Comparative Reactivities of Aortic Strips from Hypertensive and Normotensive Rats to Epinephrine and Levarterenol

By SAMUEL MALLOV, M.S., PH.D.

With the technical assistance of Irma Tiffe

Rats were rendered hypertensive by two different technics. Isometric tensions developed by strips of aorta from these rats, when exposed to various concentrations of epinephrine and levarterenol, were compared with those developed by aortic strips from normotensive controls. Almost all of the aortic strips from the DCA-salt hypertensive rats responded with smaller contraction tensions to both pressor agents than did the corresponding control strips from the normotensive animals. The same was true for most of the strips obtained from the renal hypertensive rats. These results do not lend support to the hypothesis that vascular hyperreactivity is the basis of many forms of hypertension.

The suggestion has appeared in the literature, from time to time, that the increased arteriolar resistance present in essential hypertension may be due to an enhanced sensitivity of the vascular smooth muscle to pressor agents normally found in the circulation or released by nerve endings. A number of studies of human hypertensives, and also of animals rendered hypertensive by experimental means, have yielded data tending to support such a hypothesis. The work presented in this paper was done in order to determine whether the development of experimental hypertension in animals is indeed associated with an increased vascular reactivity. It was considered advisable to study vascular strips in vitro, so that circulatory, nervous, and similar influences present in the whole animal could be eliminated. Aortic smooth muscle was chosen for convenience, with the realization that the responses of aortic and arteriolar smooth muscle to drugs may not necessarily be alike. During the course of this study, the results of a similar investigation were published by Redleaf and Tobian. These authors found that if aortic hypertrophy is taken into consideration, and conditions of comparison carefully controlled, the development of DCA-salt hypertension in rats is almost always, and renal hypertension often, associated with decreased rather than increased contractile responses of aortic smooth muscle to epinephrine and levarterenol.

Methods

Male albino rats of the Wistar strain were rendered hypertensive by two different procedures. In the first, rats weighing 80 to 100 Gm. at the start were injected intramuscularly at weekly intervals with 5 mg. of desoxycorticosterone trimethylacetate, and given 1 per cent NaCl in place of drinking water. They were fed Purina laboratory chow ad libitum. Control rats receiving no special treatment were maintained on Purina chow and water ad libitum. The animals were killed in pairs (1 normotensive control and 1 hypertensive rat) after various intervals of time ranging from 14 to 50 days. In the second procedure, groups of rats were rendered hypertensive by tying figure-eight ligatures around the left kidneys, and removing the right kidneys one to two weeks later. These animals and their controls weighed 230 to 250 Gm. at the start of the experiment. All rats were maintained on Purina chow and water ad libitum. Pairs of rats, composed of 1 control and 1 hypertensive rat, were killed 49 to 105 days after the second operation. Blood pressures were determined twice weekly by means of the micronephric manometer method of Friedman and Freed on nonsedated animals. Rats with systolic pressures not exceeding 130 mm. Hg were considered normotensive, while those

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Supported by a grant from the American Heart Association.

Received for publication September 29, 1958.

*We would like to express our gratitude to Dr. Robert Gaunt, Director of Research, Ciba Pharmaceutical Products, Inc., Summit, N.J., for supplies of Percorten Trimethylacetate.
with pressures of 150 mm Hg and higher were considered hypertensive.

The rats were killed by exsanguination after the induction of pentobarbital anesthesia. Sections of aorta extending from the aortic arch to the origin of the renal arteries were then removed, and placed in beakers of Krebs-Ringer bicarbonate solution containing 0.01 M glucose. The cylindrical strips of aorta were then dissected free of extraneous fat and connective tissue, and opened longitudinally to form rectangular sections approximately 40 mm long. A strip from a normotensive rat was then placed on top of a strip from a hypertensive animal (and vice versa in alternate experiments), and both strips were placed on a section of cork covered by filter paper which was wet with the Krebs-Ringer solution. Rectangular sections of the aortas of equal lengths (30 to 35 mm.) and widths were now prepared, and fastened to the cork by means of needles piercing the extremities. Incomplete transverse cuts were then made alternately from each side, in both sections simultaneously, by means of a razor blade used in cleaver fashion, so that sections were obtained as described by Vick, Ederstrom and Vergeer. This method of cutting sawtooth-shaped strips of aorta permitted us to obtain two strips each time that were exactly alike in length, width, and shape, and that could differ only in thickness.

Each pair of strips from 1 control and 1 hypertensive rat was then placed in a muscle bath containing the Krebs-Ringer bicarbonate solution and glucose, and sometimes NaNO₂ (10⁻⁴) and calcium disodium versenate (10⁻⁵), and maintained at 37 C. If NaNO₂ was present, after 10 to 20 minutes, the bath was washed out several times with solution not containing the nitrite, and the strip allowed to remain in such solution. The bath fluid was aerated with a mixture of 5 per cent CO₂ in oxygen. One end of each strip was attached to a glass rod by means of a stainless steel wire. The elongation of the cut strips permitted the circular muscle layers to exhibit an increased vertical component of tension when stimulated to contract. The strips were allowed to relax in the bath for periods of 2.5 to 3 hours before any drug was added to the bath fluid. After this time, basic tension was readjusted to 2 Gm., solutions of either 1-epinephrine bitartrate or 1-norepinephrine bitartrate (levaterenol)* were added in various concentrations, and the isometric contractions of the strips were recorded by means of a Grass polygraph. Each recording was allowed to continue until the maximum contractile force had been manifested for several minutes. The bath fluid was then replaced several times with fresh Krebs-Ringer bicarbonate solution, and the aortic strips allowed to relax for another 1.5 to 2 hours before a second addition of drug was made. Con-

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<th>DCA-salt hypertensive</th>
<th>Renal hypertension</th>
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<tr>
<td>Blood pressure of</td>
<td>199±5.0</td>
<td>162±2.3</td>
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<td>hypertensive rats</td>
<td>(28)</td>
<td>(27)</td>
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<td>Blood pressure of</td>
<td>124±1.2</td>
<td>126±0.6</td>
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<td>control rats</td>
<td>(28)</td>
<td>(27)</td>
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<td>Per cent difference in</td>
<td>-43.2±4.98</td>
<td>-18.4±6.96</td>
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<td>maximum tension exerted</td>
<td>(28)</td>
<td>(27)</td>
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<td>by aortic strips</td>
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<td>from hypertensive rats</td>
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<td>in response to</td>
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<td>-18.1±9.89</td>
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<td>levaterenol</td>
<td>(10)</td>
<td>(17)</td>
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<td>Per cent difference in</td>
<td>+21.1±3.61</td>
<td>+23.1±5.62</td>
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<td>wet tissue weight of</td>
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<td>aortic strips from</td>
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<td>hypertensive and</td>
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<td>control rats</td>
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<tr>
<td>Micrograms of muscle</td>
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<td>14.8±0.414</td>
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<td>hypertensive rats</td>
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<td>As above, for</td>
<td>11.58±0.003</td>
<td>13.40±0.422</td>
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<tr>
<td>control rats</td>
<td>(20)</td>
<td>(27)</td>
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*All figures are given as mean plus standard error of the mean.
†Figures in parentheses represent number of rats or of aortic strips.
‡Maximum tension expressed as mg. tension per mg. muscle nitrogen. Per cent difference for each pair of strips calculated as H-C/C X 100, where H and C are maximum tension exerted by hypertensive and control strip respectively.
§Per cent difference for each pair of strips calculated as H-C/C X 100, where H and C are mg. wet tissue weight of hypertensive and control strip respectively.
¶Difference between values for hypertensive and control strips statistically significant (p < 0.01).
**Difference between values for hypertensive and control strips statistically significant (p < 0.02).

We wish to express our thanks to Dr. M. L. Tainter, Director, Sterling-Winthrop Research Institute, Rensselaer, N.Y., for supplies of levinepinephrine bitartrate and 1-norepinephrine bitartrate monohydrate.
Fig. 1. DCA-salt hypertension. Per cent difference in maximum tension exerted between each aortic strip from a DCA-salt hypertensive rat and its normotensive control, in response to 1-epinephrine (shaded bars) or levarterenol (black bars). The difference is calculated as \( \frac{H-C}{C} \times 100 \), where \( H \) and \( C \) represent the maximum tension exerted by the strip from the hypertensive and from the control rat respectively, each tension being expressed as milligram tension per milligram muscle nitrogen.

traction of each pair of strips was induced two or three times. When low concentrations of pressor amines were used \((10^{-6})\), calcium disodium versenate was put into the bath fluid to bind heavy metal ions and prevent the rapid deterioration of the amines. With higher concentrations \((10^{-8} \text{ to } 10^{-10})\) no versenate was used. 1-Epinephrine bitartrate and levarterenol bitartrate solutions were kept in physiological saline containing 0.01 N HCl, and the concentrations were such that not more than 0.2 ml. of solution was added to the bath at any one time.

Following the recording of the contractions, the strips were washed several times, removed from the bath, dried on filter paper, and weighed. The "muscle" component of each strip was then extracted by the NaOH extraction procedure of Lowry, Gilligan, and Katersky, and "muscle" (alkali-soluble) nitrogen of the extract was determined by sulfuric acid hydrogen peroxide digestion and direct Nesslerization.

RESULTS

Properties of the Strips. The sawtooth-shaped strips of rat aorta, prepared as described, possessed several of the properties of the spiral strips of rabbit aorta prepared by Furchgott and Bhadakrom. Thus, on placing the rat aortic strips under 2 Gm. of tension in the muscle bath, they gradually relaxed, the relaxation reaching completion or near completion after 2 to 3 hours. At the same time, the contractile responses to given concentrations of epinephrine or levarterenol also gradually increased, sensitivity reaching a maximum after 2 to 3 hours.

If either amine was added to the bath so that a moderate or large concentration was present (producing 50 to 100 per cent of maximum force of contraction), contraction permitted, the drug washed out, and the strips allowed to relax to the original tension, subsequent additions of the same concentration of drug produced smaller contractile tensions than it did initially. However, if the strips were allowed to remain in the bath for at least 1.5 to 2 hours following the original contraction and washing out, sensitivity to the drug generally, but not always, returned to normal or near normal.

If the strips were allowed to remain in Krebs-Ringer solution containing NaNO\(_2\) in a concentration of \(10^{-4}\) for 10 to 20 minutes during the initial period of relaxation, and then the nitrite solution was washed out, there was no appreciable alteration of progressive relaxation. The increased sensitivity that occurred during this period, and the subsequent contraction tensions following addition of epinephrine or levarterenol were unchanged. In our hands, unlike the situation in the case of Redleaf and Tobian's experiments, practically every strip responded with contraction to epinephrine or levarterenol, even when the strip had not relaxed in NaNO\(_2\) solution. Since our results did not appear to be affected by the prior presence or absence of nitrite, we elected not to use this reagent in most of our experiments.

Aortic Hypertrophy. Both DCA and renal hypertension resulted in aortic hypertrophy. The results are summarized in table 1. Not only did the strips of aorta from hypertensive rats tend to weigh more than the corresponding control strips (of equal length and width) from normotensive animals (21.1 to 23.1 per
Reactivities of Aortic Strips

Since the two aortic strips of each pair were sectioned exactly alike, but the pairs differed from each other somewhat in size and shape, the isometric contraction tension of each strip from a hypertensive rat was compared with that of its control from a normotensive animal, and the difference expressed as a percentage of the tension exerted by the control strip. The data for each of the pairs of strips are given in figures 1 and 2, and the mean values in table 1.

Of the 28 aortic strips obtained from DCA-salt hypertensive rats, 26 responded to various doses of 1-epinephrine with smaller maximum contraction tensions than did the corresponding strips from normotensive animals, the mean difference in maximum tension exerted by all 28 pairs being −43.2 per cent. In only two pairs of strips did the control aortas contract with greater tension than did the hypertensive rat aortas. Similarly, of the 16 pairs of these strips exposed to levarterenol, 14 aortic segments from DCA hypertensive rats reacted with less tension, and two with more, than the corresponding strips from normotensive rats. The mean difference in this case was −37.2 per cent. Of the four control rat aortas which contracted with less tension than did the corresponding hypertensive rat aortas in response to epinephrine or levarterenol, two became relatively more reactive after the strips were allowed to relax in the bath for an additional period of time, and contracted with greater tension than did the hypertensive-rat strips, when again subjected to levarterenol or epinephrine respectively.
In the case of the renal hypertensive rats, of the 27 aortic strips from hypertensive animals, 20 responded with smaller, and seven with larger contractile tensions than did the corresponding control aortic strips, in response to various doses of 1-epinephrine. The mean difference in tension between hypertensive and normotensive aortic strips for all 27 pairs was -18.4 per cent. When 17 of these pairs of strips were subjected to different concentrations of levarterenol, 13 strips from hypertensive rats contracted with less tension, and four with more, than the corresponding strips from normotensive rats, the mean difference for all 17 pairs being -18.4 per cent.

**DISCUSSION**

Our results indicate that there is no hyperreactivity of aortic smooth muscle associated with the development of either DCA-salt or renal hypertension in rats. On the contrary, there is a clear tendency towards decreased responsiveness to the physiological pressor amines, epinephrine and levarterenol, this tendency being very marked in DCA-salt hypertensive rats. Thus our data do not lend support to the hypothesis that many forms of hypertension are due to an enhanced sensitivity of vascular smooth muscle to normal pressor agents circulating in the blood or released at nerve endings. One must bear in mind, however, that the circumstances under which our data were collected do not warrant any extended conclusion. We dealt with aortic smooth muscle rather than with arteriolar muscle. We considered only two of the several types of experimental hypertension, and limited ourselves to one species and two physiological pressor agents. Above all, there may be no similarity in the etiologies of experimental and of human essential hypertension.

We have no explanation for the decreased aortic reactivity seen in the hypertensive animals, but think that such decreased reactivity is probably one of the consequences of the hypertension itself. It may be one of the functional accompaniments of the morphological deterioration of the vascular walls in hypertension. The tendency towards decreased reactivity was much more marked in the aortas of the DCA-salt hypertensive rats, whose blood pressures in general were considerably higher than those of the renal hypertensive animals. In two instances, where the blood pressures were unusually high, the vascular deterioration was easily discernible to the naked eye. We could not, however, establish any clear correlation between the height of the systolic blood pressure and the degree of decreased reactivity, for the entire group of rats.

Redleaf and Tobian, using somewhat different techniques, found, in their study of the reactivities of rat aortic strips, that while the majority of responses of the strips from hypertensive rats to levarterenol were normal, a few showed decreased responsiveness. It may be that if they had taken into consideration the variations in smooth muscle content of their strips, they would have found a much greater degree of hyporesponsiveness than they did, inasmuch as muscular hypertrophy occurs in the vessel walls of hypertensive animals. The large variations in the sizes and contractile responses of their spirally cut strips may have also tended to mask some real differences.

**SUMMARY**

Rats were rendered hypertensive by means of (a) injection of DCA and feeding of 1 per cent NaCl solution in place of drinking water, and (b) constriction of one kidney and removal of the contralateral one. Isometric tensions developed by strips of aorta from these rats, when exposed to various concentrations of epinephrine and levarterenol, were compared with those developed by aortic strips from normotensive controls. Since hypertrophy of the aortas of the hypertensive rats occurred, as shown by the greater tissue weights and muscle (alkali-soluble) nitrogen concentrations of the strips from these rats, the results were expressed as milligram tension developed per milligram alkali-soluble nitrogen. Almost all of the aortic strips from the DCA-salt hypertensive rats responded with smaller contraction tensions to both epinephrine and levarterenol than did the corresponding control strips from the normotensive rats. The same
was true for most of the strips obtained from the renal hypertensive rats. The decreased responsiveness of the hypertensive rat strips is believed to be one of the consequences of hypertension. These results do not support the hypothesis that many forms of hypertension are due to an enhanced sensitivity of vascular smooth muscle to physiological pressor agents.

**Summario in Interlingua**

Rattos esseva rendite hypertensive per medio de (1) injection de acetato de disoxycorticosterona e substitution de un solution de 1 pro cento de NaCl pro le aqua a biber e (2) constriction de un ren e excision del altere. Le tension isometric disveloppate per pecias de aorta ab iste rattos quando exponite a varie concentrationes de epinephrina e levarterenol esesva comparate con illo disveloppate per pecias de aorta ab rattos normal de controlo. Viste que hypertrophia del aorta occurreva in le rattos hypertensive—evidente per le augmentate peso de histos e le plus alte concentration de (alcali-soluble) nitrogeno muscular—le resultatos esseva exprimite como mg de tension disveloppate per mg de nitrogeno alcali-soluble. Quasi omne le pecias aortic ab rattos hypertensive in consequentia de acetato de disoxycorticosterona e sal respondeva con plus basse tensiones de contraction a epinephrina e levarterenol que le pecias aortic ab rattos normotensive. Le mesmo valeva pro le majoritate del pecias obtenite ab rattos hypertensive post constriction renal. Le reducee responsiviitate del pecias aortic ab rattos con hypertension es interpretate como un del consequencias del hypertension. Iste resultatos non supporta le these que multe formas de hypertension es cause per un augmento del sensibilitate de musculo lisie vascular al effecto de physiologic agentes pressori.

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Circ Res. 1959;7:196-201
doi: 10.1161/01.RES.7.2.196

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