# Intrinsic Relaxation Factors and Length-Dependent Sensitivity in the Rat Aorta (43476)

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*Abstract.* **Possible mechanisms for length-dependent sensitivity may involve intrinsic relaxation factors of the arterial wall. The role of the endothelium, β-receptor activity, and atrial natriuretic factor were tested. $ED_{50}$ and $ED_{10}$ (concentrations for 50% and 10% maximum response, respectively) were significantly lower at the length of maximum force ($L_{max}$) than at 80% $L_{max}$ for phenylephrine stimulated WKY rat aorta rings. The same result was found for norepinephrine (NE)-stimulated rings before and after endothelium removal, and for NE stimulation in the presence of propranolol. The $ED_{10}$ for NE, but not its $ED_{50}$, was significantly decreased by endothelium removal at both lengths. The addition of propranolol decreased the $ED_{50}$ of NE at 80% $L_{max}$, but not at $L_{max}$. Relaxation sensitivity to atrial natriuretic factor was the same at $L_{max}$ and at 80% $L_{max}$ in phenylephrine-contracted WKY rings. For the rat aorta, it may be concluded that: (i) the mechanism for length-dependent sensitivity does not depend upon the endothelium or β-receptor activity, however, (ii) basal levels of endothelium-derived relaxing factor and β-receptor activity appear to modulate length-dependent sensitivity at low levels of activation, and (iii) sensitivity to atrial natriuretic factor relaxation does not depend upon length.** [P.S.E.B.M. 1992, Vol 201]

The dependence of smooth muscle sensitivity on length and preload has been found in many blood vessels using various chemical agonists (1–5). Length-dependent sensitivity is a factor in the comparison of vessels from different experimental groups (6) and may play a role in the control of blood flow (7, 8). As shown by Herlihy *et al.* (2) a possible mechanism for length-dependent sensitivity is a stress-induced increase in the production of eicosanoids. Changes in cell orientation (9) and alteration in the concentration of external calcium (1, 4) do not explain changes in sensitivity with length. However, a decrease in activator calcium may be the reason for decreased sensitivity at short muscle lengths (10). Other possible mechanisms may involve intrinsic relaxation factors in the arterial wall. Stretching of the artery may inhibit the basal release of endothelial-derived relaxing factor (EDRF) from the endothelium with the subsequent facilitation of contractile response at larger arterial diameters. Alternatively, the down-regulation of β-receptor function may occur with increasing length, thereby reducing the degree of receptor-mediated relaxation and increasing the contractile response.

The purpose of this study was to determine the effect of endothelium removal (i.e., removal of basal levels of EDRF) and β-receptor activation on length-dependent sensitivity in the rat aorta. In order to determine the effect of length on dose-response characteristics of a naturally occurring relaxing factor, experiments were conducted with atrial natriuretic factor.

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## Methods

**Vessel Preparation.** The rat aorta was used in all experiments. WKY rats (Taconic Farms) were sacrificed by cervical dislocation. The thoracic aorta was removed and placed in a dissection bath containing a physiological saline solution (PSS) at 37°C. The PSS was gassed with a mixture of 95% $O_2$ and 5% $CO_2$. The adventitia was carefully dissected away and a 2-mm ring was cut from the upper third of the aorta segment. The ring was mounted vertically in a 6-ml experimental bath and held by two stainless steel wire trapezes. The rings were equilibrated for 1 hr at a 1-g preload. A continuous flow of PSS at 37°C was maintained, except during the time of stimulation (6). The pH was 7.4, $PO_2$ was 481 mm Hg, and $PCo_2$ was 31 mm Hg. The PSS was composed of (in m*M*): NaCl (136), KCl (5.6), $NaH_2PO_4$ (1.2), $NaHCO_3$ (20), $CaCl_2$ (1.5), $MgSO_4$ (1.2), EDTA (0.027), and glucose (11.0). All chemicals

were obtained from Mallinckrodt, Inc., except for EDTA (Sigma, Inc.).

**Experimental Apparatus.** The distance between the mounting wires in the ring's lumen was adjusted by a micrometer (Mitutoyo) accurate to 5.0 $\mu$m. Tissue force was measured by a Statham UC2 transducer and recorded on a Gould 2400 strip chart recorder. The distance between the mounting wires, media thickness, and width of the ring were measured by video caliper (Vista Electronics 305 r), a CCTV camera (Cohu, Inc.), and a standard microcamera lens (Nikkon, Inc.). The video image was magnified 33 times for measurement of width and wire separation distance and 72 times for thickness measurements. Muscle length for each ring was defined as the internal ring circumference and calculated as the sum of twice the distance from the lower edge of the upper wire to the upper edge of the lower wire, the wire circumference, and two wire diameters.

**Determination of the Length for Maximum Response.** The rings were stretched in 0.1-mm increments from the length at the 1-g preload. Contractile responses were obtained at each length. When the peak force in each response was the same in two consecutive responses, or the response decreased, the micrometer was set at the length of maximum force ($L_{max}$) for subsequent responses. Contractile responses during the determination of $L_{max}$ were elicited by $5 \times 10^{-9}$ $M$ norepinephrine (NE) in the rings used for NE dose-response experiments, and by $5 \times 10^{-8}$ $M$ phenylephrine (PHE) in the rings used for PHE dose-response experiments and the atrial natriuretic factor (ANF) experiments.

In a separate group of WKY rats, $L_{max}$ was determined with phenylephrine and with norepinephrine in the presence of a $5 \times 10^{-5}$ $M$ concentration of propranolol. Individual responses to both agonists were obtained at each length. The order of agonist stimulation was alternated with each change in length.

**Dose-Response Procedures.** After equilibrating at $L_{max}$ for 1 hr, individual contractile responses were obtained in norepinephrine and phenylephrine dose-response experiments. The arterial ring was allowed to contract for 10 min and then relax in agonist-free PSS for 10 min before the next concentration was introduced. The initial concentration for NE and PHE was $5 \times 10^{-10}$ $M$. Subsequent responses were obtained by increasing agonist concentration in increments of 10. After a maximum response was obtained (usually between $10^{-6}$ and $10^{-5}$ $M$), the agonist was washed from the bath and replaced with a normal agonist-free PSS.

Experiments on ANF-induced relaxation responses were done with rat atrial natriuretic factor (Sigma). The aortic ring was stimulated with a $10^{-5}$ $M$ PHE and the contractile response attained its peak force. Cumulative additions of ANF to the bath were made from $10^{-10}$ to $10^{-7}$ $M$. After relaxation to the last addition of ANF was complete, the ring was washed with agonist-free PSS.

The dose-response procedures for NE and PHE stimulation and ANF relaxation were performed at $L_{max}$ and 80% $L_{max}$ with each aortic ring. The procedures were performed first at $L_{max}$ and then at 80% $L_{max}$ in half of the experiments. This order was reversed in the remaining half. The norepinephrine dose-response curves were also obtained in the presence of $5 \times 10^{-5}$ $M$ concentration of propranolol from a separate group of WKY rats.

Norepinephrine dose-response curves were obtained with aortic rings from 12 WKY rats with the endothelium intact and from a separate group of 12 WKY rats after endothelium removal. Phenylephrine dose-response curves were obtained from 12 WKY rats. The relaxation response to ANF was obtained from six WKY rats.

**Removal of the Endothelium from Vessel Rings.** The endothelium was removed prior to excising the aorta segment from the rat. A wooden stick was inserted into the lumen and then withdrawn while gently scraping the intima (11). The procedure was repeated four times, rotating the stick a quarter turn each time.

Two procedures were used to determine the successful removal of the endothelium. First, the vessels were precontracted with $5 \times 10^{-9}$ $M$ NE at the end of the procedure to determine $L_{max}$ and then the ring was exposed to $5 \times 10^{-6}$ $M$ acetylcholine for 5 min. Length change dose-response procedures were performed only on vessels that did not relax upon acetylcholine administration. The second method of showing removal of the endothelium was by histological examination of the luminal surface with a scanning electron microscope or with a light microscope. The method of silver staining endothelial cell borders was used for light microscopy (11).

**Data Analysis.** Active force is one half of the difference between the peak recorded force in the stimulated ring and the recorded force in the resting ring. Relative active force at a specific concentration was calculated as the active force divided by the maximum active force in the dose-response experiment. Active wall stress was calculated as active force divided by the reference cross-sectional area at $L_{max}$ or total force minus resting force at each length divided by the product of ring width and thickness at $L_{max}$. This stress is defined as lagrangian stress. The concentration of norepinephrine or phenylephrine necessary to elicit 50% ($ED_{50}$) and 10% ($ED_{10}$) of the maximal force of contraction was obtained by a best-fit curve to the norepinephrine and phenylephrine dose-response data. A linear regression to the logarithmic form of Hill's equation was used:

$$R = C^n/(K + C^n) \quad [1]$$

in which $R$ is relative force, $C$ is agonist concentration,

and $K$ and $n$ are constants. $ED_{50}$ and $ED_{10}$ were calculated from Eq. 1 using the best-fit values for $K$ and $n$. A significant difference between the mean $ED_{50}$ or $ED_{10}$ at each length at the 5% probability level was done with the two-tailed Student's $t$ test. The decrease in active force, or relaxation, induced by ANF was converted to a percentage of the maximal relaxation (or relative relaxation) at each length. A significant difference in the percentage of relaxation at $L_{max}$ and at 80% $L_{max}$ was tested for each concentration of ANF.

## Results

**Length-Dependent Sensitivity after Endothelium Removal.** Morphological measurements and force measurements of WKY aortic rings are given in Table I. In norepinephrine experiments, no differences were found in absolute length, media thickness, ring width, active force, or active stress at $L_{max}$ or at 80% $L_{max}$ when endothelium-removed rings were compared with control rings with intact endothelium. Although resting stress was greater in endothelium-removed rings than in control rings at $L_{max}$, it was the same at 80% $L_{max}$. Normalized dose-response curves are shown in Figure 1. The curve from denuded aortas at $L_{max}$ and at 80% $L_{max}$ was shifted to the left of their respective curves from intact aortas at low doses. At high doses, the curves moved closer together. The differences in $ED_{50}$ and in $ED_{10}$ due to length were significant for the endothelial intact vessels and for the endothelium-removed vessels (Table II). When denuded vessels were compared with intact vessels, the $ED_{50}$ were the same at $L_{max}$ and at 80% $L_{max}$, but the $ED_{10}$ were significantly lower for the denuded vessels at both lengths (Table II).

The effect of experimental order on obtaining dose-response curves at $L_{max}$ and at 80% $L_{max}$ was investigated. The difference in $ED_{50}$ between $L_{max}$ and 80% $L_{max}$ was significant, regardless of the order (i.e., whether the curve at $L_{max}$ is obtained first or second). No significant difference in $ED_{50}$ between denuded and intact vessels was found for either order of generating dose-response curves.

**Length-Dependent Sensitivity in Phenylephrine-Stimulated Vessels.** Dose-response curves from phenylephrine-stimulated WKY aortic rings are shown in Figure 2. The PHE curve at $L_{max}$ was positioned to the left of the curve at 80% $L_{max}$. The relative response at $L_{max}$ was significantly greater than at 80% $L_{max}$, and the $ED_{50}$ and $ED_{10}$ values were lower at $L_{max}$ (Table II). Maximum active stress with PHE was less at 80% $L_{max}$ than at $L_{max}$.The effect of experimental order on obtaining phenylephrine dose-response curves at $L_{max}$ and at 80% $L_{max}$ was similar to the effect of order in norepinephrine experiments.

When compared with the results with norepinephrine, the maximum active stress using phenylephrine and the corresponding resting stress were significantly higher. The resting stress was expected to be higher because the absolute length at $L_{max}$ was significantly longer with PHE than with NE. However, the sensitivity (i.e., a lower $ED_{50}$ and $ED_{10}$) was greater for NE than for PHE at their respective $L_{max}$ lengths. As shown in Figure 2, the phenylephrine dose-response curves were

**Table I.** Morphological and Mechanical Measurements from Aortic Rings

| | NE ($5 \times 10^{-9}$ M) | | PHE ($5 \times 10^{-8}$ M) |
|---|---|---|---|
| | Removed endothelium | Intact endothelium | Intact endothelium |
| Lengths (mm) | | | |
| at 1.0 $L_{max}$ | 4.86 ± 0.05[a] | 4.83 ± 0.08[a] | 5.46 ± 0.08[a,b] |
| at 0.8 $L_{max}$ | 3.86 ± 0.04 | 3.90 ± 0.06 | 4.37 ± 0.06[b] |
| Media thickness (mm) | | | |
| at 1.0 $L_{max}$ | 0.09 ± 0.001[a] | 0.10 ± 0.005[a] | 0.09 ± 0.002[a] |
| at 0.8 $L_{max}$ | 0.11 ± 0.001 | 0.12 ± 0.005 | 0.10 ± 0.007[b] |
| Ring width (mm) | | | |
| at 1.0 $L_{max}$ | 1.97 ± 0.05 | 2.15 ± 0.09 | 2.01 ± 0.07 |
| at 0.8 $L_{max}$ | 2.10 ± 0.04 | 2.28 ± 0.10 | 2.14 ± 0.14 |
| Active force (g) | | | |
| at 1.0 $L_{max}$ | 2.60 ± 0.17[a] | 3.02 ± 0.15[a] | 3.06 ± 0.14[a] |
| at 0.8 $L_{max}$ | 1.77 ± 0.11 | 2.01 ± 0.15 | 2.62 ± 0.13[b] |
| Active stress ($10^5$ dynes/cm$^2$) | | | |
| at 1.0 $L_{max}$ | 7.03 ± 0.14[a] | 6.95 ± 0.51[a] | 8.56 ± 0.30[a,b] |
| at 0.8 $L_{max}$ | 4.88 ± 0.26 | 4.60 ± 0.35 | 7.32 ± 0.25[b] |
| Resting stress ($10^5$ dynes/cm$^2$) | | | |
| at 1.0 $L_{max}$ | 7.07 ± 0.36[a,b] | 4.46 ± 0.37[a] | 9.39 ± 0.46[a,b] |
| at 0.8 $L_{max}$ | 0.42 ± 0.04 | 0.41 ± 0.09 | 1.58 ± 0.14[b] |

[a] A statistical difference in the same measurement at 1.0 $L_{max}$ and 0.8 $L_{max}$ is $P < 0.05$.
[b] Statistical difference for either the NE removed endothelium or the PHE intact endothelium group compared with the NE intact endothelium group is $P < 0.05$ ($n = 12$).

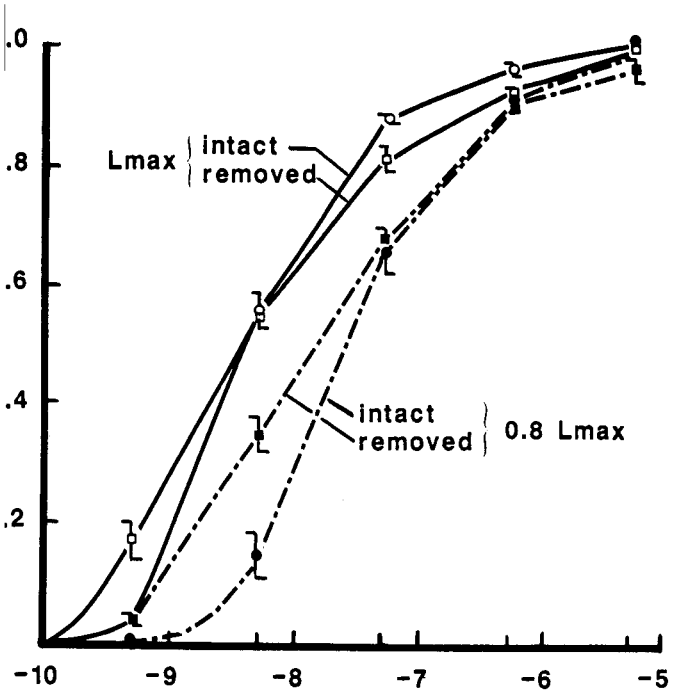


**Figure 1.** Dose-response curves of WKY aortas with and without endothelium. The relative response at $L_{max}$ is significantly greater ($P < 0.05$) than at 80% $L_{max}$ (with or without the endothelium intact) with $5 \times 10^{-10}$, $5 \times 10^{-9}$, $5 \times 10^{-8}$, and $5 \times 10^{-7}$ $M$ norepinephrine. Open symbols are results at $L_{max}$. Closed symbols are results at 0.8 $L_{max}$. Squares are results with the endothelium intact and circles are results after removal of endothelium. Each symbol is a mean value ± SE.

positioned significantly to the right of the norepinephrine dose-response curves at $L_{max}$ and at 80% $L_{max}$.

**Length-Dependent Sensitivity with Norepinephrine and β-Receptor Blockade.** When stimulated in the presence of propranolol, the norepinephrine $ED_{50}$ is significantly less at $L_{max}$ ($7.3 \pm 1.2 \times 10^{-9}$ $M$) than at 80% $L_{max}$ ($17.9 \pm 3.9 \times 10^{-9}$ $M$). These $ED_{50}$ values were less than the $ED_{50}$ with NE alone at 80% $L_{max}$, but not at $L_{max}$. $L_{max}$ obtained by norepinephrine stimulation in the presence of propranolol (5.76 ± 0.19 mm) was the same as $L_{max}$ with phenylephrine, but significant longer than $L_{max}$ obtained with NE only (Table I).

The maximum active stress with NE and propranolol ($8.1 \pm 0.4$ $10^5$ dynes/cm$^2$) was the same as that obtained with PHE, but was greater than that obtained with NE after endothelium removal (Table I).

**Effect of Length on Relaxation Sensitivity to Atrial Natriuretic Factor.** The relative relaxation induced by various concentrations of ANF at $L_{max}$ and at 80% $L_{max}$ is shown in Figure 3 for rat aortic rings contracted with $10^{-5}$ $M$ phenylephrine (endothelium intact). The relaxation dose-response curves were the same at $L_{max}$ and at 80% $L_{max}$. The respective $ED_{50}$ and $ED_{10}$ values were 4.80 ± 1.29 n$M$ and 0.42 ± 0.16 n$M$ at $L_{max}$ and 5.62 ± 1.43 n$M$ and 0.70 ± 0.31 n$M$ at 80% $L_{max}$. The difference in values at $L_{max}$ and at 80% $L_{max}$ was not significant.

## Discussion

The results have shown that removal of the endothelium does not cause a loss of length-dependent sensitivity in the rat aorta. In other studies, Herlihy *et al.* (2) reported that the presence or absence of endothelium exerted no apparent effect on preload-dependent $ED_{50}$ in the rabbit aorta. Hwa and Bevan (12) found that stretch-dependent tone is still present in endothelial-deprived rabbit ear arteries. However, removal of the endothelium does alter norepinephrine length-dependent sensitivity at low levels of activation. This can be observed from the $ED_{10}$ and $ED_{50}$ values in Table II. The average $ED_{50}$ values with and without the endothelium differed only by a factor of 1.36 at both lengths and were not significantly different. In contrast, average $ED_{10}$ values at both $L_{max}$ and 80% $L_{max}$ without endothelium were approximately twice those with endothelium, and this difference was significant. This suggests that the effect of endothelium basal activity on $ED_{10}$ is approximately two times greater than its effect on $ED_{50}$. This result is consistent with the hypothesis that a tonic type of EDRF release (13) by the endothe-

**Table II.** Length-Dependent Sensitivity in Intact (NE and PHE) and Denuded (NE Only) Aortic Rings

| | NE | | PHE |
|---|---|---|---|
| | Removed endothelium | Intact endothelium | Intact endothelium |
| $ED_{50}$ ($10^{-9}$ $M$) | | | |
| at 1.0 $L_{max}$ | 6.73 ± 1.31[a] | 9.13 ± 0.90[a] | 39.20 ± 4.53[a,b] |
| at 0.8 $L_{max}$ | 23.50 ± 3.14 | 31.96 ± 3.29 | 77.50 ± 3.91[b] |
| $ED_{10}$ ($10^{-10}$ $M$) | | | |
| at 1.0 $L_{max}$ | 3.03 ± 0.96[a,b] | 6.35 ± 0.84[a] | 41.54 ± 5.05[a,b] |
| at 0.8 $L_{max}$ | 16.30 ± 2.62[b] | 29.72 ± 3.40 | 92.80 ± 5.19[b] |

[a] Statistical difference between 1.0 $L_{max}$ and 0.8 $L_{max}$ is denoted by $P < 0.05$ ($n = 12$). $ED_{50}$ and $ED_{10}$ calculated from a best-fit curve.
[b] Statistical difference for either the NE removed endothelium or the PHE intact endothelium group compared with the NE intact endothelium group is denoted by $P < 0.05$.

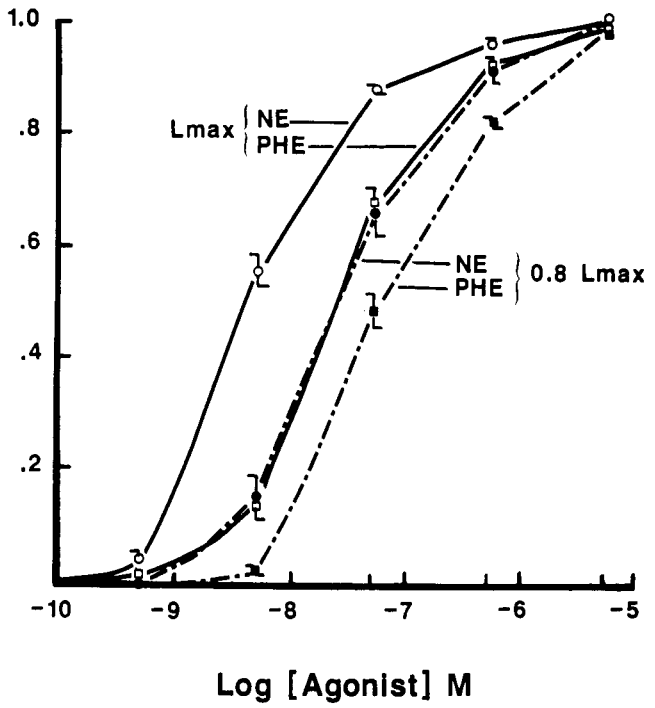


**Figure 2.** Dose-response curves of WKY aortas with phenylephrine and norepinephrine (endothelium intact). The relative response at $L_{max}$ is significantly greater ($P < 0.05$) than at 80% $L_{max}$ for both agonists with $5 \times 10^{-9}$, $5 \times 10^{-8}$, and $5 \times 10^{-7}$ *M*. Open and closed symbols are the same as in Figure 1. Circles are results with NE and squares are results with PHE.

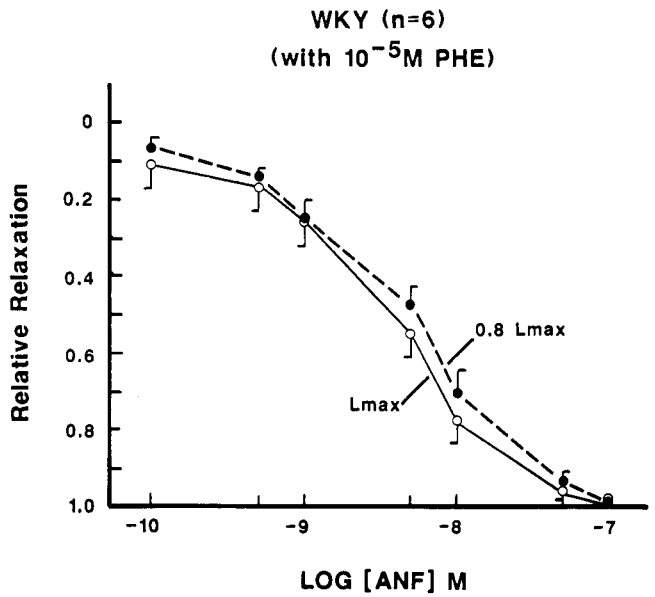


**Figure 3.** The relaxation dose-response curve with ANF at $L_{max}$ and 80% $L_{max}$ in PHE-contracted rings. When $ED_{10}$ or $ED_{50}$ is compared at $L_{max}$ and 80% $L_{max}$, there is no significant difference in their respective values for the WKY aortas. Open and closed symbols are the same as in Figure 1.

lium is independent of arterial circumference and its depressing effect is more prominent at low levels of activation than at high levels. Tesfamariam *et al.* (14) reported that EDRF is spontaneously released and tonically active in rabbit carotid artery and may be an important modulator of adrenergic vasoconstriction. The difference in $ED_{10}$ values with and without endothelium may also be due to NE-induced release of EDRF (15). However, if this were true we would expect a similar magnitude of difference in $ED_{50}$ values before and after removal of the endothelium. In addition, the resting stress at $L_{max}$ was significantly greater after removal of the endothelium (Table I). This indicates that endothelium basal activity normally inhibits basal tone and could alter the $ED_{10}$ of NE.

The elimination of $\beta$-receptor stimulation by use of phenylephrine, a pure $\alpha$-agonist, did not cause a loss of length-dependent sensitivity in the rat aorta. Similar results for preload sensitivity were shown by Herlihy and Berardo with rat aorta (1) and with rabbit aorta (2) and by Raffa and Tallarida (16) with rabbit aorta. In addition, our experiments show that $ED_{50}$, $ED_{10}$, and maximum active stress are greater with phenylephrine than with norepinephrine. The smaller active stress of NE could be due to autoinhibition (i.e., activation of $\beta$-receptors), because the maximum active stress with NE and propranolol is the same as with PHE but significantly greater than NE alone after removal of the endothelium. These results demonstrate that norepinephrine is a more potent agonist than phenylephrine when compared at their respective maximum response lengths.

Previously, we have shown that $L_{max}$ varies with the concentration level of a given agonist (4). In this paper, $L_{max}$ was different for two agonists, norepinephrine and phenylephrine, that activate the $\alpha$-receptor. In a second series of experiments, $L_{max}$ was the same with phenylephrine and with norepinephrine in the presence of propranolol (which blocks the $\beta$-receptor activation). These results suggest that the length of maximum response depends upon the specific receptor(s) that is activated as well as on the agonist concentration.

Although a specific mechanism for length-dependent sensitivity in smooth muscle has not been identified, experimental evidence for several possibilities has been obtained. In large arteries, length-dependent sensitivity exists for both receptor and nonreceptor agonists (1, 17). However, this does not appear to be true for small resistance size arteries that do not exhibit length-dependent sensitivity with potassium stimulation (18). Herlihy *et al.* (2) have presented strong evidence that lipoxygenase metabolites play a role in the change of sensitivity with preload. Arner and Hellstrand (19) have shown that the sensitivity of the contractile system in smooth muscle is length dependent. Length-dependent sensitivity in smooth muscle is not affected by extracellular calcium (1, 4) or by changes in cellular orientation with length (9). In contrast, length-dependent activation in heart muscle is due in part to transsarcolemmal calcium influx (20, 21), sensitivity of the contractile system to calcium (22), and calcium-triggered

release of calcium from the sarcoplasmic reticulum (23). In view of the variety of cellular processes that are length dependent, it is possible that many mechanisms are involved. The summation of many mechanisms and the relative importance of each mechanism may vary with the type of muscle and the specific tissue being investigated. In addition, our results suggest that other extracellular processes (i.e., tonic release of EDRF) can modify the length-sensitivity relationship.

Our results indicate that sensitivity of the rat aorta to an intrinsic relaxation agent, such as atrial natriuretic factor (24), does not normally depend upon muscle length. When muscle length was increased, Foley (25) found an increased relaxation sensitivity in NE-contracted femoral artery strips to adenosine, but a decreased sensitivity in acetylcholine-contracted rabbit coronary artery strips. A preliminary study by Herlihy and Berardo (26) found that relaxation sensitivity of potassium-contracted rat aortic strips to sodium nitrate decreased with increasing preload. Whether the sensitivity to a relaxation agent increases, decreases, or remains the same with increased muscle length may depend in part upon the selection of contractile agonist.

In conclusion, the dependence of norepinephrine sensitivity upon muscle length is not due to the basal release of EDRF or to activation of $\beta$-receptors, and the sensitivity of smooth muscle to an intrinsic relaxation factor such as atrial natriuretic factor does not depend upon length. However, length-dependent sensitivity can be modified by basal levels of EDRF at low levels of activation (i.e., low doses or short muscle lengths) and by $\beta$-receptor activation.

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