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Differences in sensitivity of rat mesenteric small arteries to agonists when studied as ring preparations or as cannulated preparations

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1 Pharmacological experiments on vascular tissue are normally performed on isometric ring or strip preparations. The aim of this study was to compare the isometric characteristics with the characteristics obtained if vessels were examined under the more physiologically appropriate isobaric condition.

2 Rat mesenteric small arteries were mounted either on two steel wires for isometric force measurement (wire-myograph) or cannulated for measurement of the internal diameter under isobaric conditions (pressure-myograph).

3 The passive pressure-diameter characteristics of the small arteries were similar on the wire- and pressure-myograph (using the Laplace relation to convert wall tension-internal circumference data from the wire-myograph to effective pressure-diameter characteristics).

4 In cumulative concentration-response experiments with noradrenaline and phenyelphrine, the threshold concentration was 8–10 times lower, and the EC_{50}-concentration was 4–5 times lower, in the pressure myograph compared to the wire-myograph. Thus vessels were not only more sensitive on the pressure myograph, but the slopes of the concentration-response curves were less steep. Similar experiments with vasopressin also showed this difference in the threshold-concentration and slope, but EC_{50} concentrations were similar.

5 Cumulative concentration-response experiments with K^+ showed no difference either in EC_{50} or in slope on the wire- and pressure-myographs.

6 On the wire-myograph, some vessels were stretched longitudinally (to mimic the longitudinal stretch which had to be used in the pressure-myograph to avoid buckling). Such stretch did not affect the passive characteristics.

7 The differences between the EC_{50} determined on the wire- and pressure-myographs as regards noradrenaline and phenylephrine were eliminated when neuronal noradrenaline uptake was inhibited by denervation. However, the slope of the concentration-response curves on the wire-myograph was not affected by denervation.

8 When vessels were exposed to cocaine (3 μM) the noradrenaline concentration-response curves were the same on the wire- and pressure-myographs as regards both EC_{50} and slope.

9 On the wire-myograph, the calcium antagonist, methoxyverapamil, (D600) reduced the maximal contractile effect of noradrenaline by 50%, but on the pressure-myograph D600 did not affect the maximal response.

10 The present results show that results obtained from vascular tissue under isometric conditions may differ substantially from the characteristics which would be obtained under isobaric conditions.

Keywords: Isometric; isotonic; myograph; concentration-response curve; amine uptake; calcium antagonist

Introduction

In vitro investigation of the contractile properties of intact vascular segments are performed by use of two principally different methods (Mulvany & Aalkær, 1990; Halpern & Kelley, 1991). Vessels can be mounted either as isometric preparations (on a 'wire-myograph', e.g. Mulvany & Halpern, 1977), when force development is measured at a certain diameter, or as cannulated isobaric preparations where the vessel is exposed to a transmural pressure and allowed to change diameter ('pressure-myograph', e.g. Halpern & Osol, 1985). Evidence is accumulating that certain characteristics of the vessels are critically dependent on which method is used. In particular, it has been suggested that the spontaneous basal tone (McCarron *et al.*, 1991; Vanbavel *et al.*, 1991) and myogenic behaviour (Halpern *et al.*, 1987; VanBavel *et al.*, 1991), which exists in most resistance arteries *in vivo*, are more easily reproduced *in vitro* in pressurized vessels than in wire-mounted vessels.

Indirect evidence for differences between wire- and pressure-myographs is also found in the literature as regards the concentration-response relations for e.g. noradrenaline (NA). Thus, for small rat mesenteric arteries, the NA pEC_{50}-values ($pEC_{50} = -\log(EC_{50}\ (M))$) on wire-myographs are reported in the range 5.52–5.96 (Whall *et al.*, 1980; Mulvany *et al.*, 1982; Nyborg & Mikkelsen, 1985; Nielsen & Mulvany, 1990), while the reported NA pEC_{50}-values for cannulated and pressurized rat mesenteric arteries of similar diameter are generally higher, e.g. in the range 6.3–6.45 (Marshall, 1977; Dohi & Lüscher, 1990; VanBavel & Mulvany, 1994). Furthermore, it appears that the slope of the NA concentration-response curves is steeper on wire-myographs than on pressure-myographs. Such differences have also been seen on a myograph where a cannulated preparation could be exposed to either isometric or isobaric conditions using a feed-back arrangement (VanBavel & Mulvany, 1994).

The possibility that the commonly used method of investigating vascular preparations isometrically may give results which are substantially different from results obtained isobar-

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ically is potentially important: the isobaric method is clearly much closer to the *in vivo* situation than the isometric method. However, until now, there has been no systematic comparison of the two methods as regards agonist concentration-response relations. We have therefore performed concentration-response experiments using both a wire-myograph (Mulvany & Halpern, 1977) and a pressure-myograph (VanBavel *et al.*, 1990) to investigate the same preparation (a rat mesenteric small artery) and using the same solutions and protocols. The results confirmed the differences indicated above, and we have carried out further experiments to investigate possible reasons for the differences. In particular, we have investigated whether the results are specific to NA by determining the responses to other agonists (the α_1-adrenoceptor agonist, phenylephrine (PE) and vasopressin) and to high potassium solution. We have also investigated the possible influence of neuronal amine uptake. The results are consistent with the differences being due in part (a) to the effect of amine uptake on amine concentration-response relations being greater for wire-myographs than for pressure-myographs and (b) to the sensitivity to agonists being increased by force in the vascular wall. Some of these results have been presented previously in brief (Buus & Mulvany, 1992).

Methods

Male Wistar rats, aged 12–16 weeks, were killed with CO_2. Part of the intestinal tract was removed and kept in a physiological saline solution (PSS, for composition see below). Segments from a second or third order branch of the mesenteric superior artery were dissected and mounted in either a pressure-myograph or a wire-myograph, as explained below.

Pressure-myograph

Vessels mounted in the pressure-myograph (VanBavel *et al.*, 1990; 1991) were cannulated at both ends with small glass cannulae having an outer diameter of 110–150 μm and secured with 15 μm thin sutures. The cannulated segment was superfused with PSS, bubbled with 95% O_2 and 5% CO_2, at a flow rate of 10 ml min^{-1} from a 100 ml reservoir. The lumen was perfused at 10–100 μl min^{-1} with PSS containing 0.5% albumin and 40 mg l^{-1} of the fluorescent dye FITC-dextran (fluorescein isothiocyanat-dextran, molecular weight 487 kDa), the luminal pressure being controlled by adjustment of the height of the reservoirs feeding and receiving the perfusate. This flow rate is substantially lower than that which induces flow-mediated vasodilatation (Tesfarmariam *et al.*, 1985), as confirmed by our observation that stopping the flow did not affect lumen diameter. A pressure transducer connected to the feeding reservoir was used to record luminal pressure continuously. The flow rate of the perfusate was adjusted by maintaining a small difference in the height of the feeder and receiver reservoirs. It was important to choose segments without any side branches to avoid leakage of FITC-dextran or albumin from the lumen to the superfusate. After mounting, a transmural pressure of 80 mmHg (110 cmH_2O) was applied to the vessel, which was then lengthened until any bends in the vessel disappeared. The vessel chamber was placed under a microscope (Leitz Orthoplan) which was equipped with a fluorescence attachment. The luminal cross-sectional area was continuously measured by means of a fluorescence technique as described by VanBavel *et al.* (1990). In brief, the FITC-dextran present in the lumen was excited with a halogen light source. Excitation light was filtered to pass 400–800 nm with the aid of a dichroic mirror and the intensity of fluorescence light emitted from the vessel lumen was measured with a photomultiplier tube. The system was calibrated as follows. The temperature of the chamber was raised to 37°C, and the vessel diameter was set in turn to three or four different diameters (by adjustment of luminal pressure). For each diameter, measurements were made (a) of the intensity and (b) of the internal diameter by direct observation through the microscope by use of an ocular micrometer. Intensity was linearly related to the cross sectional area, as found previously (VanBavel *et al.*, 1990). Calibration was checked several times during each experiment.

Wire-myograph

Vessels were threaded on two 40 μm diameter stainless steel wires and mounted on a wire-myograph (model 500A, JP Trading) allowing direct determination of the vessel wall force while the internal circumference was controlled (Mulvany & Halpern, 1977). After the temperature had reached 37°C, arteries were stretched radially to their optimal lumen diameter, equal to (internal circumference)/π, for active tension development. This normalized effective lumen diameter (d_0), is an estimate of 90% of the diameter the vessel would have had, if it were relaxed and exposed to a transmural pressure of 100 mmHg (Mulvany & Halpern, 1977).

Experimental protocol

After normalization (wire-myograph) or calibration (pressure-myograph), vessels were activated for 2 min with K-PSS (125 mM) containing 10 μM NA. Vessels in the wire-myograph were accepted only if the calculated pressure against which they could contract (calculated according to the Laplace relation) exceeded 100 mmHg (Mulvany & Halpern, 1977), and vessels in the pressure-myograph were accepted only if visual inspection showed no side-branches leaking dye and the contraction was uniform along the whole segment length. With these criteria, all vessels in the wire-myograph were accepted, but 35% of the vessels in the pressure-myograph were discarded, nearly all because of dye leaking out of minute side-branches not visible during dissection. Most of the vessels in both the pressure- and wire-myographs were also tested to ensure that they relaxed to acetylcholine (1 μM) as an indication that the endothelium was still intact, and all met this criterion. The following protocols were followed.

(1) Passive pressure-diameter relationship

The vessel segment was first mounted on the pressure-myograph and the inner diameter measured at different transmural pressures. The same segment was then transferred to the wire-myograph and subjected to increasing degrees of radial distension. Full relaxation was ensured by the presence of papaverine (10 μM).

To investigate the effect of longitudinal stretch of the vessel on the wire-myograph, the normal mounting procedure was modified. The vessel was gently stretched with a forceps and clamped at both ends between the mounting wires and the metal jaws supporting the wires. After stretching, the normalization procedure was repeated and a new d_0 was found. With this modified procedure it was possible to obtain a degree of longitudinal stretch similar to that produced by the transmural pressure in the pressure-myograph.

(2) Active properties of vessels in pressure- and wire-myographs

Here concentration-response experiments with NA, PE, vasopressin and K^+ were performed on the pressure-myograph, or on the wire-myograph. Experiments in the pressure-myograph were made at a transmural pressure of 44 mmHg (60 cmH_2O). This pressure is similar to the calculated pressure at a distension of d_0 on the wire-myograph (ca. 55 mmHg, see Figure 1). NA and PE were added in a concentration range from 1 nM (NA) or 10 nM (PE) to 32 μM

in half log increments, AVP in a range from 0.039 to 5 u l^{-1} with doubling of concentrations, and K^+ (as K-PSS, see below) in seven different concentrations ranging from 5.9 mM to 125 mM. Each dose was added to the chamber for 2 min. To ensure that the experiments with PE involved only α_1-adrenoceptors, these were done in the presence of the α_2-antagonist yohimbine (0.1 μM). K^+ experiments were made in the presence of phentolamine (1 μM) to avoid the effect of NA released from depolarized sympathetic nerve endings.

(3) Investigation of a possible difference in the influence of sympathetic nerves in pressurized and wire-mounted arteries

(a) *Cocaine experiments* After the initial concentration-response curve with NA, cocaine (3 μM) was added and a new curve generated. To investigate an influence of the vessel diameter on NA reuptake a group of similar experiments were made at distensions of both d_0 and $0.5 \times d_0$.

(b) *Denervation experiments* After the initial NA concentration-response curve, vessels were denervated *in vitro* with 6-hydroxydopamine (457 mg l^{-1}). This was done in buffer-free PSS vigorously bubbled with N_2 (Aprigliano & Hermsmeyer, 1976), followed by a 2 h recovery period in standard PSS, after which a new concentration-response curve was obtained.

(4) Action of the calcium channel blocker D600 in pressurized and wire-mounted vessels

Vessels were set either to d_0 or to $0.4 \times d_0$. In each case, after the initial concentration-response curve with NA, 1 μM methoxyverapamil (D600) was added to the vessel chamber and a new curve was generated.

Time control experiments were not performed, since the aim of the studies was to compare the responses of wire-mounted and cannulated preparations when subjected to otherwise identical protocols. It should be noted, however, that for rat mesenteric small arteries both when wire-mounted (Mulvany *et al.*, 1982) and when cannulated (Van-Bavel & Mulvany, 1994), agonist concentration-response relations are repeatable for many hours.

Drugs and solutions

Drugs used were noradrenaline-HCl (NA, Sigma), D600 (methoxyverapmil, Knoll AG), vasopressin (Sandoz, where 1 u l^{-1} = ca. 2.5 nM), albumin (bovine, Sigma), fluorescein isothiocyanate-dextran (0.011 mol (mol glucose)$^{-1}$, Sigma), cocaine chloride (Pharmacy, Aarhus University Hospital), 6-hydroxydopamine (Sigma), phentolamine (Ciba-Geigy), phenylephrine (PE) and papaverine (SAD, Denmark), and yohimbine (Sigma). All drugs were dissolved in distilled water except D600 which was dissolved in 96% ethanol. The physiological saline solution was of the following composition (mM): NaCl 119, NaH_2CO_3 25, KCl 4.7, $CaCl_2 \cdot 2H_2O$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.17, KH_2PO_4 1.18, Na_2EDTA 0.026 and glucose 5.5. K-PSS was similar to PSS except that NaCl was exchanged for KCl on an equimolar basis. The pH of the PSS was stabilized with HEPES ([N-(2-hydroxyethyl) piperazine-N'-[Z-ethane sulphonic acid]) at pH 7.40. Buffer-free PSS used for denervation consisted of (mM): NaCl 137, $MgCl_2$ 1, KCl 2.69, EDTA 0.03, $CaCl_2$ 1.8, glucose 7.8 and glutathione 0.02, pH 4.9.

Calculations and statistical considerations

The passive tension-diameter curves obtained on the wire-myograph were fitted to an exponential function: $T = a \times \exp(b \times d)$, where T is the passive tension, d the internal diameter, a and b constants. Based on the Laplace equation the effective pressure ($p = 2 \times T/d$) could be calculated (Mulvany & Halpern, 1977). For Figure 1, the diameters at various effective pressures on the fitted passive tension-diameter relation were determined; average diameters for the vessels investigated at each of these pressures were calculated. For Figure 2, the characteristics before and after stretch were determined by calculating the wall tensions and diameters at each distension (which ranged between 0.44 d_0 and 1.22 d_0); average tensions and diameters for the vessels investigated at each of these distensions was calculated.

All concentration-response curves were analyzed by iterative nonlinear regression analysis. Each regression line was fitted to a sigmoid equation: $R/R_{max} = A^n/(A^n + (EC_{50})^n)$ where R_{max} is the maximal response developed to the agonist, A is the concentration of agonist, EC_{50} is the concentration required for half maximum contraction and n is a constant which indicates the maximal slope of the concentration-response curve. The coefficients of determination for the fitted curves were $r^2 = 0.99$ both on the wire-myograph and the pressure-myograph. In each curve, the agonist concentration (A) corresponding to a certain degree of contraction (R/R_{max}) was calculated from the fitted curve; concentration-response data in the figures are therefore given with horizontal error bars. Sensitivities to NA, PE, K^+ and vasopressin are expressed as pEC_{50} equal to $-\log(EC_{50}(M))$ or, for vasopressin, $-\log(EC_{50}$ (u l^{-1})).

Results are given as mean ± s.e.mean. Differences between means were analyzed with Student's paired or unpaired two-tailed *t* test, as appropriate. In Table 2, to allow comparison between several group means, we used one way analysis of variance (one-way ANOVA) followed by Fisher's LSD (Least Significance Difference) test, having previously ensured that the data were normally distributed. The level of significance in all tests was set at $P < 0.05$.

Results

Passive properties of vessels

The passive pressure-diameter relationship of six vessels was determined first on the pressure-myograph and then on the wire-myograph. As shown in Figure 1 the relations were identical, even though on the pressure-myograph the vessels had to be stretched longitudinally by 76 ± 6% to keep the vessel straight when subjected to the highest pressure (80

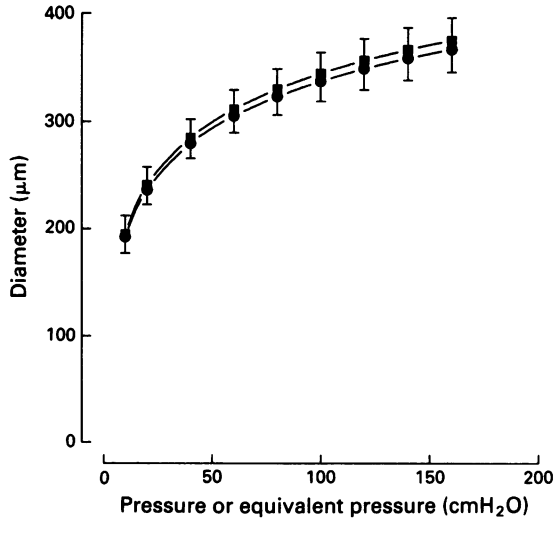

Figure 1 Pressure-diameter relation for six pressurized vessels (●) and equivalent pressure-diameter relation (■) obtained when these vessels were mounted on the wire-myograph. Equivalent pressures are calculated from the passive diameter-tension curves determined for each vessel as described in the text. Error bars show s.e.mean.

mmHg). Longitudinal stretch of vessels did not affect the normalised diameter: unstretched, d_0 was 290 ± 7 μm (n = 15); when stretched longitudinally by 56 ± 2%, d_0 was 281 ± 6 μm. Furthermore, as shown on Figure 2 longitudinal stretch did not influence the passive tension-diameter relationship.

Concentration-response experiments

Figure 3 shows typical traces obtained in NA concentration-response experiments, and Figure 4a and Table 1 show the average results. As indicated by the pEC_{50}-values in Table 1, the concentration needed to elicit half maximum response is almost 5 fold higher in the wire-myograph than in the pressure-myograph. The slope of the concentration-response curve was shallower in the pressure-myograph, consistent with the lower maximum slope (Table 1) and a 10 fold lower threshold concentration (measured as 10% of maximum responses, Figure 4a).

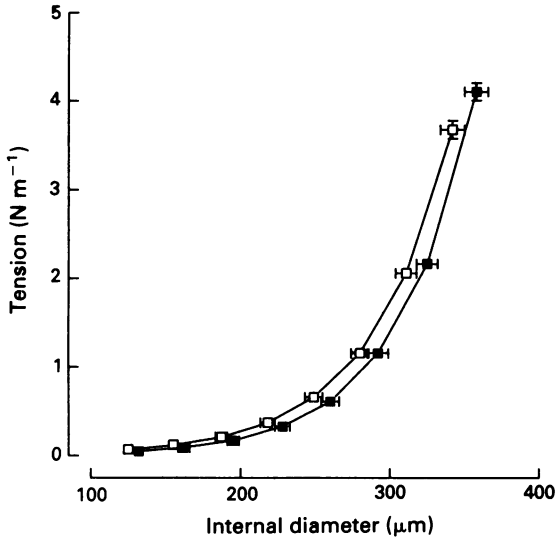

Figure 2 Effect of longitudinal stretch on passive diameter-tension characteristics of 15 vessels when mounted on the wire-myograph: unstretched (vessel length 1.92 ± 0.06 mm, ■) and when these vessels were stretched longitudinally (vessel length 2.92 ± 0.02 mm, □). Both curves are obtained from the fitted exponential passive diameter-tension curves as explained in the text. S.e.mean are shown where these exceed the size of the symbol.

To ensure that the findings concerning NA were mediated through α_1-adrenoceptors, concentration-response experiments with PE in the presence of α_2-adrenoceptor blockade (yohimbine, 0.1 μM) were also performed. These experiments also showed an increased sensitivity and lower maximum slope in the pressure-myograph compared to the wire-myograph (Table 1, Figure 4b). By contrast, experiments with K^+ (Table 1, Figure 4c) showed no significant difference in pEC_{50}-values or maximum slope. Concerning vasopressin, although the maximum slope and threshold concentration were lower on the pressure-myograph compared to the wire-myograph, there was no significant difference in the pEC_{50}-values (Table 1, Figure 4d). However, it should be noted that the effect of this drug was nearly an all-or-none response making the curve-fitting uncertain.

Inhibition of neuronal uptake by cocaine

Results from experiments with cocaine are outlined in Table 2a and Figure 5a. The increase in sensitivity to NA caused by cocaine is less in the pressure-myograph compared to the wire-myograph (Figure 5a), such that the initial differences in pEC_{50}-value and maximum slope disappeared. Cocaine did not affect the maximal active tension development in the wire-myograph (4.93 ± 0.26 N m^{-1} before and 4.47 ± 0.24 N m^{-1} after incubation with cocaine) or the maximal degree of contraction in the pressure-myograph (contracted to 22 ± 3% of relaxed diameter in the absence and to 23 ± 3% in the presence of cocaine). In ten experiments, we compared the effect of cocaine with vessels held at d_0 (257 ± 12 μm) and at 0.5 × d_0. There was a significant difference in the shift in NA-pEC_{50} caused by cocaine at these two distensions, being 0.67 ± 0.07 and 0.43 ± 0.06, respectively.

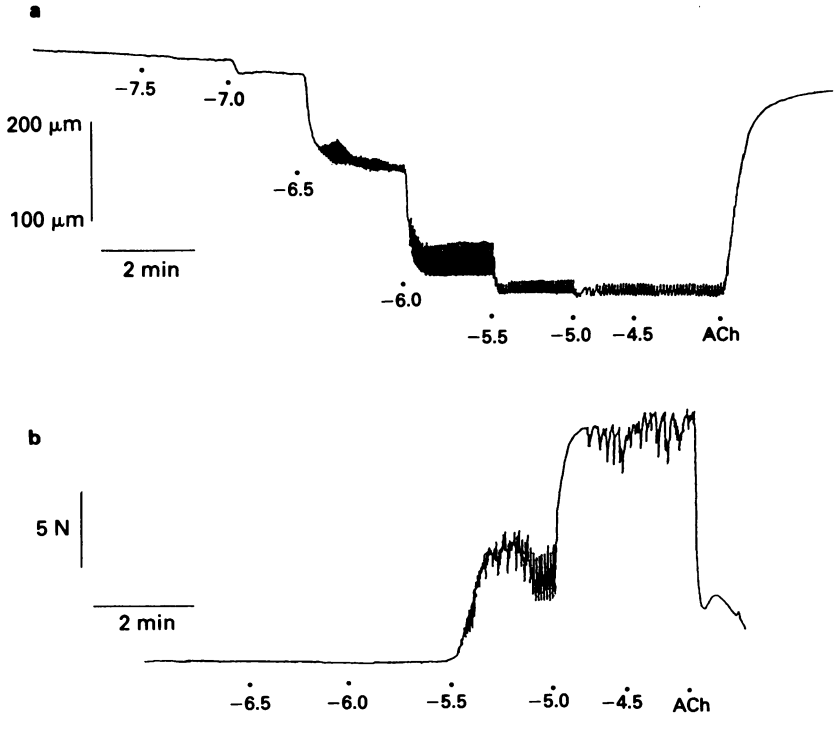

Figure 3 Traces showing noradrenaline (NA) concentration-response curves for a pressurized vessel (a), and a wire-mounted vessel (b). NA concentratons are given as log concentrations (M). ACh: acetylcholine 1 μM.

Table 1 Maximal contractions, pEC_{50} values and maximum slopes for concentration-response experiments

Agonist	*Experimental condition*	n	*Max. active tension* ($N\,m^{-1}$)	*Min. diameter* (% of relaxed diameter)	*pEC_{50}*	*Maximum slope*
Noradrenaline	Pressure-myograph	9		23 ± 4	6.42 ± 0.08[a]	1.65 ± 0.15[a]
	Wire-myograph	8	3.85 ± 0.25		5.77 ± 0.05[b]	2.60 ± 0.24[b]
Potassium	Pressure-myograph	7		30 ± 6	1.41 ± 0.02[a]	5.72 ± 0.79[a]
	Wire-myograph	7	3.36 ± 0.52		1.43 ± 0.01[a]	6.30 ± 0.20[a]
Vasopressin	Pressure-myograph	6		39 ± 7	0.54 ± 0.07[a]	3.49 ± 0.67[a]
	Wire-myograph	7	3.58 ± 0.47		0.38 ± 0.12[a]	6.18 ± 0.92[b]
Phenylephrine	Pressure-myograph	4		24 ± 4	6.00 ± 0.10[a]	2.29 ± 0.29[a]
	Wire-myograph	6	3.58 ± 0.29		5.65 ± 0.08[b]	3.94 ± 0.41[b]

Different superscripts designate within each group $P<0.05$ for pressure- v. wire-myograph by t test. $pEC_{50} = -\log (EC_{50}$ (M)) or for vasopressin $-\log (EC_{50}$ u $l^{-1})$). n is the number of vessels in each group. Values show mean ± s.e.mean.

Table 2 A Effect of cocaine (3 μM) on pEC_{50}-values and maximum slopes on vessels in the pressure- and wire-myographs

Experimental condition	n	*pEC_{50}* − Cocaine	*pEC_{50}* + Cocaine	*Maximum slope* − Cocaine	*Maximum slope* + Cocaine
Pressure-myograph	10	6.24 ± 0.07[a]	6.54 ± 0.07[c]	1.98 ± 0.21[a]	1.86 ± 0.13[a]
Wire-myograph	12	6.50 ± 0.03[b]	6.38 ± 0.09[c]	3.48 ± 0.17[b]	2.29 ± 0.23[a]

B Effect of *in vitro* denervation on pEC_{50}-values and maximum slopes on vessels in the pressure- and wire-myographs

Experimental condition	n	*pEC_{50}* Innervated	*pEC_{50}* Denervated	*Maximum slope* Innervated	*Maximum slope* Denervated
Pressure-myograph	6	6.16 ± 0.11[a]	6.56 ± 0.10[c]	1.72 ± 0.21[a]	1.50 ± 0.19[a]
Wire-myograph	8	5.85 ± 0.08[b]	6.67 ± 0.12[c]	3.07 ± 0.31[b]	3.35 ± 0.49[b]

In A and B different superscripts indicate differences between pEC_{50} and maximum slope values, respectively ($P<0.05$ by ANOVA followed by Fisher's LSD). n is the number of vessels. $pEC_{50} = -\log (EC_{50}$ (M)). Values show mean ± s.e.mean.

Two experiments on the wire-myograph provided indirect verification that PE was also taken up by the nerves, as observed in other preparations (Rawlaw *et al.*, 1980). After blocking reuptake with 3 μM cocaine, pEC_{50}-values for PE increased 0.58 and 0.44, respectively.

Inhibition of neuronal uptake by denervation

Following *in vitro* denervation, there was no difference in pEC_{50} for NA between wire- and pressure-myographs (Table 2b, Figure 5b), since, as with cocaine, denervation caused a greater change in sensitivity in the wire-mounted vessels. However, the denervation did not affect the maximum slope, so that the difference in the maximum slope remained. The denervation procedure did not alter the maximal force development of the vessels in the wire-myograph (maximal active tensions were 3.53 ± 0.32 $N\,m^{-1}$ before and 3.67 ± 0.33 $N\,m^{-1}$ after denervation) or in the pressure-myograph (contracted to 21 ± 3% of relaxed diameter before and 22 ± 3% after denervation).

D600

The effect of D600 (1 μM) on the NA concentration-response relation was investigated on the pressure-myograph ($n = 4$) and in the wire-myograph at distensions of d_0 ($n = 6$) and $0.4 \times d_0$ ($n = 6$). Results are shown in Figure 6. In all three cases, D600 was able to elicit a decrease in the pEC_{50} value for NA. In the wire-mounted vessels, D600 inhibited the maximal active tension, which declined from 4.99 ± 0.07 $N\,m^{-1}$ to 2.46 ± 0.25 $N\,m^{-1}$ and from 0.93 ± 0.03 $N\,m^{-1}$ to 0.18 ± 0.02 $N\,m^{-1}$ at distensions of d_0 and $0.4 \times d_0$, respectively. By contrast, D600 did not affect the maximal contraction in the pressure-myograph (26 ± 1% of the relaxed diameter before and 28 ± 2% after adding D600). However, in both the pressure- and wire-myographs, D600 (1 μM) eliminated vasomotion (i.e. rhythmic changes in the tension or diameter responses to the noradrenaline activation). At the end of each experiment, the vessel was stimulated with 125 mM K^+ in the presence of 1 μM D600 and 1 μM phentolamine: in both the wire- and pressure-myographs, D600 inhibited the sustained phase of the response to K^+.

Discussion

The main results are as follows. First, the passive pressure-internal diameter relationship of vessels tested on the wire-myograph (isometric conditions) was similar to that obtained on the pressure-myograph (isobaric conditions). Second, vessels on the pressure-myograph were 5–10 fold more sensitive to the α-adrenoceptor agonists NA and PE and ca. 3 fold more sensitive to threshold concentrations of vasopressin than on the wire-myograph; furthermore, the maximum slopes determined on the pressure-myograph were smaller than on the wire-myograph. By contrast, the characteristics with K^+ activation were similar. Third, as regards NA, inhibition of neuronal reuptake eliminated the difference in the pEC_{50}. Fourth, D600 decreased the maximal response to NA only in wire-mounted vessels.

Passive properties

Our finding that there was no difference in the pressure-diameter relationship between wire-mounted (calculated on the basis of the Laplace relation) and pressurized vessels suggests that lengthening caused by the intravascular pressure does not affect internal diameter. This interpretation is supported by the finding that longitudinal stretch of vessels on the wire-myograph did not affect the tension-diameter

relation (Figure 2). In contrast, Lew & Angus (1992) found that, at lower pressures, the pressurized vessels had larger diameters than the wire-mounted vessels. However, their pressurized vessels were cannulated only at one end, tied at the other end, and then pressurized like a balloon. Thus, at the lower pressure, the degree of longitudinal extension would have been less than under our conditions (where vessels were held at a fixed longitudinal extension), and this may explain the difference. In this respect, however, it should be emphasized that although under our conditions the wire- and pressure-myographs gave the same pressure-diameter relation, the longitudinal stretch used in the pressure-myograph will certainly reduce the wall thickness, as observed by Lew & Angus (1992), since the deformation involved is isovolumetric (Carew et al., 1968).

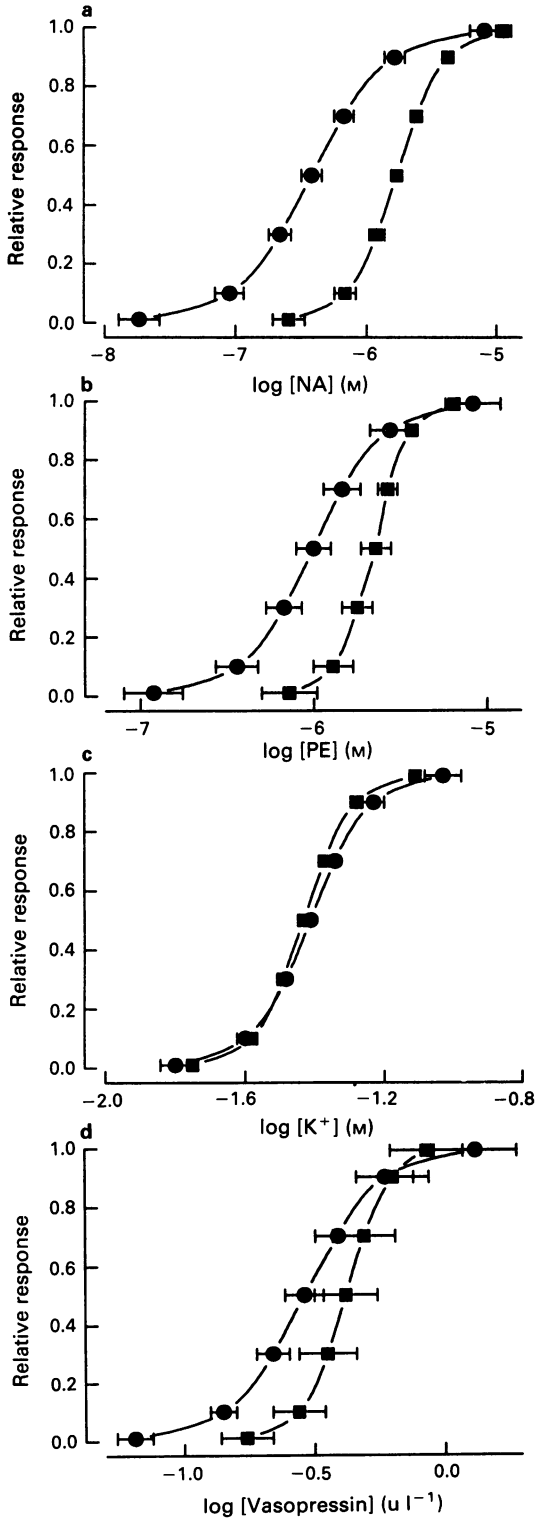

Figure 4 Concentration-response curves for (a) noradrenaline (NA), (b) phenylephrine (PE), (c) potassium (K^+) and (d) vasopressin, for (●) pressurized and (■) wire-mounted vessels. Responses are given as fraction of the maximal active tension (wire-myograph) or the maximal change in diameter (pressure-myograph). Analysis of the curves is shown in Table 1. S.e.means are shown where these exceed the size of the symbol.

Agonist responses in wire-mounted and pressurized vessels

The differences in the concentration-response relations seen in wire-mounted and pressurized vessels were of two types: changes in the pEC_{50} and changes in the maximum slope. These will be considered separately.

Changes in pEC_{50} On the basis of the experiments where the neuronal amine pump was inhibited either by cocaine or by denervation (Figure 5, Table 2), one reason for the lower pEC_{50} seen with NA activation on wire-myographs compared to pressure-myographs appears to be due to the amine pump being more effective in right-shifting the NA concentration-response relation in wire-mounted compared to pressurized vessels. The reason for this is not clear. It seems unlikely to be due to differences in adventitial v. luminal access (Venning & De la Lande, 1984; 1988; Tesfamariam & Halpern, 1988),

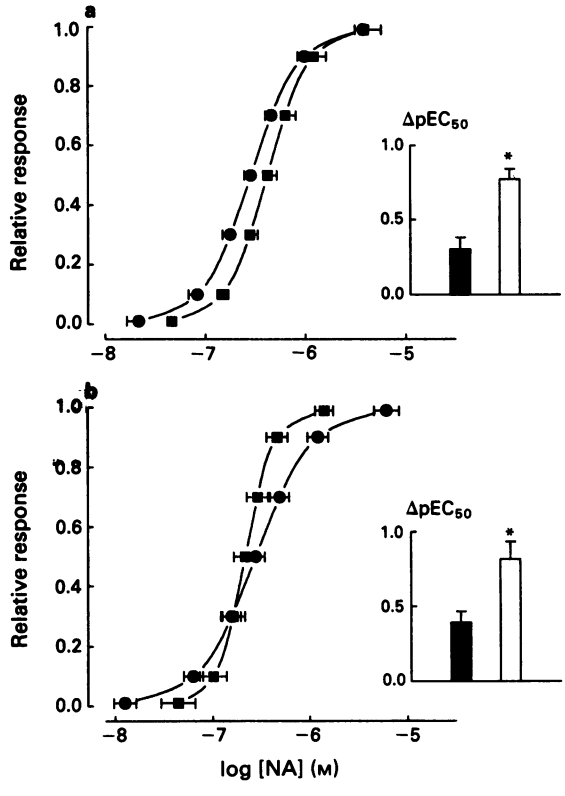

Figure 5 Effect of inhibition of neuronal amine uptake on noradrenaline (NA) concentration-response curves (a) in the presence of 3 μM cocaine and (b) after *in vitro* denervation: (●) pressurized; (■) wire-mounted vessels. Responses are given as a fraction of the maximal active tension (wire-myograph) or the maximal change in diameter (pressure-myograph). Insets show the increase in pEC_{50}-value: (a) after adding cocaine and (b) after denervation; solid columns represent pressurized and open columns wire-mounted vessels. Diameters of relaxed pressurized vessels were 323 ± 9 μm ($n = 16$) and the effective diameters of wire-mounted vessels were 280 ± 10 μm ($n = 20$). *$P < 0.05$ (unpaired t test). Analysis of the curves is shown in Table 2. S.e.means are shown where these exceed the size of the symbol.

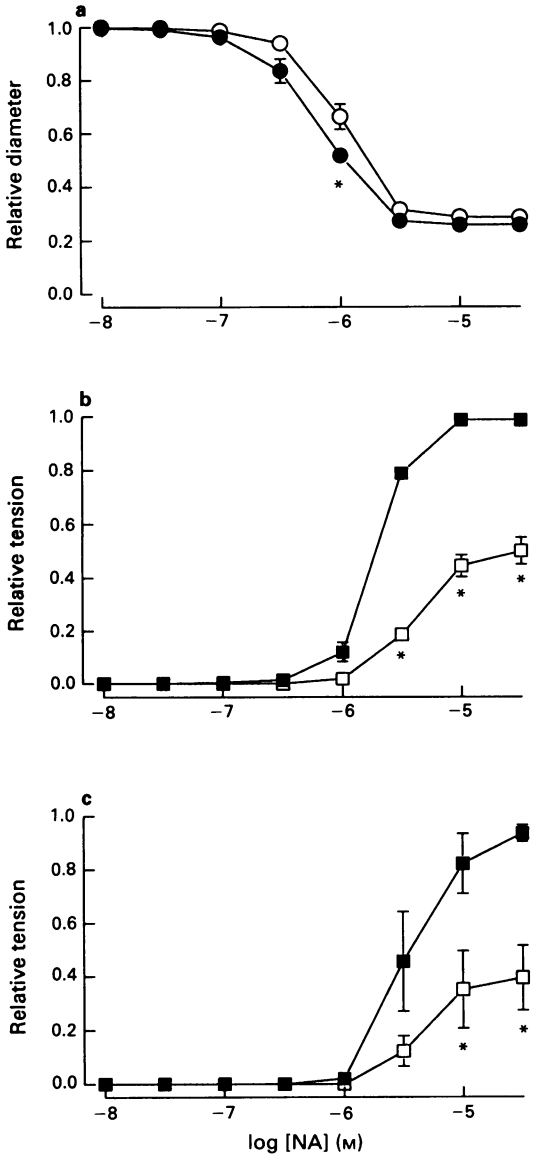

Figure 6 Concentration-response curves for noradrenaline (NA) in absence (closed symbols) and presence (open symbols) of 1 μM methoxyverapamil (D600). (a) Pressurized vessels (mean relaxed diameter 371 ± 25 μm, $n = 4$), where relative diameter given as fraction of the diameter of relaxed vessel. (b) Wire-mounted vessels at a distension of d_0 (294 μm, $n = 6$) and (c) $0.4 \times d_0$ (90 μm, $n = 6$); responses in (b) and (c) are given as fraction of the initial vessel response to the maximal concentration of noradrenaline. $^*P < 0.05$ (paired t test). S.e.means are shown where these exceed the size of the symbol.

since in both the wire- and the pressure-myographs, the NA had direct access to the adventitial surface. One possibility is that the longitudinal stretch imposed in the pressure-myograph results in the media surface area per amine pump being increased, so that the NA had freer access to the medial surface. Another possibility could be related to the observation that the effect of cocaine on the NA concentration-response relation was decreased when vessels were held at reduced diameter on the wire-myograph: in the pressure-myograph, the diameter is also greatly reduced with NA-activation. Further work is required to establish the true reason, but in any event the findings suggest that a major reason for the difference in NA pEC_{50}-values of wire-mounted and pressurized vessels is related to differences in the effectiveness of the neuronal amine pump under these conditions. This hypothesis is supported by the findings (a) that the pEC_{50} for PE (which also appears to be taken up by the amine pump, judging by the ability which we observed of cocaine to increase the pEC_{50} for PE) was also greater in pressurized vessels than for wire-mounted vessels, and (b) that the pEC_{50}-values for vasopressin and for K^+ (neither of which should be affected by the amine pump) were similar for pressurized and wire-mounted vessels.

Other mechanisms may also be responsible for differences in sensitivity on the wire- and pressure-myographs, for the NA threshold concentrations in the denervated and cocaine-exposed vessels were still slightly lower in the pressurized vessels than in the wire-mounted vessels (Figure 5), as were the vasopressin threshold concentrations (Figure 4). Such increased sensitivity is not observed when vessels are held under quasi-isobaric conditions on a wire-myograph in which the vessel distension is controlled by a motor to provide a constant pressure according to the Laplace relation (McPherson, 1992). Furthermore, threshold sensitivity in vessels held on a pressure-myograph under isometric conditions is similar to the sensitivity on such a myograph under isobaric conditions (VanBavel & Mulvany, 1994). Thus, it appears that the increased intravascular pressure itself may be causing the increase of threshold sensitivity to agonists, either directly through the increase in transmural pressure, or indirectly through the longitudinal extension caused by the increased transmural pressure. The present experiments do not show the mechanisms which are involved, but it must have some specificity, since no difference in the K^+ threshold was observed (Figure 4). The similarity of the results from NA and from PE (Figure 4, Table 1) indicate that neither α_2- nor β-adrenoceptors are involved.

Changes in maximum slope Under isobaric conditions, when a vessel contracts in response to activation, the equilibrium wall tension decreases according to the law of Laplace. This is a positive feedback mechanism, and thus isobaric activation might be expected to be an all-or-none phenomenon. That this does not happen in practice (e.g. with NA, Figure 4) is in part due to the vessel moving down the active tension-diameter curve (VanBavel & Mulvany, 1994). However, it is also likely that a myogenic mechanism is involved for, as Johnson (1980) has argued, it appears that wall tension is a regulator of vascular tone, such that a decrease in wall tension will reduce the level of activity. In isometric experiments, where resting wall tension has been varied by stretch, there is now substantial evidence that the sensitivity of vessels to α-agonists is reduced at low resting wall tensions, both in wire-mounted vessels (Price *et al.*, 1981; Nilsson & Sjøblom, 1985; McPherson, 1992) and in pressurized vessels (Lombard *et al.*, 1990; Meininger & Faber, 1991), as well as *in vivo* (Meininger & Trzeciakowski, 1988). It therefore seems plausible that the comparatively gradual maximum slope of the concentration-diameter curve, seen with agonist activation under isobaric conditions, is due to the decrease in wall tension which results from activation having a negative feedback action on vessel tone (VanBavel & Mulvany, 1994). By contrast, under isometric conditions, the increase in wall tension which accompanies activation will have a positive feedback action on vessel tone. Thus, it has been suggested (VanBavel & Mulvany, 1994) that the reason for the maximum slope being smaller for pressurized vessels compared to wire-mounted vessels may be the wall tension-regulating system proposed by Johnson (1980).

The present experiments provide some clues to a mechanism which could account for Johnson's (1980) proposed wall tension regulating system. On the basis that this system is responsible for the difference in the maximum slopes for wire-mounted and pressurized vessels, it appears that it is active not only for NA, but also for PE and vasopressin (Figure 4, Table 1). However, it does not appear to be active for K^+, where the maximum slopes for wire-mounted and pressurized vessels are the same. Furthermore, the sensitivity of wire-mounted vessels to K^+ activation is independent of

distension (Nilsson & Sjøblom, 1985). Moreover, the maximum slope of the NA concentration-response relation of wire-mounted vessels is decreased if the experiment is performed in a solution containing 30 mM K^+, which would limit any depolarizing effect of NA (Mulvany *et al.*, 1982). We can therefore hypothesize that the shallow slope of the agonist concentration-response curve of pressurized vessels is because under these conditions the agonist-induced depolarization is less than on wire-myographs. This hypothesis is supported by the finding that the verapamil analogue, D600 (which would block calcium influx through channels opened by depolarization) inhibited NA responses of wire-mounted vessels, but had little effect on pressurized vessels (Figure 6). The hypothesis does not explain, however, why cocaine (3 μM) caused a reduction of the maximum slope of wire-mounted vessels (Table 2a), a phenomenon which has been previously ascribed to high concentrations of noradrenaline causing saturation of the amine pump (Langer & Trendelenburg, 1969). Furthermore, we have found (Nilsson, Flatman & Mulvany, unpublished observations) that this concentration of cocaine does not affect the relation between force production and membrane potential in wire-mounted vessels, which argues against the maximum slope being dependent on this relation. More work, including measurements of membrane potential on a pressure-myograph, is required to elucidate this question.

Lack of action of D600 in pressurized vessels

Although the lack of action of D600 on the NA response of pressurized vessels could be explained as described above if such vessels did not depolarize in response to noradrenaline, that possibility remains to be tested. We have therefore considered other possibilities. The lack of action on pressurized vessels contrasts with the potent effect on isometric responses, as shown here (Figure 6), and previously (Nyborg & Mulvany, 1984). The lack of effect was not due to the D600 inactivating in the pressure-myograph chamber, since the vasomotion which was seen with NA activated vessels, disappeared in the presence of D600, as has been observed with other calcium antagonists (Gustafsson & Nilsson, 1991). Furthermore, the observed difference in action cannot be explained by an influence of the vessel diameter on the action of D600, since the response to NA was still reduced by about 50% at a low distension in the wire-myograph (Figure 6c). In support of our findings, an *in vivo* study on arterioles (De Clerck *et al.*, 1989) found no reduction in maximal vasoconstriction to NA, but only a reduction in sensitivity after treatment with verapamil. Further studies are required to determine the reason for this substantial difference between the responses of wire-mounted and pressurized vessels to a calcium-antagonist.

Perspectives

In summary, the results indicate that pressurized mesenteric vessels have a higher sensitivity to α-agonists (expressed as a pEC_{50}) than wire-mounted vessels under circumstances of the same initial smooth muscle cell length. This appears to be due to a smaller neuronal uptake of agonists in the pressurized vessel. But this only explains part of the difference as all the agonists, except potassium, showed a smaller threshold concentration in the pressure-myograph indicating that other factors are of importance. Furthermore, wire-mounted vessels have a steeper agonist concentration-response relation than pressurized vessels, while the calcium antagonist, D600, was a potent inhibitor of NA responses in wire-mounted vessels, but had little effect in pressurized vessels. Since the *in vivo* situation corresponds more closely to an isobaric condition than to an isometric condition, the results presented here raise the possibility that responses obtained under isometric conditions may in part be mediated by mechanisms which are less important under *in vivo* conditions. Thus, although isometric experiments are convenient to perform, our results provide additional cause for caution in interpreting them in terms of the *in vivo* situation.

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References

APRIGLIANO, O. & HERMSMEYER, K. (1976). In vitro denervation of the portal vein and caudal artery of the rat. *J. Pharmacol. Exp. Ther.*, **198**, 568–577.

BUUS, N.H. & MULVANY, M.J. (1992). Noradrenaline sensitivity of rat mesenteric small arteries determined on a pressure myograph and a wire myograph. *Acta Physiol. Scand.*, **146** (suppl 608), 54 (abstract).

CAREW, T.E., VAISHNAV, R.N. & PATEL, D.J. (1968). Compressibility of the arterial wall. *Circ. Res.*, **23**, 61–68.

DE CLERCK, F., LOOTS, W., VOETEN, J. & JANSSEN, P.A.J. (1989). Differential effects of verapamil and flunarizine on epinephrine-induced vasoconstriction and on spontaneous vasomotion of arterioles in skeletal muscle in the rat in vivo. *J. Cardiovasc. Pharmacol.*, **13**, 76–83.

DOHI, Y. & LÜSCHER, T.F. (1990). Aging differentially affects direct and indirect actions of endothelin-1 in perfused mesenteric arteries of the rat. *Br. J. Pharmacol.*, **100**, 889–893.

GUSTAFSSON, H. & NILSSON, H. (1991). Rhythmic contractions of isolated small arteries. In *Resistance Arteries, Structure and Function*. ed. Mulvany, M.J., Aalkjær, C., Heagerty, A.M., Nyborg, N.C.B. & Strandgaard, S. pp. 196–198. Amsterdam: Elsevier Science Publishers.

HALPERN, W. & KELLEY, M. (1991). In vitro methodology for resistance arteries. *Blood Vessels*, **28**, 245–251.

HALPERN, W. & OSOL, G. (1985). Blood vessel diameter measurement. *Prog. Appl. Microcirc.*, **8**, 32–39.

HALPERN, W., OSOL, R. & OSOL, G. (1987). Activation induces myogenic-like diameter responses to dynamic pressure changes in isolated and pressurized small mesenteric arteries in the rat. In *Vascular Neuroeffect or Mechanisms*. ed. Bevan, J.A., Majewski, H., Maxwell, R.A. & Story, D.F. pp. 15–22. Washington DC: JRL Press Ltd.

JOHNSON, P.C. (1980). The myogenic response. In *Handbook of Physiology – The Cardiovascular System*. ed. Bohr, D.R., Somlyo, A.P. & Sparks, Jr, H.V. pp. 409–442. Bethesda: The American Physiological Society.

LANGER, S.Z. & TRENDELENBURG, U. (1969). The effect of a saturable uptake mechanism on the slopes of dose-response curves for sympathetic amines and on the shifts of dose-response curves produced by a competitive antagonist. *J. Pharmacol. Exp. Ther.*, **167**, 117–142.

LEW, M.J. & ANGUS, J.A. (1992). Wall thickness to lumen diameter ratios of arteries from SHR and WKY: comparison of pressurised and wire-mounted preparations. *J. Vasc. Res.*, **29**, 443–449.

LOMBARD, J.H., ESKINDER, H., KAUSER, K., OSBORN, J.L. & HARDER, D.R. (1990). Enhanced norepinephrine sensitivity in renal arteries at elevated transmural pressure. *Am. J. Physiol.*, **259**, H29–H33.

MARSHALL, J.M. (1977). The effect of uptake by adrenergic nerve terminals on the sensitivity of arterial vessels to topically applied noradrenaline. *Br. J. Pharmacol.*, **61**, 429–432.

McCARRON, J.G., QUAYLE, J.M., HALPERN, W. & NELSON, M.T. (1991). Cromakalim and pinacidil dilate small mesenteric arteries but not small cerebral arteries. *Am. J. Physiol.*, **261**, H287–H291.

McPHERSON, G.A. (1992). Assessing vascular reactivity of arteries in the small vessel myograph. *Clin. Exp. Pharmacol. Physiol.*, **19**, 815–825.

MEININGER, G.A. & FABER, J.E. (1991). Adrenergic facilitation of myogenic response in skeletal muscle arterioles. *Am. J. Physiol.*, **260**, H1424–H1432.

MEININGER, G.A. & TRZECIAKOWSKI, J.P. (1988). Vasoconstriction is amplified by autoregulation during vasoconstrictor-induced hypertension. *Am. J. Physiol.*, **254**, H709–H718.

MULVANY, M.J. & AALKJÆR, C. (1990). Structure and function of small arteries. *Physiol. Rev.*, **70**, 921–961.

MULVANY, M.J. & HALPERN, W. (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.*, **41**, 19–26.

MULVANY, M.J., NILSSON, H. & FLATMAN, J.A. (1982). Role of membrane potential in the response of rat small mesenteric arteries to exogenous noradrenaline stimulation. *J. Physiol.*, **332**, 363–373.

NIELSEN, H. & MULVANY, M.J. (1990). The divergence in the excitation-contraction coupling of rat mesenteric resistance arteries lies distal to the receptor site. *Eur. J. Pharmacol.*, **179**, 1–7.

NILSSON, H. & SJÖBLOM, N. (1985). Distension-dependent changes in noradrenaline sensitivity in small arteries from the rat. *Acta Physiol. Scand.*, **125**, 429–435.

NYBORG, N.C.B. & MULVANY, M.J. (1984). Effect of felodipine, a new dihydropyridine vasodilator, on contractile responses to potassium, noradrenaline and calcium in mesenteric resistance vessels of the rat. *J. Cardiovasc. Pharmacol.*, **6**, 499–505.

NYBORG, N.C.B. & MIKKELSEN, E.O. (1985). In vitro studies on responses to noradrenaline, serotonin, and potassium of intramyocardial and mesenteric vessels from wistar rats. *J. Cardiovasc. Pharmacol.*, **7**, 417–423.

PRICE, J.M., DAVIS, D.L. & KNAUSS, E.B. (1981). Length-dependent sensitivity in vascular smooth muscle. *Am. J. Physiol.*, **241**, H557–H563.

RAWLAW, A., FLEIG, H., KURAHASHI, K. & TRENDELENBURG, U. (1980). The neuronal and extraneuronal uptake and deamination of ^{3}H-(−)-phenylephrine in the perfused rat heart. *Naunyn-Schmied. Arch. Pharmacol.*, **314**, 237–247.

TESFAMARIAM, B. & HALPERN, W. (1988). Asymmetry of responses to noradrenaline in perfused resistance arteries. *Eur. J. Pharmacol.*, **152**, 167–170.

TESFAMARIAM, B., HALPERN, W. & OSOL, G.K. (1985). Effects of perfusion and endothelium on the reactivity of isolated resistance arteries. *Blood Vessels*, **22**, 301–305.

VANBAVEL, E., GIEZEMAN, M.J.M.M., MOOIJ, T. & SPAAN, J.A.E. (1991). Influence of pressure alterations on tone and vasomotion of isolated mesenteric small arteries of the rat. *J. Physiol.*, **436**, 371–383.

VANBAVEL, E., MOOIJ, T., GIEZEMAN, M.J.M.M. & SPAAN, J.A.E. (1990). Cannulation and continuous cross-sectional area measurement of small blood vessels. *J. Pharmacol. Methods*, **24**, 219–227.

VANBAVEL, E. & MULVANY, M.J. (1994). Role of wall tension in the vasoconstrictor response of cannulated rat small arteries. *J. Physiol.*, (in press).

VENNING, M.G. & DE LA LANDE, I.S. (1984). Effects of uptake and surface of entry on the responses of the rat caudal artery to noradrenaline, adrenaline and methoxamine. *Blood Vessels*, **21**, 149–155.

VENNING, M.G. & DE LA LANDE, I.S. (1988). Role of sympathetic nerves in disposition and metabolism of intraluminal and extraluminal noradrenaline in the rabbit ear artery. *Blood Vessels*, **25**, 232–239.

WHALL, C.W., MYERS, M.M. & HALPERN, W. (1980). Norepinephrine sensitivity, tension development and neuronal uptake in resistance arteries from spontaneously hypertensive and normotensive rats. *Blood Vessels*, **17**, 1–15.

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