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Hyperresponsiveness of Renal Hypertensive Rats to Intravenous Angiotensin II.* (28863)

J. R. GASKELL, R. K. JONES AND A. P. SHAPIRO (Introduced by Melvin Hess)
Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

Although bioassay techniques have demonstrated increased circulating blood levels of angiotensin in patients with malignant hypertension and with renal artery obstruction, investigators have not been able to correlate circulating angiotensin levels with essential hypertension(1). Thus, attention has been directed toward the problem of intrinsic vascular sensitivity to angiotensin and to the question of angiotensin inactivators and their effect on rate of degradation.

Studies on angiotensin inactivating mechanisms have yielded conflicting results, with controversy arising between *in vivo* and *in vitro* observations. Thus, *in vivo* studies with I^{131}-labeled angiotensin have indicated a prolonged half-life in hypertensive plasma(2), yet *in vitro* studies with this compound have shown an increased rate of destruction in hypertensive plasma(3). The validity of these data have been questioned recently by studies of Khairallah *et al* using tritiated angiotensin II which indicate that the iodine labeled compound may not give a true picture of the half-life of the polypeptide(4). However, Hickler, using a bioassay technique in normotensive rats, has estimated the *in vitro* biological half-life of angiotensin by incubation with human hypertensive and normotensive plasma and demonstrated that angiotensin inactivation occurs more quickly in the hypertensive plasma(5). He suggests that this may represent an enzymatic adaptation to chronic angiotensinemia. Wood, on the other hand, found an increased pressor response to angiotensin in the offspring of hypertensive individuals when he reinfused their blood after it had been incubated with angiotensin (6); from these data, he suggested an impaired angiotensin degradation in these subjects.

Using bioassay techniques in rats, Blaquier has found that extracts of kidneys from normotensive and hypertensive rats show no difference in angiotensinase activity(7), while Bing actually describes decreased inactivation of angiotensin by hypertensive renal tissue

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(8). The recent studies of Khairallah and associates(9) which demonstrate that a number of nonspecific enzymes destroy angiotensin may provide a partial explanation for these discrepant results.

Failure to take into account the intrinsic pressor responsivity of the organism may also account for these conflicting observations. Accordingly, the present experiment was designed to study the role of preexisting renal hypertension in the rat on the pressor response to intravenous angiotensin II as measured by maximum elevation of blood pressure and duration of response.

Materials and methods. Male albino Carworth rats weighing 80 to 100 g were subjected to a right nephrectomy, followed one week later by the placing of a silver clip on the left renal artery. A rat was judged hypertensive after 2 consecutive systolic pressures were found to be above 140 mm Hg; 9 hypertensive rats were made available for the study beginning approximately 4 weeks after surgery. Blood pressures were measured during light ether anesthesia by the tail phonomicrophone technique, using the Infraton amplifier and recording the pulse on a direct writing Sanborn electrocardiograph(10). All rats were maintained on rat chow and tap water *ad libitum.*

Bioassays were performed on 9 normotensive controls and 9 hypertensive rats; comparative assays of rats of equal weights were performed on the same day. In preliminary studies in normal rats, a response curve for graded doses of angiotensin II† injections was developed. The dose employed in the experiment, 0.1 μg, was in the linear range of this curve and was injected by percutaneous cannulation of a tail vein. This technique made possible multiple trials during a single experimental period. The recorder was started before angiotensin injection and was permitted to run continuously during and following injection. Baseline pressures were recorded as the average of 3 consecutive pressures taken during a 2-minute period before injection. Following injection, pressures were taken at 15-second intervals. Duration of pressor response was then ascertained by observation of the recorder tape and was designated as the time in seconds for the blood pressure to return to within 10 mm Hg‡ of baseline pressure. All animals were subjected to 4 or 5 assays over a 6-month period with each assay considered the mean of 4 angiotensin injections during a single experimental period.

Results. The recording tape illustrating the source of the pressor response curve for a single injection of angiotensin in a hypertensive animal is demonstrated in Fig. 1. Baseline pressure of 170 mm Hg was followed by a maximum pressor response to 210 mm Hg after injection of 0.1 μg of angiotensin II. Return to baseline was observed 94 seconds following injection.

Comparison of average maximum pressor response of hypertensive and normotensive animals is presented in Fig. 2. Maximum response and standard error for hypertensives was 36 $\pm$ 1.30 mm Hg and for normotensives was 27 $\pm$ 0.98 mm Hg ($p < .001$).

Fig. 3 illustrates average duration of pressor responses. For the hypertensive rats it was 89 $\pm$ 3.10 seconds, for normotensives, 60 $\pm$ 1.97 seconds ($p < .001$).

Discussion. These results indicated increased responsiveness to angiotensin in the renal hypertensive rat as measured by maximum pressor response and duration of response. A number of factors, including vascular reactivity, homeostatic depressor effects, and angiotensin inactivating mechanisms deserve consideration in interpreting these findings.

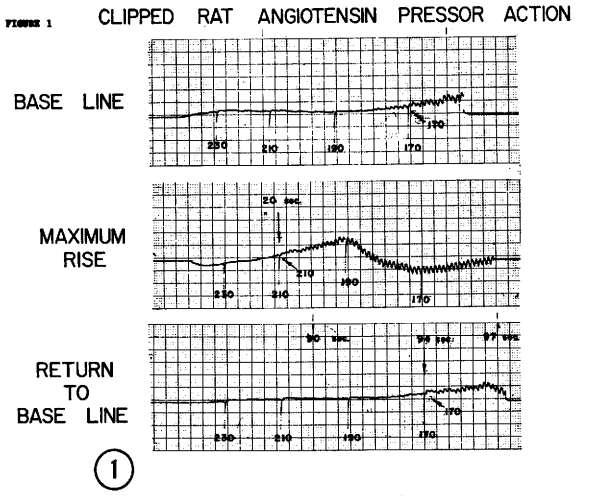
If the renal hypertensive rat had adapted to elevated angiotensin levels by the development of angiotensinase it would be expected that response to exogenous angiotensin would be minimized. Hyperresponsivity in the present study might have resulted from a lack of specificity of such an angiotensinase to synthetic angiotensin II. However, other alternatives might fit more reasonably with these data.

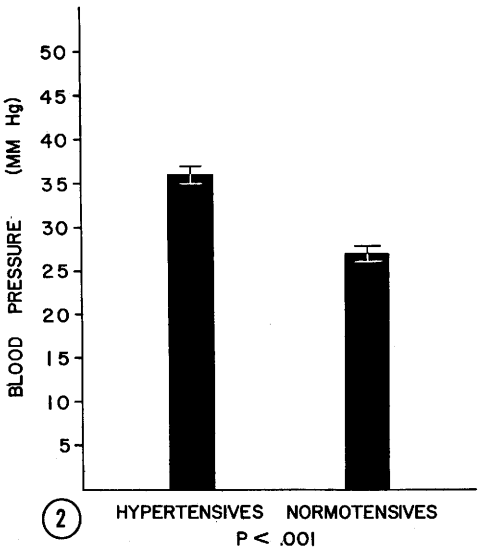
As recently reviewed by Mendlowitz(11),

† Angiotensin, as synthetic valyl-5-angiotensin II, supplied by Ciba Pharmaceutical Co., N. J.

‡ Standard deviation of baseline pressure.

several lines of evidence have suggested an increased vascular sensitivity in the hypertensive animal to vasopressor agents, including angiotensin. Furthermore, an increased pressor response to angiotensin injection has been observed in hypertensive subjects(12). Attempts to explain increased reactivity have included an evaluation of the role of sodium content of arterial tissues(13). While this subject remains unresolved, the obvious possibility applicable to the present experiment is that stimulation of aldosterone by endogenous angiotensin may lead to sodium retention and increase arteriolar reactivity to exogenous angiotensin as well as to other vasopressors.





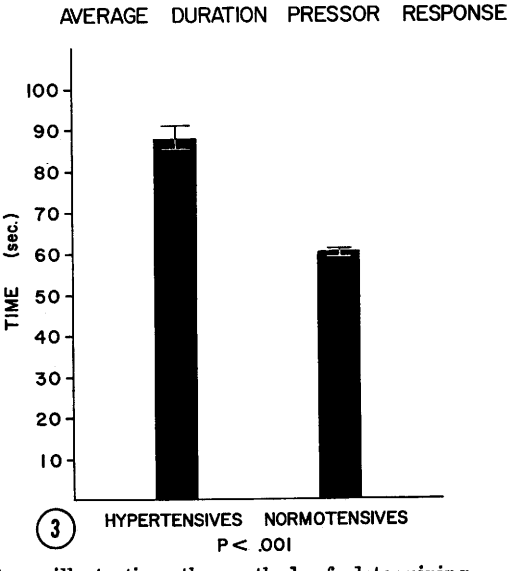


FIG. 1. Selected segments from recorder tape illustrating the method of determining hypertensive response in one hypertensive rat. Systolic pressure is determined from appearance of pulse as cuff is deflated. Vertical markers on recording indicate cuff pressure. The recorder runs continuously during injection and duration of response is determined from time markers on top margin of tape the speed of which has been calibrated previously.

FIG. 2. Maximum pressor response, mean and standard error, for hypertensive and normotensive rats.

FIG. 3. Duration of pressor response, mean and standard error, for hypertensive and normotensive rats.

The suggested role of the kidney in protecting against the development of hypertension through release of depressor substances may also be applicable(13). Thus, a depletion or exhaustion of these materials in chronic renal hypertension might lead to increased responsiveness to pressor agents. In addition, impairment of the ability of the sympathetic nervous system to buffer a pressor response in the chronic hypertensive state could account for the data.

Summary. Renal hypertensive rats were found to have a pressor response to angiotensin which exceeded that of normal rats in both magnitude and duration. The results would appear to be in keeping with an intrinsic increase in pressor reactivity of the hypertensive organism, but are not compatible with the production of a specific angiotensinase as an adaptive mechanism to prevent chronic angiotensinemia.

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Role of Histamine in Producing the Eosinophilia of Magnesium Deficiency.* (28864)

GERALD F. HUNGERFORD

Department of Anatomy, University of Southern California School of Medicine, Los Angeles

Magnesium deficiency results in a blood and tissue eosinophilia(1,2). The blood eosinophilia is especially prominent in the acute phase of the deficiency syndrome when there is also a marked dermal hyperemia. It has been reported that mast cells degranulate in the acute phase of magnesium deficiency (2,3). The release of endogenous histamine may, therefore, be responsible for the blood eosinophilia as well as the dermal hyperemia.

However, a blood eosinophilia does not occur in rats after administration of commercial histamine. It has, in fact, been reported that histamine administration stimulates the pituitary-adrenal system(4) resulting in a blood eosinopenia.

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The present experiments were designed to explore the effect of magnesium deficiency, histamine, heparin, serotonin and the pituitary-adrenal system on rat blood eosinophil levels.

Methods and materials. Male, Holtzman rats, weighing 100-150 g were placed on purified diets, either with complete salts or deficient in magnesium. The composition of these diets has been reported(2). Direct eosinophil counts were made from tail vein blood using the Speirs-Meyer diluent(5) in a Fuchs-Rosenthal hemocytometer 0.2 mm deep.

Groups and experimental conditions are presented in Tables I and II. Histamine phosphate (Lilly Co.) injections were calculated as the base. Administered pharmaceutical