Increased Vascular Reactivity in Experimental Hypertension

By David B. Gordon, Ph.D., and Alfredo Nogueira

The etiology of essential hypertension is unknown. Its pathogenesis is also not clear, but it is generally agreed that the basic defect is an excessive resistance to blood flow due to a diminished internal caliber of arterial vessels, especially of the systemic arterioles. Implicit in this concept is the idea that a compensatory action of the heart occurs, the heart beating more forcibly so that blood flow is essentially normal throughout the body, while the arterial blood pressure is abnormally high. The same situation occurs in experimentally produced hypertension.

There are, at present, two popular points of view concerning the possible cause of the reduced caliber of the arterioles. One, which may be called the mechanical theory, assumes that through hypertrophy or swelling of the vascular wall, the internal diameter of the vessels is reduced. The other, which we may term the contractile theory, assumes that the smooth muscle cells in the blood vessel wall contract more forcibly and thus actively reduce the internal diameter of the vessels.

If one accepts the second point of view, then the important question arises: what is the cause of the excessive vascular constriction? Many different hypotheses have been proposed, and a great deal of experimental evidence pro and con has accumulated without attainment of conclusive and convincing proof. One of the more recent and more promising of these hypotheses is that of excessive reactivity, i.e., the idea that the smooth muscle of the blood vessels contracts more because it reacts more to the normal nervous and humoral influences acting upon it. Since the normal neurohumoral stimulant to vascular smooth muscle is largely norepinephrine released from sympathetic nerve endings, this hypothesis involves specifically a hyperreactivity of vascular smooth muscle to norepinephrine.

There is a considerable and growing body of evidence that in human essential hypertension, as well as in certain types of experimental hypertension, the blood vessels are hyperreactive to norepinephrine. However, one drawback to the acceptance of the hyperreactivity theory has been the failure to show such a hyperresponsiveness in isolated blood vessels from hypertensive animals, when they are exposed to norepinephrine in vitro. Thus, Redleaf and Tobian reported that strips cut from the aortae of hypertensive rats were actually less reactive, on the average, than those from normal rats. Similarly, Mallow found no greater reactivity to norepinephrine of strips of aorta from desoxycorticosterone-treated or renal hypertensive rats than equivalent strips from normotensive rats. The only positive results are those of Vick, Ederstrom, and Vergeer who tested strips cut from aortae of salt-fed rats and found them slightly more reactive than strips from normal rats. However, they did not ascertain whether or not their salt-fed rats were hypertensive.

The idea occurred to us that the negative results of Tobian and of Mallow might have been due to the fact that they tested their strips at a very low initial tension. It seems plausible that if the smooth muscle of blood vessels shows a length-tension relationship such as skeletal muscle does, then the optimal...
initial length (and corresponding initial tension) might be higher for the hypertensive vessels than for the normal. It was decided to reinvestigate the response of hypertensive isolated aortic strips to norepinephrine, paying particular attention to the effect of initial tension.

Methods

The animals used were rats of the Holtzman strain. Hypertension was produced by constriction of one renal artery by a silver clip or by complete ligation of one renal artery, leaving the opposite kidney intact. Blood pressures were measured at weekly intervals by the microphonic tail method of Friedman and Freed using light ether anesthesia. The last blood pressure was taken one day before using the animal for aortic strip testing.

For measurement of the responsiveness of the aortic smooth muscle to norepinephrine, the procedure used by Redleaf and Tobian was followed, with certain important modifications. Helical strips were cut from the thoracic aorta of rats, placed in cold Krebs-Ringer solution, and gently trimmed free of surrounding connective tissue. After cutting to a width of 2 mm., one end of the strip was tied to an adjustable glass support and immersed in warm Krebs-Ringer solution in a constant temperature bath at 36°C. Bubbled through the Krebs-Ringer solution were 95 per cent O₂ and 5 per cent CO₂. The other end of the strip was tied by a nylon or cotton thread to a calibrated stiff-spring (in preliminary experiments) or to a Statham strain-gauge tension recorder connected to a Grass polygraph. The spring or the strain gauge was calibrated by hanging known weights from it. The tension applied to the strip was adjusted to the desired initial tension (usually 2 Gm.; equals 10 Gm. per cm. width of strip). A period of 1½ hours was allowed to insure complete relaxation of the strip. Five to 10 minutes prior to each addition of norepinephrine, ethylene diamine tetra-acetic acid (Versene) was added to the bath (final concentration \(10^{-5}\) M) to prevent breakdown of norepinephrine. Then \(d\)-norepinephrine hydrochloride was added to the bath (final concentration \(7.8 \times 10^{-6}\) M). A rapid initial rise in tension occurred, followed by a more gradual rise leading to a sustained plateau. The tension developed by the strip was recorded for a period of 15 minutes, and the maximum attained was used as a basis for comparison. The bath was then rinsed twice with fresh Krebs-Ringer solution and the strip promptly relaxed. After adjusting to a new and slightly higher initial tension, the procedure was repeated. Each cycle was timed so that successive additions of norepinephrine were made at 45-minute intervals, thus alternating 15 minutes of contraction and 30 minutes of relaxation. The initial tension was increased once in each cycle by a small increment until the optimum initial tension was passed, as indicated by a reduction in the active tension developed by the strip.

The length of the strip corresponding to each initial tension used was measured; at the end of the experiment, the width of the strip corresponding to each of these lengths was measured by means of an optical micrometer. These data were used to calculate the initial tension in terms of grams per centimeter width of strip.

In addition to the use of a series of initial tensions for testing the response of each strip, other differences between our technique and that of Redleaf and Tobian should be mentioned. One is the fact that our animals were killed or deeply anesthetized by a large intraperitoneal dose of pentobarbital, instead of by exsanguination prior to opening the thorax and removing the thoracic aorta. The dose used was 60 mg./rat or approximately 200 mg./Kg. body weight. The purpose of this large dose of barbiturate was to prevent or reduce the release of intrinsic epinephrine or norepinephrine and thus to protect the aorta from the combined effect of vasoconstrictor stimulation and reduced intraluminal pressure. Probably because of the use of this technique, the need for sodium nitrate as a relaxing agent prior to testing the strip was avoided.

One other difference is our production of aortic strips of uniform width (2 mm.) by means of a "cookie-cutter" type of device consisting of two razor blades held firmly in a plastic holder with a 2-mm. spacer between the blades. This made it possible to compare results directly from one strip to another.

The length of the strips (relaxed) varied from 21 to 32 mm. Under tension the strips elongated to as much as 54 mm. In many experiments, we measured the thickness of the relaxed aortic strip by focusing a microscope on the lower and then the upper surface of the strip and measuring the movement of the microscope lens by means of an appropriate calibration on the microscope. Most strips were approximately 0.1 mm. in thickness.

Results

PRELIMINARY EXPERIMENTS

In a preliminary series of experiments, aortic strips from four chronic (four to five
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months) renal hypertensive rats were compared with similar strips from four normal rats. In these experiments a stiff spring connected to a lever which traced on a kymograph was used as the recording device. This provided an almost isometric contraction, shortening being limited to less than 2 per cent of the initial length of the strip.

The results showed that a length-tension relationship does apply to aortic smooth muscle and that there is an optimum length (and corresponding optimum initial tension) at which the maximum response to norepinephrine occurs. Furthermore, in accord with our expectations, the optimum for the hypertensive strips was higher than that for the normotensives; the average figures being 36 Gm./cm. and 19 Gm./cm., respectively. The major finding was that all four strips from the hypertensive rats gave a greater tension development than the normotensive rats. The average values for additional tension developed upon exposure to norepinephrine were hypertensive, 819 mg.; normotensive, 569 mg.

SECOND SERIES OF EXPERIMENTS

Aortic strips from 10 normotensive female rats were compared with similar strips from 10 hypertensive female rats. The hypertension was of fairly short duration (2½ to 7 weeks). The response of 10 normotensive male rat aortic strips was also measured. The technique differed from the preliminary experiments only in that the tension recording device was a Statham strain gauge, and the contraction produced by norepinephrine was thereby made more truly isometric. The maximum shortening was less than 0.2 per cent of the strip length.

This change in procedure did make a difference; the absolute tension developed was considerably greater, and also the value of the optimum tension was increased. In addition, the difference in optimum tension between the hypertensive and normotensive aortic strips was apparently reduced. The average optimum tension, using the strain-gauge recording was hypertensive, 42 Gm.; normotensive, 36 Gm. However, we wish not to emphasize this finding since, in subsequent experiments, we have found that a large difference in optimum tension can still persist even even with isometric recording if the technique is somewhat altered. Figure 1 shows the response of a hypertensive and normotensive rat aortic strip at different initial tensions.

The major finding was in accordance with the results of the preliminary experiments. The aortic strips from the hypertensive rats, when exposed to norepinephrine, developed a definitely greater tension than strips from the normotensive rats. The difference between the two groups was highly significant ($P < 0.001$). The average values were: hypertensive females, 1610 mg.; normotensive females, 1029 mg.; and normotensive males, 1138 mg. The average and individual results for the female rats are listed in table 1. Figure 2 shows the active tension developed by the strips as a function of the blood pressure of each rat. The difference between the groups is obvious, and even within each group there seems to be some tendency toward increased reactivity with increased blood pressure.

An important additional observation in the second series of experiments was that the
**TABLE 1**

Comparison of Hypertensive and Normotensive Female Rats

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Blood pressure</th>
<th>Days after operation</th>
<th>Maximum response (mg.)</th>
<th>Optimal initial tension (Gm./cm.)</th>
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Discussion

The results of our experiments indicate that in experimental renal hypertension the smooth muscle of the aorta is hyperreactive to norepinephrine. We recognize, as have previous users of the aortic-strip technique, that results obtained with aortic smooth muscle do not necessarily apply to arteriolar smooth muscle. However, unless evidence to the contrary is forthcoming, it seems reasonable to assume that the portion of the arterial tree which was tested is representative of both arterial and arteriolar smooth muscle. There is some evidence that in human essential hypertension, even nonvascular smooth muscle may be hyperreactive to norepinephrine.

These positive results strongly support the hyperreactivity theory of hypertension. It is now apparent that isolated blood vessels, as well as vessels tested in vivo, do exhibit an increased responsiveness to norepinephrine, at least in experimental renal hypertension. The broader question of whether the hyperreactivity of the vascular system causes the
hypertension, or merely accompanies it, remains to be answered.

It should be pointed out that there is no real conflict between our results and those of Redleaf and Tobian \(^3\) or of Mallov \(^1\). At the lowest initial tensions which we used in our experiments (less than 10 Gm./cm. width of aortic strip) the difference between the hypertensive and normotensive strip was minimal and sometimes reversed (i.e., normotensive more reactive). Redleaf and Tobian regularly used an initial tension of approximately 3.3 Gm./cm. width, so that their experiments were unlikely to show a greater responsiveness of the hypertensive strip. The same applies to Mallov's experiments. He did not use strips of uniform width so that it is not possible to calculate the exact tension per unit width, but on the basis of the absolute tension applied (2 6m.) an approximation of 6 to 10 Gm./cm. may be estimated. The initial tensions which we used cover the physiological range, i.e., the actual circumferential tension which may be calculated to exist in the aorta of the living rat, applying LaPlace's law: \( T = P \times R \). For a rat with a mean blood pressure of 100 mm. Hg and an aortic diameter of 0.3 cm., this would be 20.4 Gm./cm., and for a rat with a blood pressure of 200 mm. Hg the tension would be correspondingly doubled.

**Summary**

The responsiveness to norepinephrine of aortic strips from hypertensive rats and from normotensive rats was compared. The strips were set up in a bath of Krebs-Ringer solution, and isometric tension development upon addition of norepinephrine was recorded. The hypertensive strips produced the greatest tension, indicating a greater reactivity to norepinephrine. It was found that the initial tension exerted upon the strip greatly influenced its response, there being an optimal initial tension at which the greatest active response was obtained. These results support the hypothesis that hypertension may be due to an excessive reactivity of vascular smooth muscle.

References

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