Response of the Main Pulmonary Artery of Dogs to Neuronally Released Versus Blood-Borne Norepinephrine

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ABSTRACT

The effects of sympathetic nerve stimulation and of norepinephrine infusions on the dynamic elastic properties of the main pulmonary artery were studied in 20 anesthetized, open-chest dogs. Pulmonary arterial pressure and diameter were linearly related between 5 to 45 mm Hg and at heart rates of 40 to 200 beats/min. Sympathetic nerve stimulation changed the stiffness (pressure-diameter slope) of the pulmonary artery by +35%; infusion of norepinephrine (0.25 µg/kg/min) by only +14%. Diameter intercepts, determined by extrapolation of the linear pressure-diameter line, were unchanged during stimulation, but decreased significantly during the infusion of norepinephrine. Local application of norepinephrine to the wall duplicated the changes produced by sympathetic nerve stimulation.

Histologically, the arrangement of smooth muscle in the outer layers of the media of the artery suggested linkage of smooth muscle with continuous elastic fibers, whereas smooth-muscle cells of the deeper layers of the media appeared to connect with each other, and elastic fibers were discontinuous. The topical application of elastase to the outer wall effected a loss of elastic fibers in the outer layers of the media. After elastase, neither stimulation nor norepinephrine infusions increased stiffness; diameter intercepts decreased with both.

The observations are consistent with the idea that stiffening of this artery during nerve stimulation is produced by contraction of smooth muscle attached to elastic fibers and that the anatomical arrangement for stiffening is located in the outer layer of the media, where the sympathetic nerve endings are located. In contrast, blood-borne norepinephrine stimulates predominantly the inner layers.

ADDITIONAL KEY WORDS

dynamic arterial elasticity

In a previous study, in which we used pulsatile flow to perfuse, in situ, the isolated lobe of the dog lung, we found quantitative and qualitative differences between the response of the pulmonary circulation to injected norepinephrine and to stimulation of the sympathetic nerves to the lobe (1). During sympathetic nerve stimulation, the large pulmonary arteries became stiffer, whereas calculated pulmonary vascular resistance either remained unchanged or increased slightly. In contrast, injections of norepinephrine produced smaller changes in the stiffness of the large pulmonary arteries and an increase in calculated pulmonary vascular resistance. These results were interpreted to

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mean that norepinephrine released during stimulation of the sympathetic nerves had acted mainly in the larger arteries, where the sympathetic nerve endings are concentrated (2), and that blood-borne norepinephrine was acting mainly on the pulmonary arterioles and much less on the large arteries.

The present study was undertaken to determine by direct measurements the effects of blood-borne and neurally released norepinephrine on the dynamic elastic properties of large pulmonary arteries. For this purpose, we used simultaneous measurements of pressures and radial dimensions in the main pulmonary artery. We also related the elastic behavior to the histological appearance of the main pulmonary artery before and after disruption of the elastic network by elastase. Finally, by comparing the changes in the dynamic elastic properties induced by blood-borne norepinephrine with those induced by neurally released norepinephrine, we developed a hypothesis concerning the structural-functional relationships within the wall of the main pulmonary artery.

Methods

A. EXPERIMENTAL PROCEDURE

Twenty dogs, weighing 13.8 to 26.0 kg, were sedated with phencyclidine hydrochloride (Sernylan, Parke, Davis & Co.) and anesthetized with alphaxalone (1.5 mg/kg iv, and 80 mg/kg iv, respectively). The dogs were ventilated by a volume respirator (Harvard Apparatus Co.) and a cuffed endotracheal tube. The expiratory tube from the respirator was submerged under 4 cm water; the airway pressure was monitored by a lateral pressure tap in the endotracheal tube, connected to a Statham P23Db transducer and tidal volume was adjusted to give airway pressures from +4 cm water at deflation to +15 cm water at peak inflation. Systemic arterial blood pressure was continuously monitored with a Statham P23Db strain gauge connected to a polyethylene cannula in either the carotid or femoral artery.

After left thoracotomy, the left upper lobe was removed to promote access to the main pulmonary artery. The pericardium was then opened, and dissection was carefully done and kept to a minimum to preserve the innervation of the pulmonary vasculature. To record pulmonary arterial pressure, either a vinyl catheter (10 cm long, i.d. 0.112 cm) was inserted into the left upper lobar arterial stump and directed toward the main pulmonary artery, or a needle (5 cm long, 20 gauge) was passed through the right ventricular outflow tract and directed into the main pulmonary artery; pressure was determined with a Statham P23Db transducer. The end-hole of the catheter or of the needle was plugged and several side holes were made near the tip. The dynamic response of the catheter and needlemanometer systems was flat to at least 20 cps.

Change in pulmonary arterial outer diameter was measured with a strain-gauge arch caliper (3), which measures displacement. The feet of the caliper were sutured securely with 5-0 silk on atraumatic needles so that the caliper spanned opposite sides of the wall of the main pulmonary artery. The caliper response was ±0.005 cm and its output was linear with strains encountered in these experiments. At the conclusion of each experiment, it was calibrated with a micrometer mounted on a bronze stand. The stability of the caliper output was checked at the conclusion of each experiment; if the baseline before and after the experiment varied by more than 0.01 cm, the data were not used. At the amplification used, the diameter measurement could be read to within ±0.005 cm.

Except for the experiments involving the intravenous administration of phenoxybenzamine and the topical application of norepinephrine, which were expected to produce lasting changes in the behavior of the vessel wall, data are reported only for those experiments in which the values for pressure in the pulmonary artery and the diameter of the main pulmonary artery returned to control values after the test period. All measurements of pulmonary arterial pressures and diameters were made during expiration and represent the average of ten complexes. All data were recorded photographically with a multi-channel oscillograph. The simultaneous pulmonary arterial pressure and diameter were also displayed as x-y plots on an oscilloscope and recorded photographically. A representative tracing is shown in Figure 1.

Sympathetic Stimulation.—The left stellate ganglion was stimulated in 12 dogs by a bipolar electrode and square wave pulses (4 msec, 12 cps, 12 v) produced by a Grass S-4 stimulator. Since our anatomical observations had suggested that stimulation of the left stellate ganglion would not reach all the adrenergic efferents to the main pulmonary artery, hypothalamic stimulation was undertaken. In a series of preliminary experiments on dogs of body weights comparable to those used in the experiments with left stellate stimulation, approximate stereotaxic coordinates were determined systematically for regions in the hypothalamus from which intense tachycardia, systemic
NEURONAL AND BLOOD-BORNE NOREPINEPHRINE

Simultaneous main pulmonary arterial pressure (PpA) and diameter (DpA) measurements (dog 19). Similarity in the form of the pulses is evident (top); the x-y plot of the same quantities reveals the linear relationship (bottom).

hypertension, and large increases of femoral arterial blood flow could be elicited (4). Femoral arterial blood flow was measured with a Biotronex 610 flowmeter and 4-mm cuff transducer. The hypothalamic points proved to be located 21 to 29 mm rostral to the interaural plane and 1 to 3 mm lateral to the midsagittal plane; the vertical coordinate, which was variable, was established for each animal by gradually lowering the electrode into the brain with the stimulator on until maximal circulatory effects could be obtained with the smallest voltage. In eight dogs, complete experiments involving hypothalamic stimulation were successfully done with bipolar electrodes constructed from blunt, 22-gauge hypodermic needles; rectangular pulses (1 to 1.5 msec in duration, 80 to 100 cps, 2 to 5 v for 10 to 30 sec) were delivered by a Grass S-4 stimulator. At the end of the experiments, the brain was fixed by perfusion of the carotid arteries with 10% formaldehyde until blood was cleared, and the brain was placed in 10% formalin for 2 weeks. The position of the electrode tip was then established.

Norepinephrine and Other Drugs.—In 18 dogs, norepinephrine was administered intravenously at the rate of approximately 0.25 µg/kg/min for 1 to 3 minutes; four of the dogs also received 200 µg of norepinephrine as bolus injection intravenously. In eight of the dogs, 1 ml of isotonic saline containing 100 µg of norepinephrine was slowly dripped on the surface of the main pulmonary artery, at the site where the diameter and pressure measurements were being made. As a control intervention for all norepinephrine administrations, isotonic saline was given in equivalent volumes by the same route.

Phenoxybenzamine¹ (7.5 to 10 mg/kg) was administered intravenously in a single dose in eight of the dogs. That this dose was sufficient for complete adrenergic blockade was shown by the consistent disappearance of the systemic arterial and pulmonary arterial pressor response to 200 µg of norepinephrine. Fifteen to 20 minutes after the end of the infusion, atropine (0.5 mg/kg) was given intravenously as a bolus injection to five of the dogs. Propranolol (2 mg/kg) was given intravenously in a single dose to four of the dogs; these four dogs received both atropine and propranolol.

Elastase.—In two dogs, pledgets of cotton soaked in 31 mg/ml of elastase² were applied for one hour to the outside of the main pulmonary artery. Approximately three-fourths of the circumference of the main pulmonary artery was exposed to elastase, since the pericardium was not dissected posteriorly. There was no attempt to prevent the adjacent tissue from coming into contact with the elastase. The dynamic elastic behavior of the main pulmonary artery in response to sympathetic nerve stimulation and to intravenously administered norepinephrine was tested before and one hour after the elastase was applied. In one additional dog, papain³ was substituted for the elastase.

Histology.—At the end of each experiment, a 5 to 6 mm-long segment of the pulmonary artery, across which the caliper had been attached, was exercised and mounted on a glass cylinder at approximately the mean in-vivo diameter and length. The wall thickness was then measured across the axis spanned by the caliper by a Vernier caliper. The seven specimens, including two treated with elastase and one with papain, were fixed while mounted on the glass cylinder in 10% formalin. After fixation for 24 hours, sections were taken, embedded in paraffin, and stained with either the Orcein-Van Geison stain or with hematoxylin and eosin.

8. CALCULATIONS

Pulmonary arterial pressure (PpA) and pulmonary arterial diameter (DpA) were related as follows:

¹ Courtesy of Smith, Kline & French Co.
² Elastase from pancreas, crystallized and suspended, Sigma Chemical Co.
³ Crude extract from papaya, Sigma Chemical Co.
1. The slope of the pressure-diameter plot was expressed as the change in the outer diameter divided by pressure change (\( \Delta D/\Delta P \), in cm/mm Hg). A decrease in this slope represents an increase in stiffness.

2. The pressure-diameter plot was extrapolated to the zero pressure intercept and expressed as the diameter intercept (\( D_R \)) according to the formula:
\[
D_R = D_0 - (\Delta D/\Delta P)P
\]
where
- \( D_0 \) = diameter at end diastole (cm),
- \( P \) = diastolic pressure (mm Hg).

In this paper, \( D_R \) will be used to describe the pressure-diameter relationship at zero pressure as though the pulmonary artery were a perfect elastic structure. Although the pressure-diameter relationship is nonlinear at pressures higher than 45 mm Hg, the relationship was linear over the lower pressure range.

3. Measurements of pressure, diameter, and wall thickness allowed the determination of the dynamic elastic modulus (\( E \)) in dynes/cm², based on Bergel's formula and assumptions (5) as simplified by Patel et al. (6). According to the formula:
\[
E_{dyn} = (1 - \sigma^2) \left( \frac{\Delta P}{\Delta R} \right) (R_0^2/h),
\]
where
- \( \sigma \) = Poisson's ratio taken as 0.5,
- \( h \) = wall thickness (cm),
- \( \Delta R \) = outer radius change (cm),
- \( R_0 \) = outer radius at end diastole (cm),
- \( \Delta P \) = pulse pressure (dynes/cm²).

4. Pulse wave velocity (\( c' \), in cm/sec) was calculated by the Moens and Korteweg formula:
\[
c' = \sqrt{\frac{E\rho}{2R}}
\]
where
- \( \rho \) = density of blood in g/cm³ as 1.06,
- \( R \) = mean radius (cm).

The fluid was assumed to be incompressible, the flow to be inviscid, and the effect of blood velocity was ignored.

A fundamental assumption in our studies is that the cross-sectional configuration of the vessel is circular. There is some evidence based on postmortem corrosion casts that the main pulmonary artery has an elliptical configuration with a ratio of major to minor axis of approximately 1.2 (7). If this is true in vivo, then there will be an error of varying magnitude depending on the axis in which the caliper is placed. Nonetheless, as long as the caliper position remains fixed as in the present experiments, and as long as there is no shift in the axis between the control and test periods, a valid comparison can be made of the effects of successive interventions on wall stiffness during the course of a single experiment.

**Results**

Stimulation of the left stellate ganglion resulted in modest increments in heart rate, which never exceeded 10% of the control rate, and variable increments in mean aortic pressure ranging from 5 to 30% of control levels. During hypothalamic stimulation, heart rate more than doubled and the increments in mean aortic pressure were usually much larger (i.e., more than 30% of control levels) than during stellate stimulation. Blood flow in the femoral artery usually increased during hypothalamic stimulation by a factor of 3 to 5 over control levels. However, even though the two types of stimulation differed in their systemic effects on heart rate and aortic pressure, the pressure-diameter changes induced in the main pulmonary artery were identical. Consequently, in the following sections no distinction will be made between the two types of stimulation, insofar as their effects on pressure-diameter relationships of the main pulmonary artery are considered.

**Dynamic Elastic Properties of the Main Pulmonary Artery.** In all experiments except those involving the infusion of norepinephrine, the plots of pulmonary arterial pressure vs. diameter were linear as illustrated in Figure 1. This linear relationship persisted over the entire range of heart rates (40 to 200 beats/min) and pulmonary artery pressures (5 to 45 mm Hg) that were observed in the present experiments. But even though the pressure-diameter relationship in the pulmonary artery was linear, the slopes and intercepts varied with the interventions. Values for the diameter-pressure (\( \Delta D/\Delta P \)) slopes and for the diameter intercepts are shown for all experiments in Table 1 and are summarized in Table 2.

For the control period, the values for \( \Delta D/\Delta P \) averaged 11.13 \times 10⁻³ cm/mm Hg; the corresponding mean diameter intercept was 1.605 cm. During sympathetic nerve stimulation the \( \Delta D/\Delta P \) slopes decreased to a mean of 7.18 \times 10⁻³ cm/mm Hg. By statistical analysis of paired data, \( \Delta D/\Delta P \) during sympathetic nerve stimulation was significantly less than during the control periods (P < 0.001). However, the diameter intercept during sympathetic nerve stimulation did not differ significantly from that of the control.
TABLE 1

Elastic Properties of Main Pulmonary Artery: Control and Experimental

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Wt (kg)</th>
<th>AD/AP × 10⁻³ (cm/mm Hg)</th>
<th>Diameter intercept (cm)</th>
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<td></td>
<td>C</td>
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<td>NE</td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13.8</td>
<td>7.14</td>
<td>3.40</td>
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<td>3</td>
<td>15.0</td>
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</tr>
<tr>
<td>5</td>
<td>19.0</td>
<td>13.20</td>
<td>6.46</td>
</tr>
<tr>
<td>7</td>
<td>20.1</td>
<td>9.00</td>
<td>6.74</td>
</tr>
<tr>
<td>9</td>
<td>16.6</td>
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<td>24.6</td>
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<td>18.4</td>
<td>7.14</td>
<td>3.80</td>
</tr>
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</tr>
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<td>18.3</td>
<td>7.28</td>
<td>4.00</td>
</tr>
<tr>
<td>18</td>
<td>16.0</td>
<td>8.62</td>
<td>4.00</td>
</tr>
<tr>
<td>19</td>
<td>21.0</td>
<td>10.10</td>
<td>6.36</td>
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<td>14.6</td>
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<td>18.1</td>
<td>15.95</td>
<td>9.15</td>
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<td>23*</td>
<td>26.0</td>
<td>10.40</td>
<td>6.00</td>
</tr>
<tr>
<td>24*</td>
<td>16.2</td>
<td>7.89</td>
<td>6.40</td>
</tr>
<tr>
<td>25*</td>
<td>19.7</td>
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<td>11.88</td>
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<td>27*</td>
<td>19.3</td>
<td>13.63</td>
<td>9.58</td>
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<td>28*</td>
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<td>10.65</td>
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<tr>
<td>29*</td>
<td>18.0</td>
<td>21.15</td>
<td>12.46</td>
</tr>
<tr>
<td>30*</td>
<td>17.0</td>
<td>19.54</td>
<td>13.81</td>
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</table>

C = control; SNS = during sympathetic nervous stimulation; NE = during infusion of norepinephrine; PBZ = after phenoxybenzamine. See page 252 for definition of terms.

*Hypothalamic stimulation was used to elicit sympathetic nerve stimulation.

TABLE 2

Summary of Paired Data

<table>
<thead>
<tr>
<th>C</th>
<th>SNS</th>
<th>NE</th>
<th>PBZ</th>
<th>No. of pairs</th>
<th>SD*</th>
<th>P†</th>
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<tbody>
<tr>
<td>Δ D/Δ P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cm/mm Hg</td>
<td>11.13</td>
<td>7.18</td>
<td></td>
<td>20</td>
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<td>× 10⁻³</td>
<td>11.43</td>
<td>9.45</td>
<td></td>
<td>18</td>
<td>1.29</td>
<td>&lt;.001</td>
</tr>
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<td></td>
<td>7.38</td>
<td>9.45</td>
<td></td>
<td>18</td>
<td>1.67</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Dₚ</td>
<td>1.605</td>
<td>1.610</td>
<td></td>
<td>20</td>
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<td>1.555</td>
<td></td>
<td>18</td>
<td>0.025</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>1.605</td>
<td>1.555</td>
<td></td>
<td>18</td>
<td>0.025</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>1.600</td>
<td>1.605</td>
<td></td>
<td>8</td>
<td>0.020</td>
<td>&gt;.30</td>
</tr>
</tbody>
</table>

Abbreviations same as in Table 1. See page 252 for definition of other terms.

*Standard deviation of the differences.
†For a paired t-test.

state; indeed, it was almost the same (within 0.005 cm) in 15 out of 20 experiments and within 0.015 cm in the remaining 5. A representative experiment shown in Figure 2 illustrates the change in the slope of the pressure-diameter relationship even though the zero pressure intercept on the diameter ordinate was almost the same. The response developed rapidly, as illustrated in Figure 3. The slope decreased progressively in successive beats although the diameter intercept remained the same (Fig. 4). The average decrease in the slope of ΔD/ΔP during sympathetic nerve stimulation was 35%.

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Effect of sympathetic nerve stimulation on the pulmonary arterial pressure ($P_{pa}$) vs. diameter ($D_{pa}$) relationship is shown from a representative experiment (dog 17). During sympathetic nerve stimulation, the diameter of the main pulmonary artery is smaller despite higher pressures (top); (x-y plots) the flatter slope during sympathetic nerve stimulation is associated with approximately the same diameter intercept (bottom). SNS = sympathetic nerve stimulation.

During the infusion of norepinephrine, both the slopes of $\Delta D/\Delta P$ and the diameter intercepts decreased significantly from control. However, decreases in slope were less than during sympathetic stimulation. Figure 5 compares the two responses and Figure 6 shows the changes in slope and in diameter intercept during successive cycles while norepinephrine was being infused. The average decrease in the slope of $\Delta D/\Delta P$ during the norepinephrine infusions was 14% from control. This decrease in the slope of $\Delta D/\Delta P$ with norepinephrine is of the same order of magnitude as described by Patel et al. (8), who reported a decrease of 16% in the slope of $\Delta D/\Delta P$ when norepinephrine was infused in amounts 10 times as great as those in this study. Although not shown in Table 2, bolus injections of 200 $\mu$g norepinephrine resulted in slightly greater slope changes in $\Delta D/\Delta P$ than did the infusions, but the values never approached those found with sympathetic stimulation.

During the norepinephrine infusions, the rate of decrease in diameter after peak systole was less than the rate of decrease in pressure; this discrepancy caused the pressure-diameter loop to open. Figure 5 illustrates an example of diastolic lag in diameter change during norepinephrine infusion. The opening of the pressure-diameter loop indicates an increase in the viscosity of the vascular wall similar to that seen in muscular systemic arteries by Peterson (9). Figure 7 illustrates an instance of bigeminal rhythm during norepinephrine infusion in which the extra beat occurred early, slightly after the inscription of the dicrotic notch of the preceding beat. In this example, the opening of the loop is clearly manifested in an exaggerated form, possibly due to the prolonged diastolic period.

In six dogs, the effects on $AD/AP$ and diameter intercept of 100 $\mu$g of norepinephrine applied topically to the main pulmonary artery were identical with those elicited by sympathetic stimulation. But the pulse pressure remained unchanged (Fig. 8). Moreover, after the topical application of norepinephrine, the pressure-diameter relationship did not return to control values after several hours.

After the intravenous infusion of phenoxybenzamine (7.5 to 10 mg/kg) in eight dogs, the $AD/AP$ slopes increased (Fig. 9) but the diameter intercept was not significantly changed from control.

Table 3 summarizes the mean pressures, dynamic elastic moduli ($E_{dyn}$), and pulse wave velocities ($c_p$) for all the experiments. By statistical analysis of paired data, the values for $E_{dyn}$ and $c_p$ were found to change significantly ($P < 0.005$) between interventions. Most notable is the higher $E_{dyn}$...
Systemic blood pressure, pulmonary arterial pressure ($P_{PA}$), and pulmonary arterial diameter ($D_{PA}$) before and during hypothalamic stimulation (dog 22). Onset of stiffening is first evidenced by a change in diameter, which starts approximately 1 second after the start of stimulation.

and $c_0$ during sympathetic stimulation than during the intravenous administration of norepinephrine, even though the mean pressure was higher during the norepinephrine infusion.

After the intravenous injection of atropine and propranolol (0.5 mg/kg and 2 mg/kg, respectively), the values for $\Delta D/\Delta P$ and diameter intercept were the same as before the drugs were given. Nor was there any change in the responses to the subsequent sympathetic stimulation or to the intravenous administration of norepinephrine or of phenoxybenzamine.

Structural Characteristics of the Wall of the Main Pulmonary Artery.—Histological examination (Fig. 10) of the main pulmonary artery beneath the caliper showed that elastic fibers of the internal elastic lamella were continuous and parallel. In the adjacent medial layers, the elastic fibers were discontinuous and interspersed with sparse amounts of collagen and large numbers of smooth-muscle cells. In this part of the media, the orientation of the smooth-muscle cells was predominantly tangential; in areas where there was no intervening collagen or elastin, the smooth-muscle cells appeared to connect with each other. There were more elastic fibers in the outer media than in the inner; they formed a pattern of concentric circles.

Pressure-diameter plots before (A) and during hypothalamic stimulation (dog 22); arrow indicates consecutive x-y plots which slant to final plot at B. Despite the progressive decrease in slope during stimulation, the diameter intercept of the main pulmonary artery ($D_{PA}$) remained virtually unchanged.
Comparison of the effects of sympathetic nerve stimulation (SNS) and norepinephrine (NE) (dog 2). The results during sympathetic nerve stimulation are similar to those shown in Figure 2. Infusion of norepinephrine produced an intermediate slope but with a lower diameter intercept and some opening of the loop.

Pressure-diameter plots photographed before (A) and during (progressively right to B) the intravenous infusion of norepinephrine (5 μg/min) showing a lower diameter intercept with a decrease in slope (dog 22).

During the infusion of norepinephrine (5 μg/min) a run of bigeminy (top) exaggerated the usual lag in diameter behind pressure during diastole. The x-y plot (bottom) shows the wide opening of the loop (dog 25).

Topical application of 100 ng norepinephrine (dog 22) changed the slope from A to B but the diameter intercept remained similar to that produced by sympathetic stimulation in the same dog (Fig. 4).
Comparison of the effects of phenoxybenzamine (PBZ) (10 mg/kg) on the relationship between $D_{PA}$ and $P_{DA}$, $P_{PA}$, $D_{DA}$ and systemic blood pressure tracings for control periods with effects of each of three interventions illustrated for dog 9. Relationship between data during control periods and with sympathetic nerve stimulation and norepinephrine infusions are similar to those shown in previous figures. After phenoxybenzamine there is an increase in mean diameter at a lower pressure with larger fluctuations per unit pressure change during a cardiac cycle. After phenoxybenzamine, the slope is steeper than during control, sympathetic nerve stimulation, and after the infusion of norepinephrine. However, the diameter intercept is similar to that observed during the control period and during sympathetic nerve stimulation but quite different from that observed during the infusion of norepinephrine.
lamellae; these smooth-muscle cells were often oriented obliquely and longitudinally.

In Situ Enzymatic Digestion of the Wall of the Main Pulmonary Artery.—Elastase (pledgets of cotton soaked in 31 mg/ml solution) was applied topically to the wall of the main pulmonary artery in two dogs. The effects of sympathetic stimulation and of norepinephrine infusion on $\Delta D/\Delta P$ and diameter intercept were tested immediately before and 1 hour after the application of elastase (Table 4). After the elastase, diameter intercepts decreased in response to sympathetic stimulation and norepinephrine infusion, but the slopes of $\Delta D/\Delta P$ were virtually unchanged (Fig. 11). Histological examination in two dogs revealed that the elastase had destroyed the outer elastic lamellae (Fig. 12, left).

The application of papain (pledgets soaked in crude papaya extract) to the wall of the main pulmonary artery produced a vivid, red-purple discoloration of the wall and hemorrhage which persisted throughout the experiment. However, as may be seen in Table 4, the responses to sympathetic stimulation and the infusion of norepinephrine were practically unchanged. Histological examination re-

TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>$P_{H2}$ (mm Hg)</th>
<th>$E_{dyne}$ ($\times 10^5$ dyn/cm²)</th>
<th>$c''$ (cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.1 ± 0.8</td>
<td>1.70 ± 0.11</td>
<td>278 ± 9</td>
</tr>
<tr>
<td>n = 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNS</td>
<td>18.0 ± 0.8</td>
<td>2.70 ± 0.20</td>
<td>352 ± 13</td>
</tr>
<tr>
<td>n = 20</td>
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</tr>
<tr>
<td>NE</td>
<td>20.3 ± 1.0</td>
<td>2.05 ± 0.16</td>
<td>306 ± 13</td>
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<tr>
<td>PBZ</td>
<td>13.1 ± 1.0</td>
<td>1.41 ± 0.14</td>
<td>255 ± 13</td>
</tr>
<tr>
<td>n = 8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations as in previous tables. See page 252 for definition of other terms.

Values are means ± se.
revealed adventitial hemorrhages, but the outer elastic lamellae were intact (Fig. 12, right).

**Discussion**

**Dynamic Elastic Properties of the Main Pulmonary Artery**

A linear, closed-loop relationship between pressure and diameter in the main pulmonary artery, over a wide range of heart rates, was found by Patel et al. (8). They concluded that the inertial and viscous properties of the artery were probably quite small. In general, the present data support their conclusions for the normal main pulmonary artery. However, the opening of the loop during the infusion of

**Table 4**

Elastic Properties before and after In-Situ Enzymatic Digestion of the Main Pulmonary Artery

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Wt (kg)</th>
<th>ΔD/ΔP × 10^-5 (cm/mm Hg)</th>
<th>Diameter intercept (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>SNS</td>
</tr>
<tr>
<td>27*</td>
<td>19.3</td>
<td>13.63</td>
<td>9.58</td>
</tr>
<tr>
<td>After elastase</td>
<td>19.12</td>
<td>20.25</td>
<td>19.00</td>
</tr>
<tr>
<td>28*</td>
<td>20.6</td>
<td>14.87</td>
<td>10.65</td>
</tr>
<tr>
<td>After elastase</td>
<td>14.90</td>
<td>14.82</td>
<td>13.40</td>
</tr>
<tr>
<td>29*</td>
<td>18.0</td>
<td>21.15</td>
<td>12.46</td>
</tr>
<tr>
<td>After papain</td>
<td>22.50</td>
<td>12.50</td>
<td>21.15</td>
</tr>
</tbody>
</table>

Abbreviations as in previous tables.

*Hypothalamic stimulation for SNS.
norepinephrine suggests that norepinephrine, by causing contraction of the vascular smooth muscle, also increased the viscosity of the wall of the main pulmonary artery. A similar effect of norepinephrine has been described for systemic muscular arteries (9).

Measurements of pulse wave velocity in dogs have yielded values ranging from 230 to 325 cm/sec (7, 10). Bargainer (10), using direct puncture of the main pulmonary artery in open-chest dogs, found an average pulse wave velocity of approximately 275 cm/sec. This value was the average velocity of those components of the pulse wave which had a frequency between 9 and 23 cps. Attinger found a phase velocity of 230 cm/sec when lungs were inflated to 25 cm water and 325 cm/sec when the inflation pressure was 5 cm water (7). The average pulse wave velocity of 278 cm/sec found in the present study, obtained by entirely different techniques, agrees well with these earlier values.

Changes in Dynamic Elastic Properties Induced by Blood-Borne Versus Neuronally Released Norepinephrine

Although the main pulmonary artery is consistently stiffened by both sympathetic stimulation and exogenous blood-borne norepinephrine, the pressure-diameter slope is less affected by exogenous blood-borne norepinephrine. The smaller pressure-diameter slope change with exogenous blood-borne norepinephrine cannot simply be explained by differences in amount of norepinephrine since the slope change was smaller than that produced by nerve stimulation, both with small dose infusions and large dose injections. More importantly, the difference in response was qualitative as well as quantitative, as shown by the decrease in diameter intercept with exogenous blood-borne norepinephrine. Therefore the pressure-diameter slope and the diameter intercept can be changed independently.

Since norepinephrine applied directly to the vessel wall, like nerve stimulation, produced changes in pressure-diameter slope without change in diameter intercept, the difference between the response of the main pulmonary artery to exogenous blood-borne norepinephrine vs. neuronally released norepinephrine would appear to be the site reached by the norepinephrine rather than any inherent qualitative difference.

Fluorescent microscopy (11, 12) and HPNE uptake studies of blood vessels (13), including large pulmonary arteries, have demonstrated that the terminal sympathetic effector plexus
is confined to the adventitia or the adventitio-medial junction and does not enter the media. Therefore, sympathetic nerve endings lie quite distant from all smooth-muscle cells except those near the adventitia, where norepinephrine is probably released. The oblique orientation of outer medial smooth-muscle cells sandwiched between continuous elastic fibers suggests a linkage between smooth muscle and elastic fibers. Benninghofs microdissection studies (14) on the aorta described such a linkage, which Burton proposed (15) would give a mechanical advantage to the smooth muscle in resisting deformation of the arterial wall. The results after destruction of the outer elastic lamellae by elastase are interesting in this regard. After elastase, norepinephrine infusions and nerve stimulation resulted in no alteration of slope, yet led to a decrease in diameter intercept. Therefore, an outer medial elastic fiber-smooth muscle linkage appears to be necessary for stiffening of the artery, as proposed by Bader (16).

A decrease in diameter intercept would appear to result from contraction of a different group of smooth-muscle cells. The discontinuous elastic fibers in the inner media, along with the tangentially oriented smooth muscles, suggest that smooth-muscle cells attach to each other in a ring-muscle arrangement as described by Benninghoff (14). Contraction of the ring muscles would decrease the diameter intercept and have less effect on the pressure-diameter slope. Presumably the diameter intercept change with blood-borne norepinephrine, whether it arrives by diffusion from the lumen, the vasa vasorum, or both, is due to stimulation of the inner medial ring muscle.

We do not know the concentrations of norepinephrine in different parts of the vascular wall during stimulation and the infusions of norepinephrine. However, the results do seem to indicate that during stimulation diffusion of norepinephrine within the vascular wall did not occur in sufficient concentrations to affect ring muscle and that infused norepinephrine affected ring muscle as well as outer medial smooth muscle. The data thus indicate that the smooth muscle in the wall of the main pulmonary artery does not behave as a functional electrical syncytium.

It is interesting that there was a decrease in main pulmonary arterial stiffness, with no consistent change in diameter intercepts, after alpha-receptor blockade with phenoxybenzamine. Phenoxybenzamine has no direct vasodilating action; instead, it acts to block alpha receptors. The results are consistent with the hypothesis that a tonic train of sympathetic nerve impulses was stimulating smooth muscle of the outer media during control periods and that blockade of the effects of these impulses resulted in a decrease in stiffness without changes in the diameter intercepts.

The physiological role of stiffening of the large pulmonary artery in the cardiopulmonary adaptations of the body to stress is not yet established. Unfortunately, this question cannot be explored in situations such as exercise or the "defense reaction" until a way for selective, nontraumatic denervation of the major pulmonary arteries can be found. On the other hand, it has been shown that stiffening of large pulmonary arteries favorably affects the distribution of blood within the lungs (17) and helps to maintain the pulmonary blood volume within narrow limits (1), both of which are characteristic of the pulmonary circulation during exercise (18).

An additional potential role for the stiffening of the main pulmonary artery could be the adjustment of the sensitivity of pulmonary arterial baroreceptors (19) to pressure. It has been shown that increased resistance to deformation of the wall of the carotid sinus by intra-arterial pressure leads to a decrease in the carotid sinus reflex response to blood pressure change (20).

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Response of the Main Pulmonary Artery of Dogs to Neuronally Released Versus Blood-Borne Norepinephrine

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