{interaction}$ where this is <0.05. In such cases this implies that the parameter concerned develops differently with age in the two strains. Therefore where $P_{interaction} < 0.05$, we have not used the data to draw any conclusions about the development of the parameter with age, but have treated the data as in Table 1.

Results

Noradrenaline and potassium responses

Figure 1 demonstrates the effect of withdrawing Ca^{2+} from the bathing solution on the response of vessels to noradrenaline and potassium. In PSS containing 2.5 mM Ca^{2+}, the responses to noradrenaline and potassium are similar (Figure 1a, d), both consisting of an initial rapid increase in tension followed by a more gradual increase in the following 1 min. Thereafter the tension normally declined by up to 10%. If the activating solutions did not contain Ca^{2+} but 0.1 mM EGTA (Figure 1b, e) then the tension fell after the initial rapid increase in tension: at the end of a 2 min

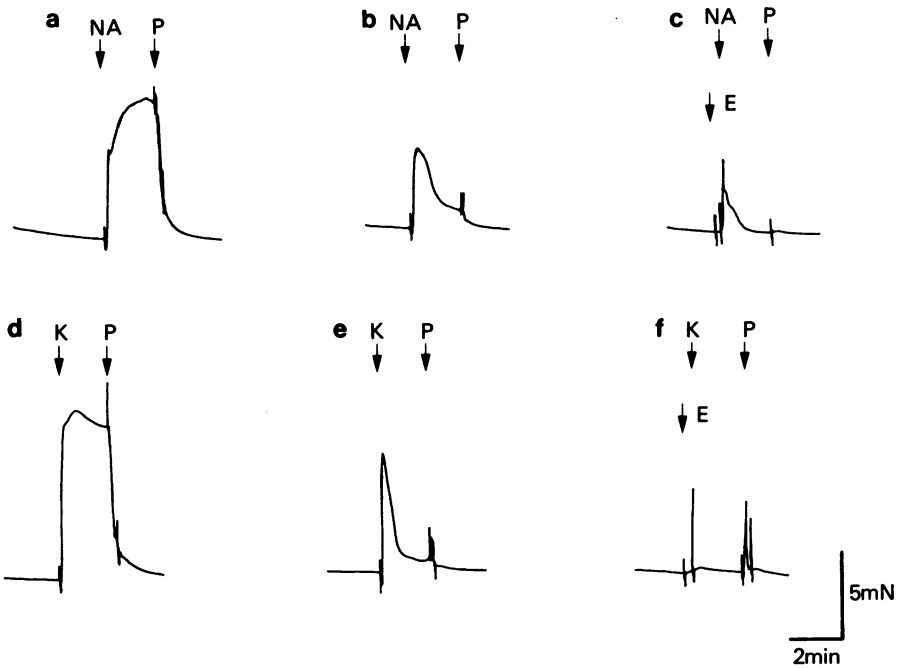

Figure 1 Effect of calcium-free solutions on noradrenaline (a–c) and potassium (d–e) responses of mesenteric resistance vessel from adult spontaneously hypertensive rat. In all cases vessels were initially held in PSS (physiological salt solution). At NA (upper responses) solution was changed to PSS containing 10 μM noradrenaline. At K (lower responses) solution was changed to K-PSS (containing 125 mM potassium). In (a) and (d) these activating solutions contained 2.5 mM calcium. For other responses the activating solutions contained no calcium, but 0.1 mM EGTA. In (c) and (f), before exposure to the activating solutions, the vessel was exposed at E for 10 s to EGTA-PSS (containing 5 mM EGTA and no calcium). Solutions were changed back to PSS at times marked P. Vessel segment length = 1.76 mm. L_0/π = 277 μm.

period by about 70% with noradrenaline stimulation and to near zero tension with potassium stimulation. If the same Ca-free activating solutions were used following a 10 s period in EGTA-PSS (containing 5 mM EGTA), very different responses were obtained (Figure 1c, f). With noradrenaline stimulation, a transient response lasting about 1 min was seen, while with potassium stimulation there was no response. Subsequent stimulations with either Ca-free noradrenaline or Ca-free potassium solutions (see Figure 3) produced no further responses. These effects were essentially the same in both SHR and WKY vessels. It therefore appeared that the vessels could be depleted of available Ca^{2+} by a 10 s immersion in 5 mM EGTA followed by activation with Ca-free noradrenaline solution (Christensen, 1979).

The responses to both noradrenaline and potassium are affected by the presence of nerve terminals in the vessel wall (Vanhoutte, 1978). These terminals take up noradrenaline so that the concentration of noradrenaline in the cleft gap surrounding the α-adrenoceptors is below that of the surrounding solution. Furthermore, the terminals release noradrenaline upon potassium stimulation. Therefore the effects of noradrenaline and potassium on the vascular smooth muscle cells are best studied by eliminating the effects of the nerve terminals. Here we have done this by adding cocaine (3 μM) to the noradrenaline activating solutions and phentolamine (1 μM) to the potassium activating solutions. Figure 2 demonstrates the effects of the drugs. Cocaine enhances the response to 2 μM noradrenaline but does not affect the response to 10 μM noradrenaline or to potassium. Phentolamine eliminates the response to noradrenaline and reduces the steady response to potassium by about 50%.

Calcium sensitivity

The calcium sensitivity of the vessels has been investigated by experiments similar to those shown in Figure 3, in which the calcium sensitivity of vessels in response to noradrenaline (Figure 3a) and to potassium (Figure 3b) have been determined. Vessels were first depleted of available activator calcium by brief immersion in EGTA-PSS followed by activation with 10 μM noradrenaline in Ca-free PSS as described

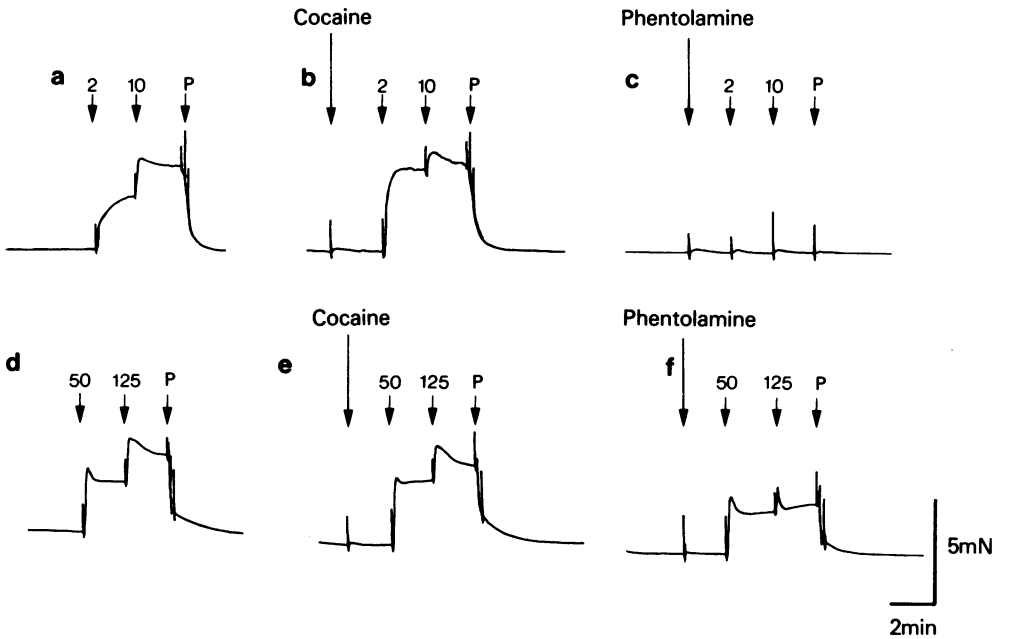

Figure 2 Effects of cocaine and phentolamine on noradrenaline (a–c) and potassium (d–f) responses of mesenteric resistance vessel from adult spontaneously hypertensive rat. In all cases vessel was initially bathed in physiological salt solution (PSS). Vessel was then challenged (upper records) with 2 μM (at 2) and 10 μM (at 10) noradrenaline in PSS, and (lower records) with 50 mM (at 50) and 125 mM (at 125) potassium (equimolar exchange of NaCl for KCl in PSS). In (b) and (d), solutions contained 3 μM cocaine from time indicated by arrow. In (c) and (f) solutions contained 1 μM phentolamine from time indicated by arrow. P indicates solution change to PSS. Vessel segment length = 1.25 mm. L_0/π = 136 μm.

above. In the noradrenaline experiments, vessels were then bathed in solutions containing increasing concentrations of calcium for 4 min periods, and were activated for the second half of each period with 10 μM noradrenaline. In the potassium solutions, vessels were stimulated constantly with 125 mM potassium while the calcium concentration was raised progressively, the vessels being held at each concentration for 2 min. In all of the paired experiments described in this paper with noradrenaline stimulation, the SHR vessels responded at a lower calcium concentration than the WKY vessels. By contrast with potassium stimulation, higher concentrations of calcium were required to elicit a response, and there was little difference in the responses of SHR and WKY vessels. Figure 3 also demonstrates our repeated observation that in response to noradrenaline stimulation vessels from both young and adult SHRs showed phasic activity (rhythmic tension variations superimposed on a steady tension), while WKY vessels rarely showed such activity. Neither SHR nor WKY vessels showed phasic activity with potassium stimulation.

Using this procedure, the calcium sensitivity of vessels from the young and adult SHRs and WKYs has been investigated. The basic mechanical characteristics of these vessels are shown in Table 2. As found in previous investigations (Mulvany *et al.*, 1980a), the lumen diameter of the SHR vessels was smaller than that of the WKY vessels at both ages, while the media/lumen ratio of the SHR vessels was greater than that of the WKY vessels. The contractile response of the vessels to NA-K-PSS was greater for the SHR vessels from the adult rats, but there was no difference in the responses from the young rats. Thus the active media stress of the vessels was the same in each age group. However because of the smaller lumen diameter of the SHR vessels, these would have been able to contract against higher pressures than the WKY vessels (active effective pressure) in each age group.

The Ca^{2+} dose-response characteristics of the vessels are shown in Figure 4, and the corresponding Ca-sensitivities (expressed as Ca-pD_2 values) are shown in Table 3. In both age groups the Ca-sensitivity of the SHR vessels (Ca-$ED_{50} \simeq 0.1$ mM) is about twice that of the WKY vessels (Ca-$ED_{50} \simeq 0.2$ mM) in response to noradrenaline. The Ca-sensitivity in response to potassium stimulation is much lower

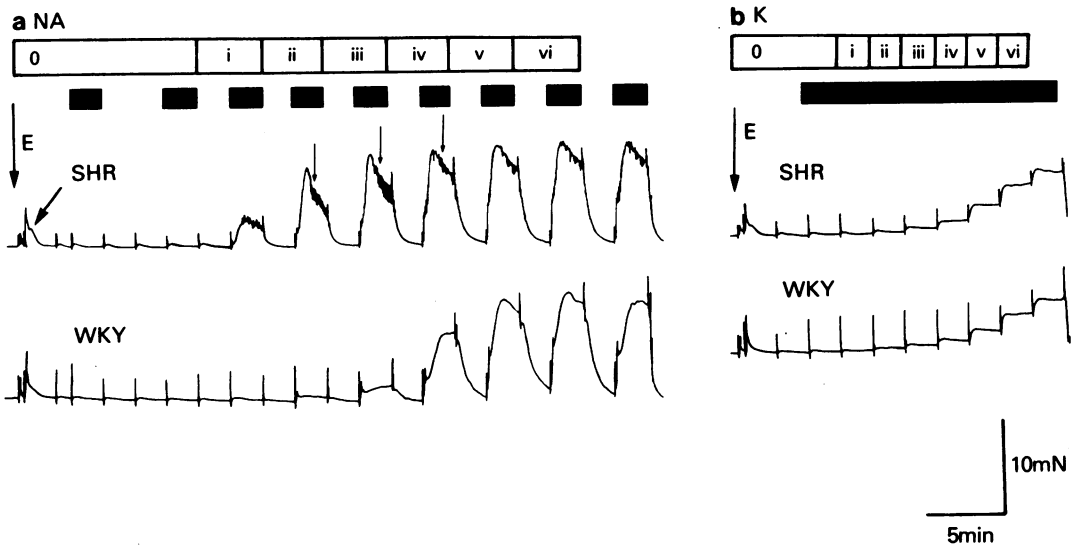

Figure 3 Calcium dose-response records from mesenteric resistance vessels when challenged with (a) noradrenaline and (b) potassium. Vessels were taken from adult spontaneously hypertensive rat (SHR, upper records) and an age-matched Wistar-Kyoto rat (WKY, lower records), and mounted on a double myograph in the same bath. The upper (open) blocks indicate the calcium concentrations: (0) 0 mM; (i) 0.05 mM, (ii) 0.1 mM; (iii) 0.2 mM; (iv) 0.4 mM; (v) 0.8 mM; (vi) 1.6 mM. Elsewhere the calcium concentration was 2.5 mM. The lower shaded bars indicate exposure to (a) 10 μM noradrenaline plus 3 μM cocaine, (b) 125 mM potassium plus 1 μM phentolamine. In both (a) and (b) vessels were depleted of calcium at E, by a 10 s exposure to EGTA-PSS and then a 2 min exposure to 10 μM noradrenaline in Ca-free PSS (containing 0.1 mM EGTA). The thickening of the trace in the top record of (a) (small arrows) is due to phasic activity (rhythmic tension variation at ~10/min). The large arrow in the top record points to a second maximum observed in the initial response to noradrenaline in Ca-free PSS. Vessel segment lengths were 1.41 mm (SHR) and 1.93 mm (WKY). L_0/π = 178 μm (SHR) and 250 μm (WKY).

Table 2 Characteristics of mesenteric resistance vessels from young (4 wk) and adult (4 months) spontaneously hypertensive (SHR) and control Wistar-Kyoto (WKY) rats

		Young	*Adult*	P_{strain}	P_{age}	$P_{interaction}$
Effective lumen diameter (μm)	SHR	148 ± 7	170 ± 9	<0.05	NS	NS
	WKY	174 ± 11	184 ± 10			
Media/Lumen ($\times 10^2$)	SHR	9.5 ± 1.1	6.5 ± 0.4	<0.05	<0.001	NS
	WKY	7.7 ± 0.8	4.5 ± 0.4			
Active wall tension (mN/mm)	SHR	2.6 ± 0.2	3.4 ± 0.3*			<0.01
	WKY	2.4 ± 0.2	2.6 ± 0.2			
Effective active pressure (mN/mm^2)	SHR	35 ± 2	41 ± 3	<0.001	NS	NS
	WKY	27 ± 2	28 ± 2			
Active media stress (mN/mm^2)	SHR	196 ± 17	311 ± 34	NS	<0.001	NS
	WKY	187 ± 14	280 ± 48			

Effective lumen diameter is $l_0 = L_0/\pi$ where L_0 is normalized internal circumference (see Methods). Media/lumen ratio = m_0/l_0, where m_0 is media thickness measured at internal circumference L_0. Active wall tension, ΔT_0, is developed force divided by twice segment length, see Methods, when maximally activated by NA-K-PSS (containing 10 μM noradrenaline and 125 mM potassium). Effective active pressure, $\Delta P_0 = \Delta T_0/(L_0/2\pi)$, that is maximum pressure which vessel would have been able to withstand. Active media stress, $\Delta\sigma_0 = \Delta T_0/m_0$, that is developed force divided by media cross-sectional area. Values give mean ± s.e.; 10 vessels in young groups and 9 vessels in adult groups. See Methods for description of analysis. Where $P_{interaction}$ is <0.05, SHR and WKY values have been compared at each age by *t* test, and there the asterisk indicates $P < 0.05$. In probability table at right, NS indicates not significant.

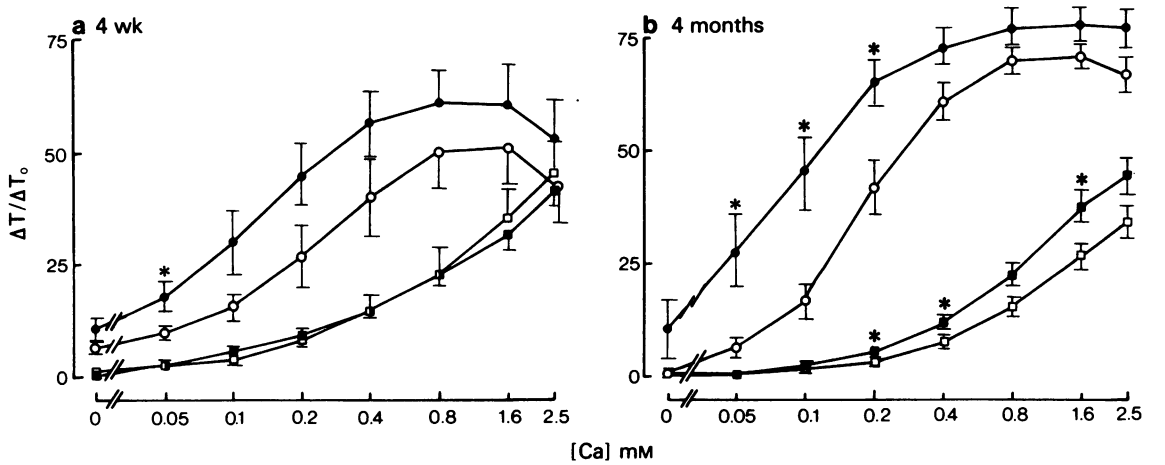

Figure 4 Calcium dose-response characteristics in response to maximal noradrenaline (circles) or potassium (squares) stimulation, determined in experiments similar to those shown in Figure 3, using mesenteric resistance vessels from spontaneously hypertensive (SHR, closed symbols) and Wistar-Kyoto (WKY, open symbols) rats. Rats were in (a) young 4 wk and in (b) adult 4 months old. Responses are expressed as fraction of vessel responses, ΔT_0, to NA-K-PSS (physiological salt solution containing 10 μM noradrenaline and 125 mM potassium). Noradrenaline stimulating solutions were x-Ca-PSS (x = 0 to 2.5 mM) containing 10 μM noradrenaline and 3 μM cocaine to prevent neuronal noradrenaline uptake. Potassium stimulating solutions contained 125 mM potassium (equimolar exchange of NaCl for KCl in x-Ca-PSS, and 1 μM phentolamine to block effects of noradrenaline released from nerve terminals by potassium. Asterisks indicate a significant difference ($P < 0.05$) between the SHR and WKY responses at the calcium concentration concerned. Vessel characteristics are shown in Table 2. Bars show s.e. mean.

($Ca\text{-}ED_{50} \simeq 0.8$ mM), and here the SHR vessels are only about 14% more sensitive to calcium. The maximum responses to noradrenaline and potassium were similar in the young vessels, but in the adult vessels the maximum responses to noradrenaline were about twice those to potassium.

A possible reason for the increased Ca-sensitivity of the SHR vessels could be that they contained greater intracellular calcium stores. One measure of the size of these stores (Devine, Somlyo & Somlyo, 1972) is the size of the response to noradrenaline in a Ca-free solution. We have therefore examined the size of the Ca-free noradrenaline responses during the calcium depletion procedure described above. The maximum amplitude of these responses expressed as a fraction of the response to noradrenaline in solutions containing 2.5 mM calcium, was larger in the adult vessels than in the young vessels, but there was no difference between corresponding SHR and WKY vessels (Figure 5). However there did seem to be a difference

Table 3 Calcium sensitivity of mesenteric resistance vessels from young (4 wk) and adult (4 months) spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats

		Young	*Adult*	P_{strain}	P_{age}	$P_{interaction}$
NA	SHR	3.96 ± 0.07 (10)	4.07 ± 0.07 (9)	<0.001	NS	NS
	WKY	3.66 ± 0.06 (10)	3.76 ± 0.06 (9)			
K	SHR	3.16 ± 0.02 (10)	3.12 ± 0.03 (9)	<0.05	NS	NS
	WKY	3.10 ± 0.04 (10)	3.07 ± 0.03 (9)			

Sensitivity expressed as $Ca\text{-}pD_2 = \log(Ca\text{-}ED_{50}(M))$, where $Ca\text{-}ED_{50}$ is concentration of calcium required to give half maximal response when vessels were activated by 10 μM noradrenaline (NA, upper pairs of values) or 125 mM potassium (K, lower pairs of numbers). Values given as mean ± s.e. (number of experiments). Noradrenaline solutions contained 3 μM cocaine to inhibit neuronal uptake of noradrenaline. Potassium solutions contained 1 μM phentolamine to eliminate effect of neuronal release of noradrenaline by potassium. See Methods for description of analysis of variance used.

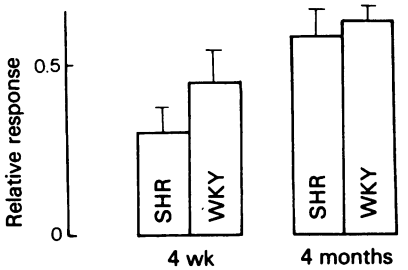

Figure 5 Responses to 10 μM noradrenaline in Ca-free PSS, following 10 s exposure to EGTA-PSS (containing 5 mM EGTA), of mesenteric resistance vessels from 4 wk and 4 month spontaneously hypertensive rats (SHR) and control Wistar-Kyoto rats (WKY). Responses are expressed as fraction of the vessel responses to 10 μM noradrenaline in PSS (containing 2.5 mM calcium). The form of the responses can be seen in Figure 1(c) and Figure 3. Bars show s.e. mean. Same vessels as those in Figure 4.

in the form of the responses. The response of most of the SHR vessels had two peaks (e.g. Figure 3), while in the WKY vessels the relaxation phase of the response was generally monotonic, particularly in the young vessels.

The effects on Ca-sensitivity of sub-maximal doses of noradrenaline and potassium were investigated in all the adult vessels. When activated with 1 μM noradrenaline, the vessels were much less sensitive to calcium, but the SHR vessels were again more sensitive ($P < 0.001$) (Ca-pD_2 = 3.27 ± 0.10) than the WKY vessels (Ca-pD_2 = 2.88 ± 0.06). Submaximal activation with potassium (50 mM) did not however affect the Ca-sensitivity to potassium stimulation (Ca-pD_2 = 3.23 ± 0.04 (SHR), 3.12 ± 0.06 (WKY)).

Noradrenaline and potassium sensitivity

The noradrenaline and potassium sensitivity of the vessels was determined in noradrenaline and potassium dose-response experiments on these vessels, the average results for the experiments with the young vessels being shown in Figure 6. For the reasons given above, the noradrenaline experiments were done in the presence of 3 μM cocaine and the potassium experiments in the presence of 1 μM phentolamine. For both young and adult vessels (Table 4) the noradrenaline sensitivity of the SHR vessels (NA-ED_{50} ≃ 0.55 μM) was greater than that of the WKY vessels (NA-ED_{50} ≃ 0.84 μM), while there was little difference in their potassium sensitivity (K-ED_{50} ≃ 43 mM). The effect of cocaine was assessed by performing noradrenaline dose-response experiments on the young vessels in the absence of cocaine, and determining differences in the resulting pD_2 values. The change in pD_2 caused by cocaine was

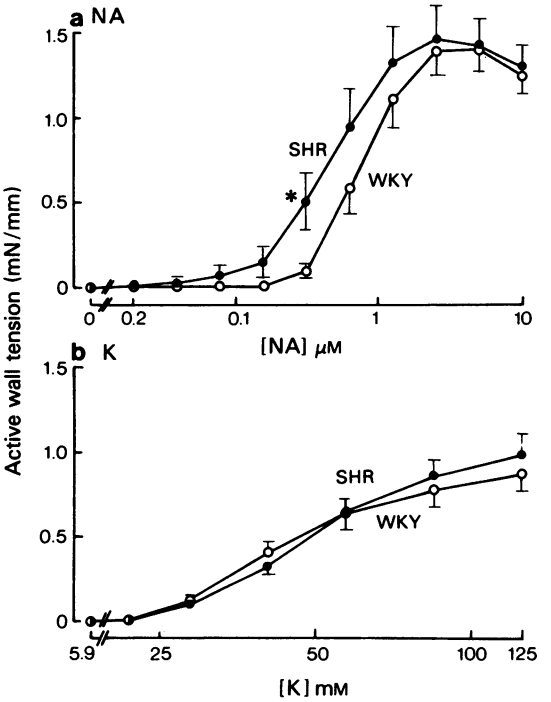

Figure 6 (a) Noradrenaline and (b) potassium dose-response curves of mesenteric resistance vessels from 10 4 wk spontaneously hypertensive rats (SHR) and 10 age matched Wistar-Kyoto rats (WKY). Responses expressed as active wall tension (measured increase in force above resting wall tension divided by twice segment length, see Methods). Noradrenaline stimulating solutions were PSS containing 3 μM cocaine to prevent neuronal noradrenaline uptake. Potassium stimulating solutions were PSS with an equimolar exchange of NaCl for KCl to give the required potassium concentration and 1 μM phentolamine to block effects of noradrenaline released from nerve terminals by potassium. The asterisk indicates a significant difference in the SHR and WKY responses at the agonist concentration concerned. Same vessels as those in Figure 4 (a). Bars show s.e. mean.

greater in the SHR vessels (ΔpD_2 = 0.65 ± 0.12) than in the WKY vessels (ΔpD_2 = 0.42 ± 0.08). These determinations were not made on the adult vessels, for we have already described very similar findings for such vessels (Mulvany *et al.*, 1980a).

Discussion

The main result of this investigation is that there are differences in the calcium sensitivity of mesenteric resistance vessels from SHRs and WKYs. These differences are found both in young (4 wk) animals and in adult (4 months) animals, that is both before and after the development of elevated blood pressure in the

SHRs. The differences in Ca-sensitivity are seen with noradrenaline stimulation but not with potassium stimulation. The experiments were performed under conditions in which the effects of noradrenaline uptake and release by the nerve terminals had been eliminated. Therefore the results suggest that there are specific differences in the noradrenaline-activated calcium handling properties of the smooth muscle cells within the SHR resistance vessels, and that these differences are not a consequence of the increased blood pressure.

Vascular supersensitivity in the SHR

The sensitivity of perfused vascular beds from the SHR to noradrenaline and potassium is generally described as greater than normal (see Yamori & Horie, 1977), but the sensitivity of isolated arterial preparations appears either to be normal or lower than normal (see Bohr, 1974; Mulvany *et al.*, 1980a). The noradrenaline and potassium responses are however substantially affected by the presence of the nerve terminals in the vascular wall (Vanhoutte, 1978). Their uptake of noradrenaline results in the noradrenaline sensitivity of the vessels being less than that of the smooth muscle cells, while the release of noradrenaline upon potassium activation could also result in the potassium sensitivity of the vessels differing from that of the smooth muscle cells. There is evidence for a greater effective innervation of the SHR vessels (Ichijima, 1969) and for a greater neuronal uptake (Whall *et al.*, 1980; Mulvany *et al.*, 1980a). Therefore if conclusions are to be drawn concerning vascular smooth muscle sensitivity of SHR vessels, it is important that the effects of neuronal uptake and release are inhibited. Under conditions of uptake inhibition, isolated tail artery (Hermsmeyer, 1976; Webb & Vanhoutte, 1979), isolated mesenteric resistance vessels (Mulvany *et al.*, 1980a), and perfused renal vascular beds (Collis & Vanhoutte, 1977; Berecek, Rascher & Gross, 1979) all show greater noradrenaline sensitivity in SHRs. By contrast however, isolated SHR and WKY portal veins show no differences in noradrenaline sensitivity with inhibition of neuronal uptake (Mulvany, Ljung, Stoltze & Kjellstadt, 1980c). There are no reports of the potassium-sensitivity of perfused vascular beds in which the nerve terminals have been inactivated, but in isolated mesenteric resistance vessels in which the nerve terminals have been destroyed *in vitro* with 6-hydroxydopamine the potassium sensitivity of SHR and WKY preparations is the same (Nyborg, Christensen & Mulvany, 1980). Thus the available evidence suggests that in general the smooth muscle of the SHR arterial vasculature has an increased noradrenaline sensitivity but a normal potassium sensitivity, which was indeed the case for the vessels used in this investigation (Table 4).

A number of recent papers, in which both perfused vascular beds and isolated vessels, have been used indicated that there are also differences in the calcium-sensitivity of the SHR and WKY vasculature, in particular in respect to noradrenaline stimulation. Folkow, *et al.* (1977) found that when perfused with low calcium solutions the precapillary vessels from hindquarter preparations retained their noradrenaline responses better than those of normotensive controls.

Table 4 Noradrenaline (NA) and potassium (K) sensitivities of mesenteric resistance vessels from young (4 wk) and adult (4 months) spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats

		Young	*Adult*	P_{strain}	P_{age}	$P_{interaction}$
NA-pD_2	SHR	6.33 ± 0.11	6.20 ± 0.03	0.01	NS	NS
	WKY	6.11 ± 0.06	6.05 ± 0.05			
$\Delta T_{NA}/\Delta T_0$	SHR	0.60 ± 0.07	0.78 ± 0.05	NS	0.05	NS
	WKY	0.63 ± 0.06	0.70 ± 0.04			
K-pD_2	SHR	1.31 ± 0.01	1.39 ± 0.04	NS	0.05	NS
	WKY	1.36 ± 0.01	1.40 ± 0.04			
$\Delta T_K/\Delta T_0$	SHR	0.38 ± 0.04	0.45 ± 0.04	NS	NS	NS
	WKY	0.38 ± 0.06	0.35 ± 0.04			

NA-pD_2 and K-pD_2 show, respectively, log (NA-ED_{50}(M)) and log (K-ED_{50}(M)). NA-ED_{50} and K-ED_{50} are, respectively, concentrations of noradrenaline and potassium required to give half maximal responses to the agonist concerned. ΔT_{NA}, ΔT_K are the response of the vessel with 10 μM noradrenaline and 125 mM potassium, respectively. The noradrenaline solutions contained 3 μM cocaine to inhibit neuronal uptake of noradrenaline. The potassium solutions contained 1 μM phentolamine to eliminate the effect of neuronal release of noradrenaline by potassium. ΔT_{NA} and ΔT_K are expressed as fraction of ΔT_0, where ΔT_0 is the response to NA-K-PSS (containing 10 μM noradrenaline and 125 mM potassium, but no cocaine or phentolamine). Values are expressed as mean ± s.e., 10 vessels in young groups, 9 vessels in adult groups. See Methods for description of analysis of variance used.

Isolated SHR portal veins were also more sensitive to calcium in response to noradrenaline stimulation than WKY preparations (Pegram & Ljung, 1980). Although the isolated SHR aorta is reported to be less calcium sensitive (Lederballe Pedersen, Mikkelsen & Andersson, 1978), other investigators (Noon, Rice & Baldessanni, 1978) found an increased tone in this preparation, which is calcium-dependent. In support of the findings of Noon *et al.*, a greater plasma-membrane Ca-flux has been found in aorta from SHRs (Zsotér, Wolchinsky, Henein & Ho, 1977) while in subcellular fractions of SHR mesenteric arteries there is an increased plasma-membrane uptake of calcium (Wei, Janis & Daniel, 1976). These noradrenaline-stimulated calcium-sensitivity differences are in contrast to the reports concerning calcium-sensitivity with potassium stimulation. We have shown previously (Mulvany & Halpern, 1977) that mesenteric resistance vessels from 5 month old SHRs have the same potassium activated calcium sensitivity. This is consistent with Finch & Haeusler's (1974) findings using a perfused mesenteric preparation, where again there was no difference in calcium sensitivity with potassium activation. Thus there appears to be a general consensus that, in agreement with the present findings, the SHR vasculature is, with certain exceptions, more sensitive to calcium when stimulated with noradrenaline but not when stimulated with potassium.

Calcium activation

The main purpose of the present investigation was to determine whether the differences in noradrenaline sensitivity of mesenteric resistance vessels could be accounted for by differences in calcium sensitivity. Noradrenaline is thought (Bolton, 1979) to raise the free intracellular calcium level, the ultimate trigger for activation, through three main pathways. First, noradrenaline reacts with the α-adrenoceptors which as the result of an as yet unknown mechanism causes the opening of calcium channels (NA-channels) (Godfraind, 1976). Second, this influx of calcium causes depolarization and the opening of another set of potential-dependent channels (potential-channels) (see Bolton, 1979). Third, noradrenaline causes release of calcium from intracellular stores (Deth & Casteels, 1977). The spread of activation throughout the entire smooth muscle is facilitated by electrical connections between the smooth muscle cells, which cause the musculature to act as an electrical syncytium (Holman & Neild, 1979). By contrast the effect of raising the potassium concentration is solely (in the absence of noradrenaline release from the nerve terminals) to cause depolarization and the opening of the potential-channels.

One reason for the increased noradrenaline sensitivity of the SHR cells could therefore be because these contain larger intracellular calcium stores (Devine *et al.*, 1972). However, it seems unlikely that these could directly explain our results, for the calcium sensitivity experiments were performed subsequent to calcium depletion. Moreover the peak responses to noradrenaline in Ca-free solutions of SHR and WKY vessels were similar. However, if calcium is required to release intracellular calcium (Keatinge, 1972) then these findings are not conclusive, and our observation that the Ca-free responses are slightly prolonged in the SHR vessels (Figure 3) may be of importance. Therefore although we do not exclude the possibility that the intracellular stores may play a minor role in determining the calcium sensitivity of these vessels, we consider that the main factor is probably the agonist-induced change in the calcium permeability of the plasma membrane.

In the current investigation with maximal noradrenaline activation the NA-channels should have been fully open. Therefore our finding that the SHR vessels were more sensitive to calcium, suggests either that the SHR NA-channels are more permeable to calcium or that there are more of them. Our further finding that with maximal potassium stimulation there is no difference in calcium sensitivity suggests that the potential channels in SHR and WKY vessels are similar. We therefore conclude from these experiments that a contributory factor to the increased noradrenaline sensitivity of the SHR vessels may be that their noradrenaline controlled calcium permeability is greater.

This hypothesis is consistent with the findings of Jones (1973) that the SHR plasma membrane is more permeable to Na and K. Indeed since the calcium channels may also be permeable to Na and K (Droogmans & Casteels, 1979) they could in themselves be the cause of the increased K and Na-permeability. Hermsmeyer (1976) has proposed that it is the increased K- and Na-permeability which is indirectly the cause of the increased noradrenaline sensitivity. The resting membrane potential of the SHR and WKY cells is the same (Hermsmeyer, 1976; Bell & Kushinsky, 1978). Therefore an increased K- and Na-permeability might be expected to cause or require an increased Na-K electrogenic pump activity in the SHR cells, and there is now experimental evidence for such an increased activity (Hermsmeyer, 1976; Webb & Bohr, 1979). Hermsmeyer proposed that noradrenaline activation would short-circuit the Na-K pump, so that noradrenaline would produce a greater depolarization in the SHR cells. If however this mechanism applies in our vessels it remains to be explained why their potassium sensitivity is the same, for by the same reasoning potassium activation could also be expected to 'short-circuit' the Na-K pump.

Aetiology

As in our previous investigation (Mulvany *et al.*, 1980a) the lumen diameter of the SHR vessels was smaller than that of the WKY vessels both before the onset of hypertension and after, while an increase in medial thickness occurred subsequent to the increased blood pressure. Therefore these findings support our previous conclusions that morphological factors contribute to the increased peripheral resistance reported for young and adult SHRs (Pfeffer, Frohlich, Pfeffer & Weiss, 1974). The differences in pharmacological characteristics were also found in the vessels from SHRs of both age groups. An increased calcium sensitivity causing an increased noradrenaline sensitivity could clearly also contribute to raising the peripheral resistance. Thus because our results indicate that this increased sensitivity is not the result of the elevated blood pressure, these pharmalogical differences appear to be intrinsic to the SHR and possibly among the factors causing hypertension in the SHR. Whether the increased calcium sensitivity of the SHR cells is a genetic defect or whether it is the result of some other genetic defect remains to be seen. We do not exclude the possibility that it is a neurogenically induced phenomenon. Our results showing that the vessels from the young SHRs had an increased cocaine shift suggests that, as in adult vessels (Whall *et al.*, 1980), there was an increased innervation of these vessels. We would therefore propose that one possible mechanism for the increased calcium sensitivity is that the increased innervation results in an increased number of α-adrenoceptor controlled calcium channels, and that it is this which is responsible for the increased noradrenaline sensitivity rather than differences in the properties of the α-receptors.

A second possibility is that the factor which is responsible for the increased rhythmic activity of SHR vessels in both age groups could also contribute to the increased noradrenaline sensitivity. One cause of the increased activity could be an increased electrical conductivity between the cells of the SHRs, for it has previously been shown that increasing electrical conductivity can cause rhythmic activity in canine tracheal smooth muscle (Kannan & Daniel, 1978). This would result in a greater than normal electrotonic spread of the depolarization induced by noradrenaline in the innervated cells, and thereby an increased noradrenaline sensitivity. Furthermore since the noradrenaline-induced depolarization is the result of calcium influx through the noradrenaline controlled calcium channels, the noradrenaline stimulated calcium sensitivity would also be increased. In contrast, since potassium depolarization affects all cells equally, increased conductivity between cells would not affect the potassium sensitivity of the potassium-activated calcium sensitivity.

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