Role of wall tension in the vasoconstrictor response of cannulated rat mesenteric small arteries

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1. We have studied the influence of mechanical loading conditions on the responses of cannulated rat mesenteric small arteries to noradrenaline, vasopressin and potassium.

2. The cross-sectional area (CSA) of vessels was continuously monitored. Isometric loading (CSA-controlled conditions) or isobaric loading (pressure-controlled conditions) was achieved by feedback adjustment of the distending pressure.

3. Noradrenaline (0·3 μM) and vasopressin (0·05 u l^{-1}) induced myogenic responsiveness, resulting in a constant or declining CSA with increasing pressure. Potassium (32 mM) induced weak myogenic responsiveness.

4. At a constant pressure of 60 cmH_2O, noradrenaline and vasopressin concentration–response curves were graded, the concentration–response curves of individual vessels being extended over two to three decades. Sensitivity to the vasoconstrictors, expressed as pD_2 values ($-\log_{10} EC_{50}$), averaged 6·45 ± 0·18 log M and 1·27 ± 0·20 log u l^{-1} for the noradrenaline and vasopressin concentration–response curves respectively. The isobaric pD_2 for K^+ was 1·54 ± 0·07 log M.

5. During CSA-controlled conditions, noradrenaline and vasopressin induced all-or-none responses to stretch. Potassium induced graded responses to stretch.

6. During CSA-controlled conditions, noradrenaline and vasopressin concentration–response curves also showed all-or-none behaviour. Almost the full response occurred through only a doubling of the concentration. pD_2 values were 6·88 ± 0·38 log M (noradrenaline) and 1·87 ± 0·43 log u l^{-1} (vasopressin). Isometric vessels were significantly more sensitive to noradrenaline and vasopressin than isobaric vessels. Isometric K^+ curves were gradual. pD_2 was 1·54 ± 0·07 log M, a value not different from the isobaric value.

7. These findings can be explained by assuming that agonist sensitivity is wall tension dependent, such that sensitivity increases with increasing wall tension. This concept accounts for partial regulation of wall tension during pressure-controlled conditions, as well as instability due to a positive feedback loop of active tension development and tension-induced sensitization during CSA-controlled conditions.

Many vascular smooth muscle preparations exhibit myogenic responses, defined as contractile responses caused by stretch or increase in transmural pressure (Johansson, 1989). This indicates that these preparations possess at least one type of mechanosensor, and such responses are commonly seen in preparations showing basal tone (Kuo, Davis & Chilian, 1988; Jackson & Duling, 1989). However, mechanosensors also appear to be involved in modulation by stretch (Tallarida, Sevy, Harakal, Bendrick & Faust, 1974; Price, Davis & Knauss, 1981, 1983; Nilsson & Sjoeblom, 1985) or pressure (Harder, 1988; Lombard, Eskinder, Kauser, Osborn & Harder, 1990; Meininger & Faber, 1991) of the responses to α-adrenergic agonists and other vasoconstrictors. The nature of these putative mechanosensors is unknown, and the sensors involved in both the myogenic response and sensitization to drugs may or may not be identical.

It has been suggested (Johnson, 1980) that the mechanosensor involved in the myogenic response is a tension sensor. Thus Johnson suggested that it is the increase in wall tension, not the pressure, which induces a constriction. This constriction then leads (according to the

Laplace relationship) to a reduction in wall tension, and thereby also to inhibition of further constriction. The net effect of this negative feedback loop on wall tension is that vessel diameter is regulated, to some extent, against changes in transmural pressure.

A consequence of this proposal is that the responses of vessels to vasoconstrictors could also be modulated by wall tension, and that this could form the basis of the stretch and pressure sensitivity of the vasoconstrictor responses mentioned above. On this basis, under isobaric conditions, the constriction resulting from agonist activation and the consequent fall in wall tension would lead to a reduced agonist sensitivity. Thus wall tension may not only be regulated against disturbances in pressure, but also against changes in agonist concentration. In contrast, under isometric conditions, the consequence of Johnson's proposal is that the feedback loop of tension control is positive, not negative, because activation then leads to an increase, rather than a decrease, in total wall tension. The presence of a tension sensor would therefore be expected to destabilize the response of isometric vessels to agonist activation.

In this paper we present evidence for the presence of a tension sensor which modulates the responses of rat mesenteric small arteries to noradrenaline and vasopressin stimulation. The work has been done using cannulated vessels in which a servo system was used to control intravascular pressure or vessel cross-sectional area (CSA). This allowed the vessels to be held in either isobaric (pressure-controlled) or isometric (CSA-controlled) mode. The responses of cannulated vessels to agonists and pressure during pressure-controlled conditions have been compared with the responses of the cannulated vessels to agonists and stretch under CSA-controlled conditions.

METHODS

Cannulation

Male Wistar rats were killed by either CO_2 inhalation or removal of the heart after anaesthesia by pentobarbitone (0·06 mg (g bodyweight)$^{-1}$). Distal segments of mesenteric arcade arteries were dissected out in physiological salt solution (PSS; composition (mM): NaCl, 119; KCl, 4·7; KH_2PO_4, 1·18; $MgSO_4$, 1·17; $NaHCO_3$, 25; $CaCl_2$, 1·6; EDTA, 0·026; glucose, 5·5; Hepes, 10; solution equilibrated with 5% CO_2, pH 7·35 ± 0·05). The vessels were cannulated using glass micropipettes and secured on the pipettes by two strands of 15 μm thread at each end. The vessels were pressurized at both ends by a voltage-to-pressure converter (Fairchild T-5200-9, Winston-Salem, NC, USA), which pressurized the reservoirs connected to both cannulas. A 1–2 cmH_2O pressure gradient was maintained between both cannulae by adjusting the height of the fluids in the reservoirs. This gradient induced a flow of the order of 10–100 μl min^{-1}. This flow was too small to induce flow-dependent dilatation or constriction, as evidenced by the lack of effect of stopping flow in preconstricted vessels. Vessels were perfused with PSS containing 40 mg l^{-1} of the fluorescing dye fluorescein isothiocyanate (FITC)–dextran (Sigma, St Louis, MO, USA; MW 500000) and 5 g l^{-1} bovine serum albumin (Sigma; fraction V).

Measurement of cross-sectional area

Luminal CSA was monitored as described elsewhere (VanBavel, Mooij, Giezeman & Spaan, 1990). In short, the FITC–dextran in the lumen was excited by a weak blue light source (wavelength 400–480 nm), and the total amount of emission light (wavelength > 515 nm) was measured using a photomultiplier tube. Constriction of the vessel results in a decrease of the volume of dye solution and thereby in a decrease of fluorescence light. The linear relation between photomultiplier current and CSA was calibrated using estimations of CSA from manual diameter measurements.

Application of isometric loading

A short description of the isometric loading technique and some initial results have been presented previously (VanBavel & Spaan, 1991). Under isometric loading conditions, pressure was continuously adjusted in order to keep the CSA signal equal to a reference CSA. Under these conditions, the increase in pressure needed to maintain the isometric state in response to agonist activation ('active pressure') reflects the activity of the vessel. The essentials of the CSA-controlled circuitry are illustrated in Fig. 1. The output of the photomultiplier is compared to a fluorescence set-point that serves as an index of the desired CSA. The difference is both amplified and integrated by the control block in Fig. 1. Output from this block is used to drive the voltage-to-pressure transducer. Due to the integrating action of the control block, the steady-state fluorescence signal will match the reference signal, while the amplifying action causes rapid adjustments in pressure due to either a step change in the reference value or a sudden change in the activity of the vessel.

Stretching of passive vessels typically took about 5 s. Attempts to stretch the vessel after development of active pressure had little success: in initial experiments we found that, due to the high wall viscosity of activated vessels, extremely high pressures were needed to perform this task within a reasonably short time. This always resulted in irregular constriction and irreversible loss of function. Therefore we applied only very slow stretches to already activated vessels, taking about 1 min to finish the stretch. This still resulted in a loss of active pressure in some cases, but the vessel remained straight and no irreversible damage appeared to have occurred.

Application of drugs

Noradrenaline-hydrochloride and acetylcholine-hydrochloride (Sigma) were kept in frozen stock solutions of 10^{-2} M in distilled water. Other drugs were phentolamine (Regitine; Ciba-Geigy, Basel, Switzerland) and lysine vasopressin (Lypressinum; Sandoz, Basel, Switzerland); stock solutions of these drugs were prepared freshly for each experiment.

All drugs were applied extraluminally only. The solution in the cannulation chamber was continuously refreshed by recirculating fluid from a 100 ml reservoir at a rate of 10 ml min^{-1}. Concentration–response curves were obtained by changing the supply reservoir for the refreshment solution with one containing the new final concentration. During these phases, the excess fluid in the cannulation chamber was not recirculated.

Protocol

The following protocol was applied with either noradrenaline (NA, $n = 9$), lysine vasopressin (LVP, $n = 5$) or potassium ($n = 4$) as a vasoconstrictor.

(1) The passive steady-state CSA was determined at distending pressures between 5 and 180 cmH_2O. This was done by stepping up and down twice between these pressures and averaging the results. Most vessels were passive in PSS. However, to be sure that no activity remained, 1 or 10 μM acetylcholine was added as a vasodilator. The passive CSA at 140 cmH_2O (CSA_0) was taken as an index of the anatomical size of the vessel.

(2) The vasoconstrictor was added to the superfusate at 20 cmH_2O. Subsequently, pressure was increased in steps to 60, 100, 140 and 180 cmH_2O and then decreased in these steps back to 20 cmH_2O. Each pressure level was maintained for 3 min. Results for the up-going and down-going phases were averaged for each vessel.

(3) Pressure was set to 60 cmH_2O, and a cumulative concentration–response curve was made. Each vasoconstrictor level was maintained for 2 min.

(4) The vessel was loaded isometrically by feedback adjustment of the pressure. The CSA was set to $0{\cdot}25 \times CSA_0$ and the vasoconstrictor was added. Subsequently the CSA was changed to 0·36, 0·49, 0·64 and $0{\cdot}81 \times CSA_0$. Each CSA was maintained for 3 min. Note that these levels are equivalent to diameters of 0·5, 0·6, 0·7, 0·8 and 0·9 times the passive diameter at 140 cmH_2O.

(5) During isometric loading, the CSA was clamped to $0{\cdot}81 \times CSA_0$. Subsequently, a cumulative concentration–response curve was constructed. Each concentration was maintained for 2 min. In some vessels, holding the CSA at $0{\cdot}81 \times CSA_0$ resulted in active responses prior to agonist stimulation. In these cases, the CSA was reduced to $0{\cdot}64 \times CSA_0$ or $0{\cdot}49 \times CSA_0$.

Steps (2) and (4) were performed with either 0·3 μM NA, 0·05 u l^{-1} LVP, or 32 mM K^+. Concentration–response curves (steps (3) and (5)) were performed for 0·01–5 μM NA (3 concentrations per decade), 0·002–2 u l^{-1} LVP (3 concentrations per decade), and 6–125 mM K^+ (9 concentrations). Experiments with potassium were done in the presence of 1 μM phentolamine in order to block possible effects of noradrenaline produced by depolarized nerves.

Steps (2)–(5) were carried out in a random order.

Data analysis

All recorded CSAs were normalized to the passive value at 140 cmH_2O (approximately 100 mmHg), i.e. CSA_0. Sensitivity to the vasoconstrictors was expressed as pTh_{10} and pD_2, $-\log_{10}$ of the concentration needed to induce 10 % and 50 % of the maximal effect, respectively. The steepness of the concentration–response curves is expressed as the percentage of the total response that is covered by a single step in the concentration. All statistical tests are two-sided paired t tests, with $P < 0{\cdot}05$ indicating significant differences. Data are presented as means ± S.D., unless otherwise indicated.

RESULTS

Eighteen vessels were successfully cannulated. The CSA_0 values of the vessels used with noradrenaline (NA, $n = 9$), vasopressin (LVP, $n = 5$) and potassium ($n = 4$) were $(64{\cdot}0 \pm 13{\cdot}3) \times 10^3$, $(65{\cdot}0 \pm 23{\cdot}3) \times 10^3$ and $(51{\cdot}6 \pm 6{\cdot}3) \times 10^3$ μm^2 respectively, equivalent to diameters of 284 ± 30, 284 ± 52 and 255 ± 31 μm. There was no significant difference between these three groups of diameters. At 60 cmH_2O, some vessels ($n = 12$) developed periods of slight basal tone, resulting in typically 10–15 % reduction in CSA, while others ($n = 6$) remained maximally dilated in the absence of vasoconstrictors.

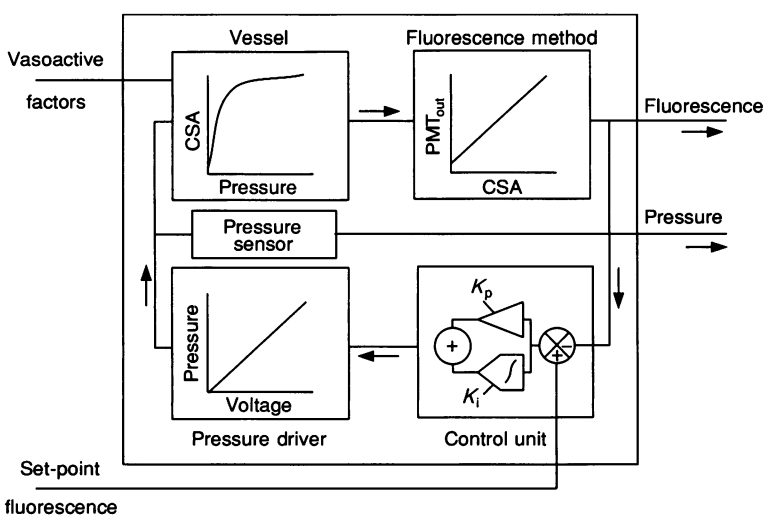

Figure 1. Technique for isometric loading of the vessel
The 'Vessel' block in this schematic representation shows luminal cross-sectional area of the vessel as a function of pressure. This CSA causes a photomultiplier signal, as indicated by the calibration curve in the 'Fluorescence method' block. The control unit compares this fluorescence signal with a set-point and drives the voltage-to-pressure converter (Pressure driver).

Pressure-controlled conditions

Addition of submaximal concentrations of NA, LVP or K^+ caused vessels to show myogenic characteristics. These results are summarized in Fig. 2, which shows both the individual steady-state pressure–CSA relationships and the mean relationships after addition of 0·3 μM NA (Fig. 2*A*), 0·05 u l^{-1} LVP (Fig. 2*B*), and 32 mM K^+ (Fig. 2*C*). For comparison, the mean passive relationships are also indicated. As can be seen, at 20 cmH_2O the vasoconstrictors induce little constriction. Above 20 cmH_2O, however, their effect becomes progressively more marked. Between 60 and 180 cmH_2O, the vessels with NA- or LVP-induced tone showed a flat or significantly declining steady-state CSA with increasing pressure, while the CSA of the vessels with K^+-induced tone continuously increased with pressure. Thus NA- or LVP-stimulated vessels were more sensitive to pressure than K^+-stimulated vessels, even though the amount of induced tone was approximately equal for all constrictors. As shown in the inset of Fig. 2*C*, K^+ did induce myogenic responses. However, these responses, in contrast to the myogenic responses in the presence of NA or LVP, were not large enough to overcome the initial passive elastic effects upon changing pressure.

Under isobaric conditions (60 cmH_2O), NA and LVP caused a graded concentration-dependent constriction of individual vessels. Figure 3*A* and *B* shows the individual concentration–response curves. The K^+ curves (Fig. 3*C*) were biphasic, starting with a reversion of the small amount of basal tone that was present at the start of measurement of these curves, followed by a concentration-dependent constriction of the vessels. The initial dilatation presumably stems from the action of K^+ on the Na^+–K^+-ATPase. Table 1 summarizes pTh_{10} and pD_2 values for the three constrictors.

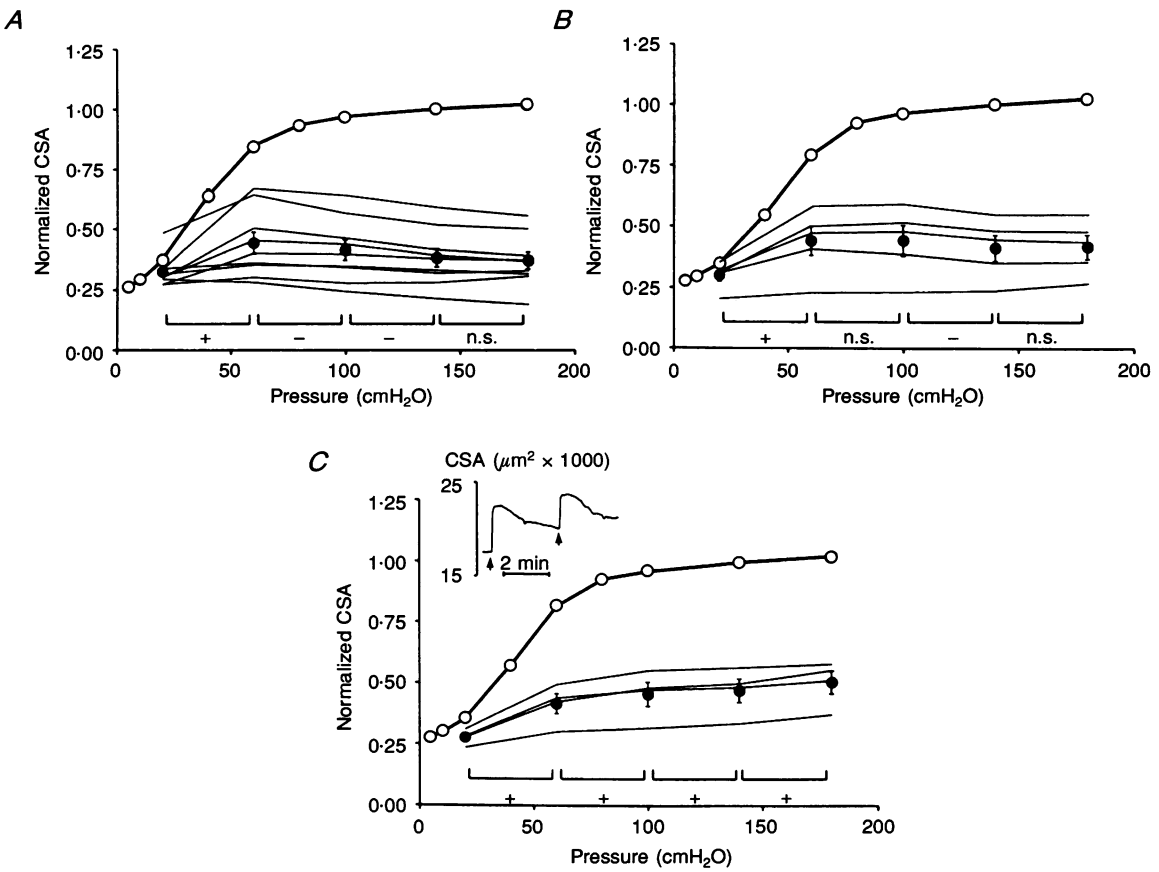

Figure 2. Cross-sectional area (CSA) as a function of distending pressure
These results are normalized with respect to the CSA when relaxed at a pressure of 140 cmH_2O (CSA_0). ●, mean CSA in presence of 0·3 μM NA (*A*; $n = 9$), 0·05 u l^{-1} LVP (*B*; $n = 5$), or 32 mM K^+ (*C*; $n = 4$). ○, mean CSA of the same groups of vessels at maximal dilatation. Thin lines, individual relations in the presence of the vasoconstrictors. The symbols + and – indicate a significant increase and decrease respectively in CSA between two consecutive pressure levels in the presence of the vasoconstrictors; n.s., no significant change of CSA. Error bars are S.E.M.; bars not shown are within symbol limits. The inset in *C* shows part of one of the experiments with K^+, in which the arrows indicate step changes of pressure from 60 to 100 and 100 to 140 cmH_2O.

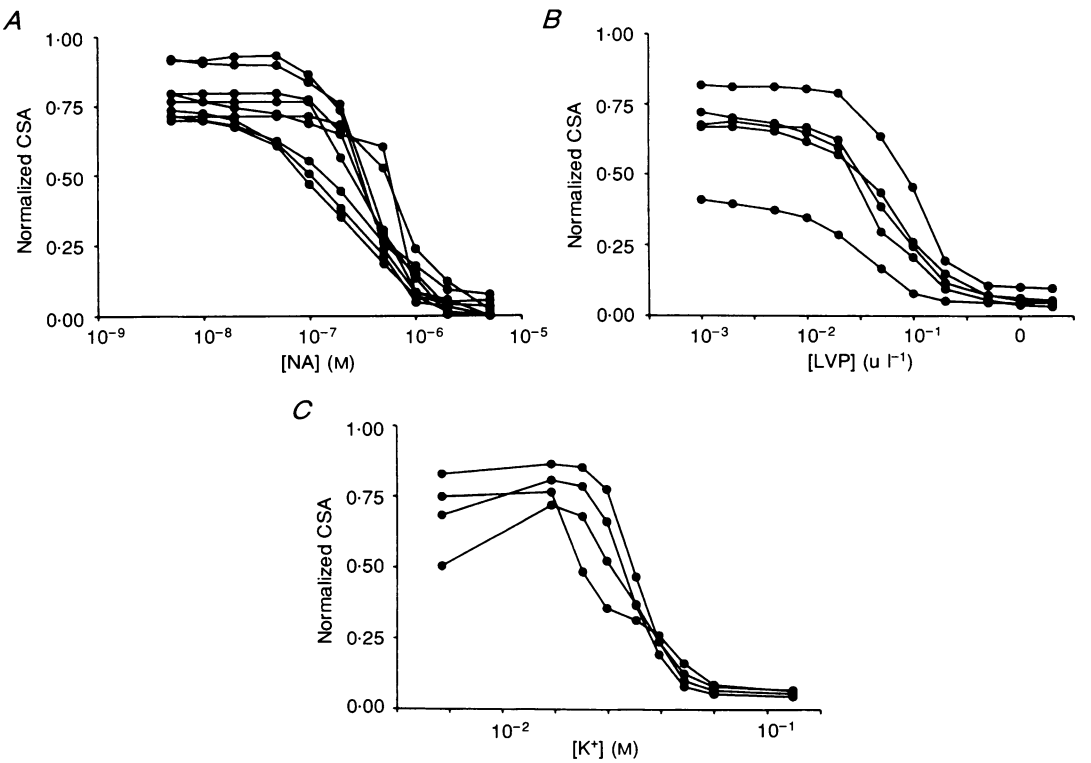

Figure 3. Isobaric concentration–response curves of individual vessels
Results are shown for NA (*A*), LVP (*B*) and K^+ (*C*) at 60 cmH_2O distending pressure. CSA has been normalized to the passive level at 140 cmH_2O (CSA_0) and is smaller than 1 at the start of the curves due to the lower pressure and, in some cases, a small amount of basal tone.

Table 1. pTh_{10} and pD_2 values (± S.D.) for NA (log M), LVP (log u l^{-1}) and K^+ (log M) under isobaric and isometric conditions

		pTh_{10}		pD_2	
	n	Isobaric	Isometric	Isobaric	Isometric
NA	9	7·10 ± 0·35	7·08 ± 0·41	6·45 ± 0·18	6·88 ± 0·38*
LVP	5	1·93 ± 0·29	2·05 ± 0·43	1·27 ± 0·20	1·87 ± 0·43*
K^+	4	1·68 ± 0·07	1·62 ± 0·05	1·54 ± 0·07	1·54 ± 0·07

*$P < 0·05$ (isobaric *vs.* isometric).

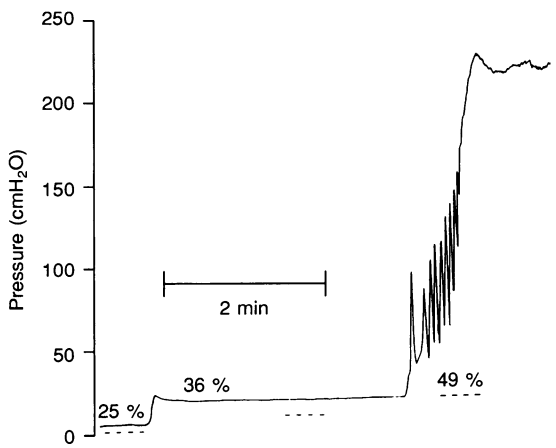

Figure 4. Example of stretch activation of a vessel in the presence of 0·3 μM NA
The ordinate shows the pressure needed to keep the vessel at the desired CSA (respectively 25, 36 and 49 % of CSA_0). The dashed lines indicate this pressure during absence of tone. The difference between these dashed lines and the response during 0·3 μM NA is the 'active pressure' which is almost absent at the two smallest distensions, but large during 49 % distension.

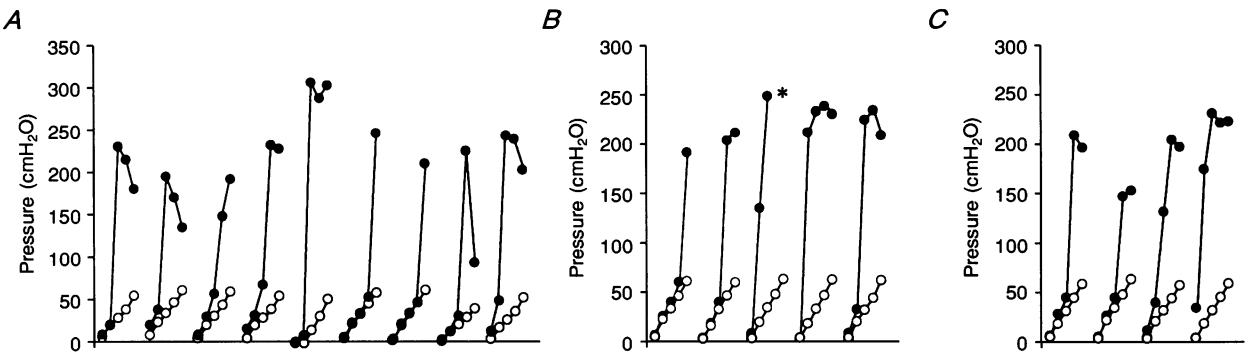

Figure 5. Stretch activation of individual vessels
These results were obtained during stimulation with 0·3 μM NA (*A*), 0·05 u l^{-1} LVP (*B*) and 32 mM K^+ (*C*). Each of the curves in these line plots reflects one vessel. The 5 points on each curve reflect the steady-state pressures at stretches of 0·25, 0·36, 0·49, 0·64 and 0·81 × CSA_0. Open circles are pressures measured at these stretches during full relaxation. *In this case stretch to 0·64 × CSA_0 resulted in irreversible damage of the vessel.

CSA-controlled conditions

Under CSA-controlled conditions, in the presence of 0·3 μM NA, 0·05 u l^{-1} LVP, or 32 mM K^+, the vessels exhibited stretch activation responses. Figure 4 shows an example of this. The CSA of this vessel was set to 0·25 × CSA_0 during application of 0·3 μM NA. The steady-state pressure needed to maintain this level of distension was only slightly higher than the pressure which was needed to distend the vessel in the passive state, as indicated by the dashed line. Also, the pressure needed to stretch the vessel to 0·36 × CSA_0 was not much greater than that in the passive state. However, when stretched to 0·49 × CSA_0, the vessel rapidly developed activity, and high pressure levels were needed to prevent vasoconstriction. In this particular case, but not in the other eight vessels stimulated with NA, transient oscillations in pressure developed that presumably reflect the NA-induced vasomotion seen in isobaric vessels (VanBavel, Giezeman, Mooij & Spaan, 1991) and the oscillations in tension seen in wire-mounted mesenteric vessels on NA stimulation (Mulvany, Nilsson & Flatman, 1982).

Figure 5 shows the steady-state responses to stretch of all vessels. In each case the passive component of the pressure needed to achieve the distensions, as determined from linear interpolation of the passive pressure–CSA relations, is indicated by the open circles. As can be seen, all vessels showed stretch activation in the presence of the vasoconstrictors. The threshold stretches at which activity developed were 0·61 ± 0·12 × CSA_0 and 0·40 ± 0·17 × CSA_0 for NA and LVP respectively. The corresponding pressures were 42 ± 18 and 30 ± 22 cmH_2O. In all vessels stimulated with NA, and four of five vessels stimulated with LVP, this response had an all-or-none character: once active pressure developed, it rose to super-physiological levels: 226 ± 40 cmH_2O with NA and 217 ± 22 cmH_2O with LVP. In contrast, a clear all-or-none response to stretch was found in only one of four potassium-stimulated vessels.

Noradrenaline and vasopressin concentration–response curves under isometric conditions, unlike isobaric concentration–response curves, were extremely steep. An example of this is illustrated in Fig. 6. At concentrations below 0·2 μM NA, the vessel did not develop activity at all, and the pressure needed to keep the CSA of the vessel at 0·81 × CSA_0 reflects the passive properties of the vessel. On stimulation with 0·2 μM NA, the vessel started to develop

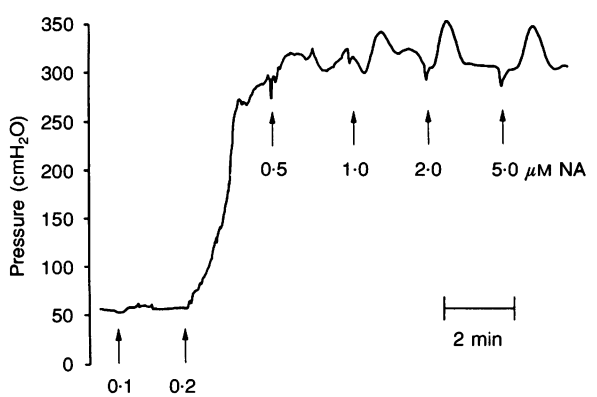

Figure 6. Example of an isometric noradrenaline (NA) concentration–response curve
The vessel was kept at 0·81 × CSA_0. Note that the full response of this vessel occurs with only a doubling of the NA concentration from 0·1 to 0·2 μM. Further increments in [NA] induce only small, transient increases in active pressure.

active pressure. Once started, the increase in pressure became faster and faster, until the pressure was well above physiological levels. Further increments in the NA concentration resulted in only small, transient increments in pressure. Consequently, almost the whole response of the vessel occurred at a single doubling of the NA concentration.

Figure 7 shows all individual isometric NA concentration–response curves. In some cases, we could not perform these measurements at $0{\cdot}81 \times CSA_0$, since spontaneous pressure developed at this stretch. In these cases, concentration–response curves were made at $0{\cdot}64 \times CSA_0$ (NA: $n = 3$; LVP: $n = 2$) or $0{\cdot}49 \times CSA_0$ (NA: $n = 1$; K^+: $n = 1$). Table 1 summarizes pTh_{10} and pD_2 values. It may be noted that although the pTh_{10} values measured under these isometric conditions were not significantly different from those seen under isobaric conditions (Fig. 3) for any of the vasoconstrictors, the pD_2 values for NA and LVP were significantly higher under isometric than under isobaric conditions. This indicates that the overall sensitivity of vessels to NA and LVP, but not to K^+, is greater under isometric than isobaric conditions.

As indicated in Fig. 7, the curves for NA and LVP were extremely steep, with respectively 86 ± 9 and 82 ± 5 % of the full response occurring following only a doubling of the concentration. Indeed, Fig. 7*A* underestimates the steepness of the NA curve: open circles in Fig. 7*A* indicate data points where active pressure started to develop just at the end of the 2 min period before further increment of the NA concentration. Thus, taking these non-stationary points into account, all vessels showed a nearly all-or-none response to an increase of the NA concentration. For LVP, we allowed for a longer period than 2 min in cases where the pressure clearly was not stable. The responses to NA and LVP started at pressures of 58 ± 17 and 72 ± 5 cmH_2O respectively, and caused pressure to rise to 228 ± 40 and 227 ± 23 cmH_2O. In four cases for NA and three cases for LVP, this maximum pressure level was compared to the active pressures induced using 5 μM NA and 125 mM K^+ as stimuli. Pressures developed after this stimulation were only 11 ± 9 and 7 ± 3 % higher than the responses to doubling, respectively, the NA or LVP concentrations.

DISCUSSION

There are four main results of this study. First, under isobaric conditions, the presence of 0·3 μM NA or 0·05 u l^{-1} LVP resulted in graded constrictor responses of mesenteric small arteries to increases in intravascular pressure, i.e. classic myogenic responses (Bayliss, 1902). On the other

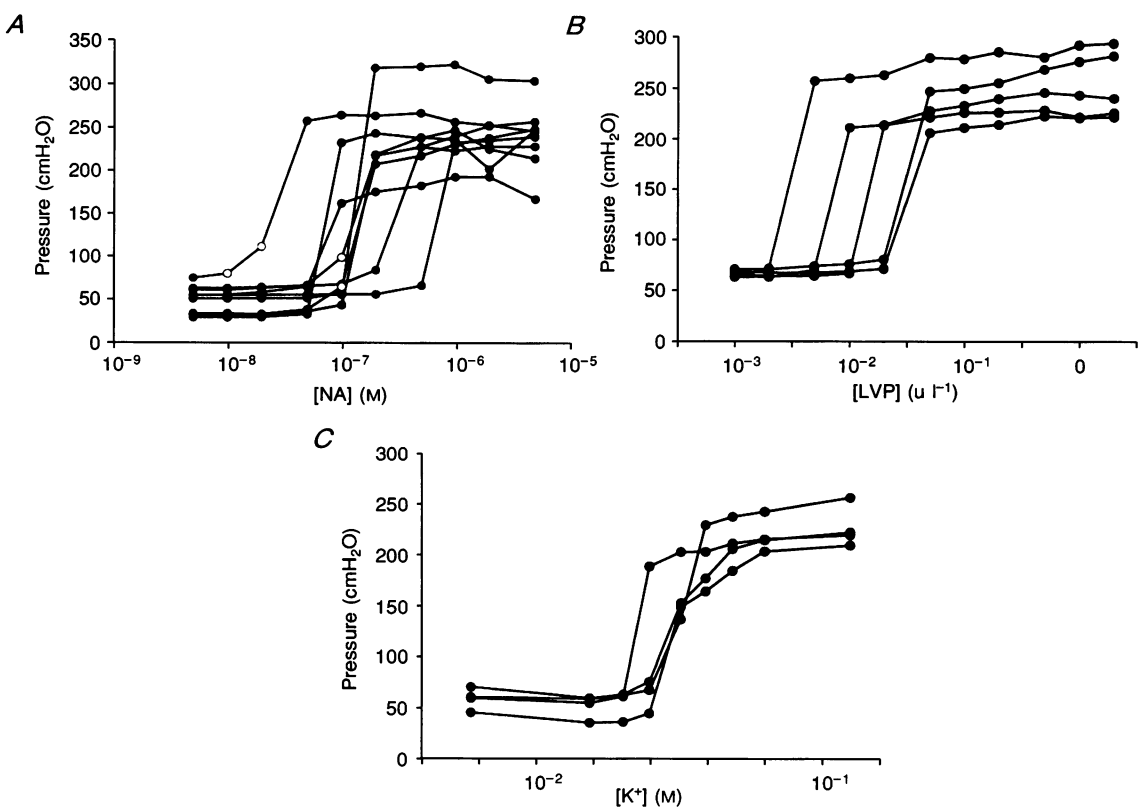

Figure 7. Isometric concentration–response curves
Responses of individual vessels are shown for NA (*A*), LVP (*B*) and K^+ (*C*). Open circles in *A* indicate measurements in which the response to the NA concentration indicated was clearly not stable after the 2 min period that was allowed before increasing the concentration further.

hand, 32 mM K^+-stimulated vessels reacted to pressure with a continuous increase in CSA. Second, under isobaric conditions, the vasoconstrictor effects of NA, LVP and K^+ were graded, the concentration–response relations of individual vessels covering two to three decades for NA and LVP. Third, under isometric conditions, the presence of 0·3 μM NA or 0·05 u l^{-1} LVP resulted in vessels exhibiting all-or-none responses to stretch. K^+ (32 mM) also induced stretch activation, but this was not a clear all-or-none effect. Fourth, under isometric conditions, the NA and LVP concentration–response relations were steep and were an almost all-or-none effect in individual vessels, in strong contrast to the graded effect of these agents on isobarically loaded vessels. There was no difference between isobaric and isometric concentration–response curves for K^+-treated vessels.

The induction of myogenic responsiveness by NA in this study confirms the results of a previous study (VanBavel *et al.* 1991) on rat mesenteric vessels, and Speden & Warren (1986) have made similar observations on cannulated rabbit ear arteries. Meininger & Faber (1991) also showed that NA induced myogenic responsiveness in rat cremaster arterioles *in vivo*, and in another study (Faber & Meininger, 1990) they demonstrated that both α_1- and α_2-agonists induce myogenic responses in that preparation. Lombard *et al.* (1990) have shown that myogenically active renal arteries are more sensitive to NA at higher pressures: 10^{-8} M NA, for instance, maximally constricted the vessels at 140 mmHg, but had no effect at 60 mmHg, thereby increasing the negative slope of the pressure–diameter relation almost 4-fold over the slope seen in the absence of NA. We have also shown, in a preliminary study, that the presence of acetylcholine resulted in myogenic responses in porcine coronary resistance vessels that lack basal tone (Goto, Giezeman, VanBavel & Spaan, 1991). Furthermore, Harder (1988) showed increased serotonin sensitivity upon pressure elevation in cannulated cat cerebral arteries (even though the raised pressure did not result in increased diameter) and an increase in the negative slope of the pressure–diameter relation upon serotonin application. It might thus be that the modulation of agonist-induced tone by pressure is a property that is common to a large number of vasoconstrictors.

The finding that all these vessels exhibit myogenic responses in the presence of agonists indicates that a mechanosensor exists in the vessel wall that modulates the agonist signal transduction pathway. The nature of this mechanosensor is unknown, but it cannot be a pure stretch sensor, for this would be inconsistent with the observed negative slopes of the steady-state pressure–diameter relations. Similar concerns are voiced concerning the myogenic responses seen in vessels having basal tone, suggesting the possibility of a common effect of pressure on both basal and agonist-induced tone.

To explain the negative slope of the pressure–diameter relation, Johnson (1980) suggested that it was wall tension, rather than wall length, which was being sensed. We suggest that a tension sensor could also account for the myogenicity which is induced by agonists, and we term this 'tension-induced sensitization'. In qualitative terms, it is easily shown that under isobaric conditions with such a mechanism, agonist activation will result in a reduction in sensitivity to the agonist. This is because activation induced by an agonist under isobaric conditions will cause a reduction in diameter, and thus (according to the Laplace relation) in the wall tension. Consequently, each increment in agonist concentration causes further desensitization (i.e. negative feedback), and the isobaric concentration–response curve of individual vessels would be expected to be extended over a large range of agonist concentrations. Under isometric conditions such a mechanism would be expected to have completely the opposite effect. In this case, activation of the vessel by NA leads to an increase, rather than a decrease, of total wall tension, and in the course of the tension development, the vessel would be expected to become more, rather than less sensitive to the agonist. Thus the mechanism would provide positive feedback under isometric conditions, resulting in steep concentration–response curves. The presence of tension-induced sensitization would also be expected to result in steep stretch activation curves.

These qualitative assessments of the consequences of the presence of tension-induced sensitization in the vascular wall correspond closely to the findings we have presented here regarding NA- and LVP-activated rat mesenteric small arteries: (a) negative pressure–diameter relations were observed over a range of pressures from 60 to 140 cmH_2O for NA and from 100 to 140 cmH_2O for LVP (Fig. 2); (b) isometric concentration–response curves for these agonists were remarkably steep, with a doubling of the concentration accounting for 86 % of the full response for NA and 82 % of the full response for LVP (Fig. 7), while about a 100-fold increment in concentration was needed to achieve the same response at constant pressure (Fig. 3); and (c) all-or-none responses to stretch were observed (Fig. 5) in the presence of NA and LVP. Our findings also corroborate reports on the responses of rat mesenteric small arteries mounted on wire myographs: under isometric conditions, the slope of the NA concentration–response curve has been shown to be steep (Mulvany *et al.* 1982), and stretch activation of basal tone has been demonstrated on wire-mounted resistance vessels (Nakayama, 1982; Hwa & Bevan, 1986). Most wire-mounted vessels, however, do not show all-or-none behaviour. A possible difference between the current study and other reports may be that, as shown by the movement of nuclei upon stimulation (Mulvany & Warshaw, 1979; Daly, Gordon & McGrath, 1992), at the subcellular level wire-mounted vessels are not truly isometric, even though the total wall circumference can be maintained close to isometric using modern strain gauges. An alternative explanation might be that a circular circumference is somehow important for the tension effect.

The shape of the isobaric concentration–response curves were approximately sigmoid, thereby apparently following normal mass action receptor theory. However, mass action cannot be the only determinant of the shape of the curve, since at least three well-established mechanical factors are also involved in defining the relation between agonist concentration and radius: (1) the radius–tension relation of the passive elements, (2) the radius–tension relation of the contractile apparatus, and (3) the relation between pressure, radius and tension, as approximated by the Laplace relationship.

These mechanical factors need to be taken into account, together with mass action, in order to make predictions about the shape of the concentration–response relation. The relations are too complex to allow qualitative reasoning, and we have therefore constructed a quantitative model of the vascular wall which includes these mechanical factors (Fig. 8). The equations of the elements are shown in the Appendix. This quantitative analysis showed that the responses which we have observed in the presence of agonists (myogenic responsiveness, a shallow isobaric concentration–response relation, an all-or-none stretch response, an all-or-none isometric concentration–response relation) could not be reproduced by the model, unless it included a feedback mechanism which allowed the level of tone to increase with increasing tension (Fig. 9, right panels). The model therefore included a tension-induced sensitization element (Fig. 8, element *c*), whereby the relation between tone and agonist concentration was shifted to the left as wall tension increased. With inclusion

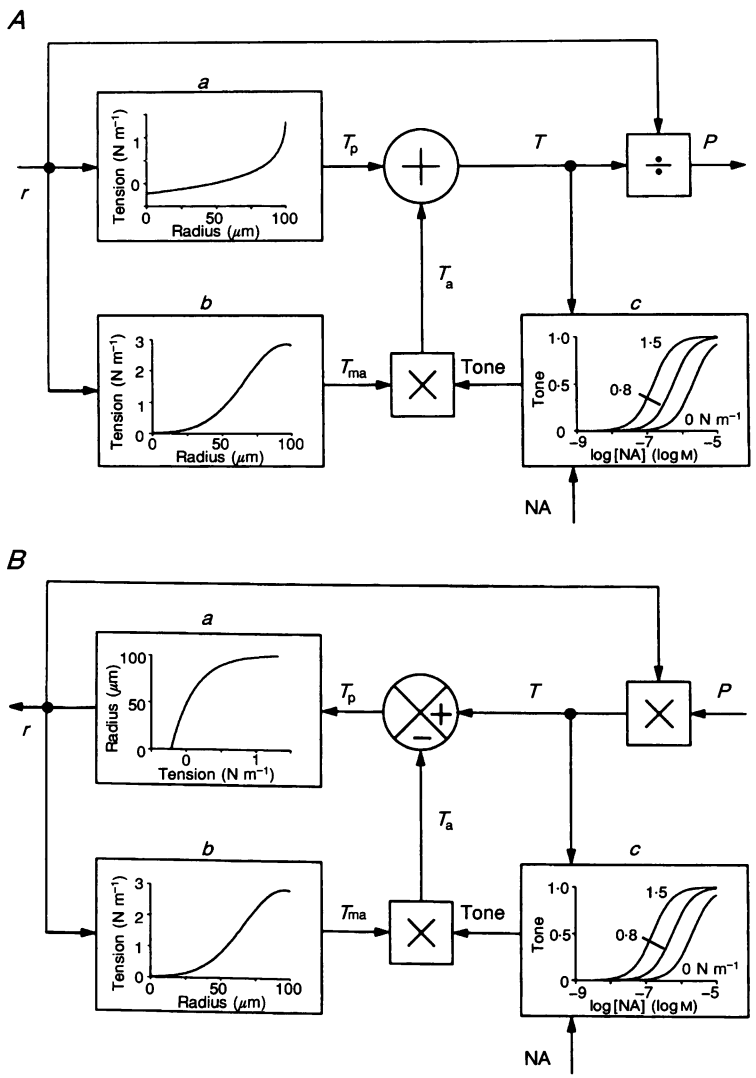

Figure 8. Model of tension-induced sensitization
This schematic representation includes the passive properties of the vascular wall (*a*), the maximum ability for active tension development as a function of the radius (*b*) and tension-induced sensitization to noradrenaline (*c*). The various blocks show steady-state input–output relations. The model is shown for isometric (*A*) and isobaric (*B*) loading conditions. *r*, radius; *P*, transmural pressure; T_p, passive tension; T_{ma}, maximal active tension; T_a, actual active tension; *T*, total tension. See Appendix for further details.

of this element all major experimental findings in this study were predicted (Fig. 9, left panels).

Specifically, the model makes the following predictions. Myogenic responsiveness in the presence of NA is predicted if tension-induced sensitization is present (Fig. 9*A*); without tension-induced sensitization (Fig. 9*B*), the vessel constricts at low pressure, but would yield when pressure is raised. The predicted isobaric concentration–response curves are shallower in the presence (Fig. 9*C*) than in the absence of tension-induced sensitization (Fig. 9*D*). The model also predicts increased NA sensitivity at higher pressures, as has been reported for other vessels (Lombard *et al.* 1990; Meininger & Faber, 1991). Finally, the model predicts the induction of all-or-none responses to stretch and NA under isometric conditions (Fig. 9*E* and *G*), responses which are not seen when tension-induced sensitization is absent (Fig. 9*F* and *H*). Furthermore, the predicted isometric concentration–response curve shifts leftward at higher distensions (Fig. 9*G*), as has been found by a number of authors, both in this preparation (Nilsson & Sjoeblom, 1985) and in others (Tallarida *et al.* 1974; Price, Davis & Knauss, 1981, 1983). We must emphasize that alternative models can be constructed that also explain the current findings. Thus the effect of wall tension on sensitivity might be far more complex than a simple shift in EC_{50} values. It is also possible that some other mechanical parameter that is

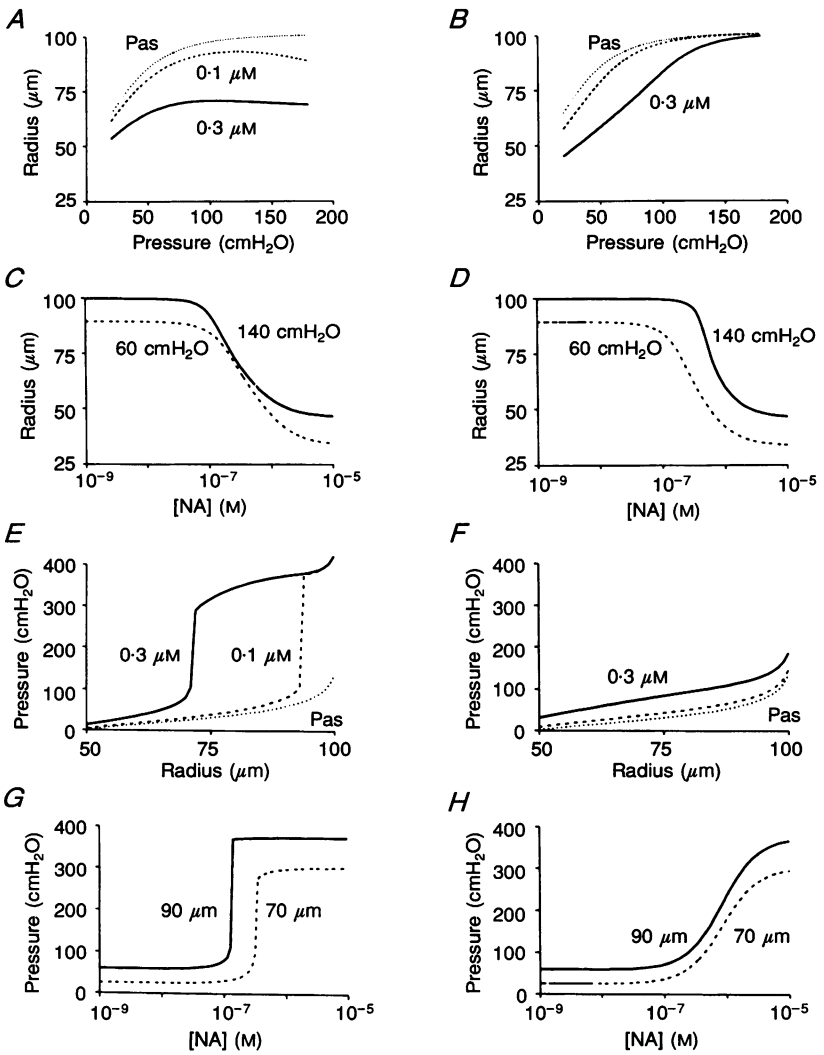

Figure 9. Predictions from the model shown in Fig. 8
The left-hand panels are the predictions when tension-induced sensitization is present. Right-hand panels show the predicted behaviour in the absence of this mechanism, with the NA sensitivity fixed to the level corresponding to a wall tension of 0·5 N m^{-1}. *A* and *B*, pressure–radius relationship for a passive vessel and at two concentrations of NA. *C* and *D*, isobaric concentration–response curves at two pressures. *E* and *F*, stretch activation curves for two NA concentrations and at maximal dilatation. *G* and *H*, isometric NA concentration–response curves at two radii.

positively related to both radius and pressure could cause the sensitization. However, such alternative models would have the same concept in common, namely that vascular reactivity depends on mechanical forces and that this dependence leads to the existence of positive and negative mechanical feedback pathways under isometric and isobaric conditions respectively.

The myogenic responses and stretch activation seen on agonist activation were also seen when potassium was applied as a vasoconstrictor, suggesting that K^+-induced tone is also under the influence of wall tension. Yet isometric all-or-none responses were not observed, and isometric and isobaric concentration–response curves were not significantly different. Thus an unstable positive feedback loop such as is shown in Fig. 8*A* seems not to exist with K^+-induced vasoconstriction. In general, the induction of instability by positive feedback depends on the open-loop gain, defined as the product of the slopes involved. Instability will only occur if the gain exceeds unity. In our model, the gain involves both the maximal active tension at any radius and the magnitude of the effect of tension on tone. Thus, for K^+, a small effect of tension on tone, as evidenced by the weak myogenic responses, may cause the gain of the positive feedback under isometric conditions to be below unity. A diverging sensitivity to K^+ under isobaric and isometric conditions might still exist, but the difference would be small and hard to detect experimentally. Alternatively, the myogenic responses induced by potassium may have a fundamentally different base from those induced by NA and LVP. In contrast to our results with NA and LVP therefore, we have no evidence that K^+-induced myogenic responses are caused by a tension rather than a stretch receptor. Further research is clearly required at this point. A reduced gain of the positive feedback loop might also explain why clear all-or-none effects do not generally appear on wire-mounted vessels, even though the steepness of agonist concentration–response curves is still greater for a wire-mounted vessel than for a cannulated vessel (Buus, VanBavel & Mulvany, 1994).

We did not aim to establish the mechanism for tension-induced sensitization in the current study, but an attractive possibility is that the mechanism involves tension-dependent depolarization of the vascular smooth muscle cells. This depolarization could be caused by the opening of stretch-dependent non-specific cation channels, which are known to exist in vascular smooth muscle cells (Davis, Donovitz & Hood, 1992), if the channels were embedded in such a way that they sense tension rather than vessel stretch. Support for this possibility comes from the observation of a depolarizing effect of distending pressure in a number of vessel types, even though myogenicity prevented distension of the vessels (Harder, 1984; Harder, Gilbert & Lombard, 1987; Smeda & Daniel, 1988). Furthermore, NA sensitivity increases upon depolarization in rat mesenteric small arteries (Mulvany, Nilsson & Flatman, 1982) and other preparations (Nilsson & Sjoeblom, 1985; Nelson, Standen, Brayden & Worley, 1988). In this respect it is perhaps relevant that the steep NA concentration–response relations of isometric, wire-mounted mesenteric small arteries under normal conditions become less steep during potassium depolarization (Mulvany *et al.* 1982); this would interrupt a positive feedback loop which included tension-induced depolarization. The potassium data in the current study also are in agreement with a role for membrane potential in sensitization: the direct, large depolarizing effect of raising K^+ would limit additional changes in membrane potential due to alterations in wall tension. Thus the effect of tension on K^+-induced tone might be less than the effect on NA- and LVP-induced tone because K^+ and wall tension share a critical part of their signal transduction pathways, namely membrane potential. Further examination of the mechanism using combined measurements of membrane potential and intracellular calcium under various mechanical conditions would help to elucidate the role of electromechanical coupling of the mechanosensor. Boonen (1992) concluded from such studies on rat mesenteric vessels that the mechanosensor involved in length-dependent sensitivity to NA contains both an electromechanical element and an element for sensitization of the contractile machinery to calcium, possibly via protein kinase C. A role for this kinase has also been suggested in the myogenic response of rat cremaster muscle arterioles (Hill, Falcone & Meininger, 1990).

Current literature suggests that the myogenic sensor is located in the smooth muscle cells and not in the endothelial cells (see Meininger & Davis, 1992). We did not test whether the endothelium mediates or modulates the effects of wall tension in the current study, and this question needs to be addressed in future studies.

In conclusion, we suggest that a tension sensor exists in the wall of rat mesenteric arteries, and that stimulation of this sensor increases NA and LVP sensitivity. In isometric vessels, where pharmacological stimulation results in an increase in tension, such a sensor would provide a positive feedback loop, causing all-or-none responses to the agonists and to stretch. By contrast, in isobaric vessels, where NA and LVP stimulation result in a decrease in tension, the sensor would provide a negative feedback loop, causing a negative pressure–diameter relation and shallow concentration–response curves.

APPENDIX

Equations used in model of vascular wall

The model (see Fig. 8) includes six elements.

(1) A passive radius–tension curve, reflecting the properties of the elastic elements in the wall. This curve has been fitted to the passive data in Fig. 2, assuming the Laplace relation to hold, and scaled to a vessel radius of

100 μm at 140 cmH_2O. The actual relation used for the model was:

$$T_p = -k \ln[(r_m - r)/(r_m - r_s)], \quad (1)$$

with T_p the passive tension and r the radius. The maximal radius, r_m, and the slack radius, r_s, were taken as 101 and 48 μm respectively. The parameter k was taken as 0·33 N m^{-1}.

(2) An active radius–tension curve at maximal activation. This curve was not determined in the current study. We therefore fitted a smooth curve to data from Mulvany & Warshaw (1979) recorded on the same vessel type, but in an isometric wire myograph:

$$T_{ma} = T_1 \exp[-b(r - r_1)^2], \quad (2)$$

with r_1 the optimal radius for active tension development, T_{ma} the maximal possible active tension at a given radius, and T_1 the value for T_{ma} at r_1. Parameters r_1 and T_1 were set to 96 μm and 2·84 N m^{-1}. Parameter b was set to 6×10^{-4} μm^{-2}. This curve provides a good fit to the data of Mulvany & Warshaw for the range of radii relevant to the current simulation, but underestimates the capability for active tension development at distensions above 110 μm.

(3) The concept of tone, defined as the ratio of actual tension, T_a, and maximal active tension at a given radius, T_{ma}. A tone level of 0 means full relaxation; a tone level of 1 means maximal activation. Thus tone is expressed by T_a/T_{ma}.

(4) NA concentration–tone relations. Tension-induced sensitization is introduced here as a parallel leftward shift of the concentration–tone relation with increasing total wall tension:

$$\text{Tone} = [\text{NA}]^h / (EC_{50}{}^h + [\text{NA}]^h), \quad (3)$$

and

$$EC_{50} = EC_{50,u} \times 10^{aT}, \quad (4)$$

with [NA] the noradrenaline concentration, h the Hill coefficient, $EC_{50,u}$ the EC_{50} of mechanically unloaded vessels, and T the total wall tension. Parameter a represents the shift in sensitivity with wall tension. Parameters h, $EC_{50,u}$, and a were taken as 1·5, 2 μM and $-0·75$ m N^{-1}, respectively. These values were chosen to match the mean Th_{10} and EC_{50} values observed at a constant pressure of 60 cmH_2O (Fig. 3), and the mean Th_{10} and EC_{50} values observed at a constant distension of $0·9r_0$ (or $0·81 \times CSA_0$, Fig. 7).

(5) The parallel arrangement of elastic and contractile elements, to give $T_p + T_a$ = total wall tension, T.

(6) The Laplace relation: T/r = transmural pressure, p. Under isometric conditions, the independent variable is r, the output variable p showing the current transmural pressure (Fig. 8*A*). Under isobaric conditions, the independent variable is p, the output variable r being the resulting radius (Fig. 8*B*).

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