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Effect of Dibenamine on Renal Hypertension in Rats.*

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(Introduced by Louis S. Goodman.)
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The sequence of events by which interference with renal hemodynamics leads to elevation of the systemic blood pressure has been carefully studied (Braun-Menendez, *et al.*,[1] and Goldblatt[2]) and has been shown to be independent of nervous mechanisms. However, scattered reports[3-5] have suggested that drugs acting to block the vasopressor action of the sympathetic nervous system may lower the blood pressure in animals with renal hypertension. Sympathectomy also reduces the blood pressure in animals with renal hypertension,[6] but the reduction is limited in extent and prior sympathectomy does not prevent the development of renal hypertension.[6-8]

The use of drugs in the study of neurogenic factors in renal hypertension has been limited by the relative ineffectiveness of the available agents. Demonstration of the high potency and specificity of the adrenergic blocking action of N,N-dibenzyl-β-chloroethylamine (Dibenamine)[9] suggested that it would be a useful tool in evaluating the role of the sympathetic nervous system in both experimental renal hypertension and human essential hypertension. The present study is concerned with the response of rats with renal hypertension to Dibenamine.

Methods. Mature, 200-300 g, Sprague-Dawley strain rats were made hypertensive by bilateral compression of the renal parenchyma according to the method of Grollman.[10] The procedure was carried out in two stages 4 to 6 weeks apart. The incidence of satisfactory hypertension obtained was never more than 30%.

Blood pressure was measured by a modification of the tail plethysmograph method of Byrom and Wilson,[11] using unanesthetized animals and a thin rubber plethysmograph membrane around the tail. A 10 mm occlusion cuff was employed and the plethysmograph was maintained at 42-44°C. This temperature provides adequate vasodilatation and obviates the necessity of heating the entire animal.[12] The rats were housed at a nearly constant temperature and blood pressure readings were made in the same room.

Dibenamine and its alcohol derivative, N,N-dibenzyl-ethanolamine, were administered orally in small lactose tablets. Oral administration was employed in order to avoid the difficulty of repeated intravenous injections and the local tissue damage which follows

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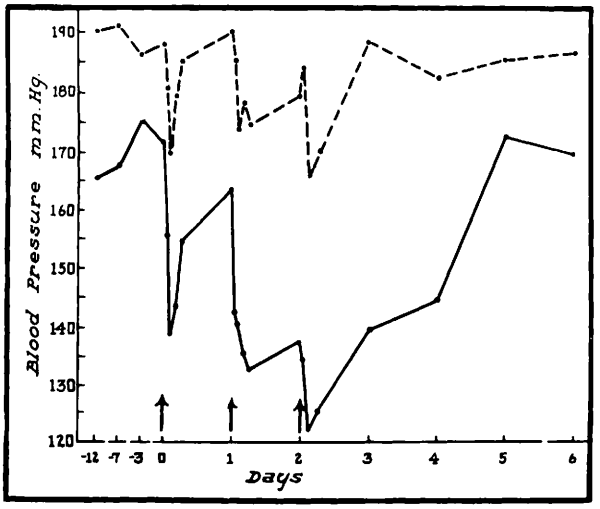

FIG. 1.
Blood pressure responses of renal hypertensive rats to Dibenamine; (•————•) average of 11 animals showing a cumulative effect and (•– – – –•) average of 6 animals with no cumulative effect. Arrows indicate oral administration of 100 mg/kg Dibenamine.

subcutaneous or intraperitoneal administration of the drug.[9]

A series of experiments in which the carotid blood pressure of rats under pentobarbital anesthesia was recorded directly demonstrated that a single oral dose of 100 mg/kg Dibenamine largely blocked and reversed the pressor response to small (1-2.5 μg/kg) intravenous injections of epinephrine but not the response to large doses. The incomplete adrenergic blocking action of this single oral dose of Dibenamine reached a maximum about 2 hours after administration and repeated daily administrations were found to have a moderate cumulative effect.

Results. Seventeen hypertensive rats were given oral doses of 100 mg/kg Dibenamine daily for 3 days and their blood pressure determined at frequent intervals for 6 days. The results of these experiments are graphed in Fig. 1. Two reasonably distinct types of response were observed and have been plotted separately. A total of 11 animals (65%) developed a progressive lowering of the blood pressure to approximately normotensive levels. In this group the average blood pressure did not return to pretreatment levels until 3 days after the last administration of Dibenamine. An early fall in pressure reaching a maximum in 2 to 6 hours occurred after each dose of Dibenamine. The maximum depressor effect tended to occur earlier after the first dose than after subsequent administrations.

The second group consists of 6 animals (35%) in which the blood pressure returned to within 20 mm Hg of the control value in less than 24 hours after each Dibenamine administration. A transient depressor action was noted after each dose of Dibenamine, but there was little or no cumulative effect. The immediate depressor action was also smaller in this group than in the group reacting with a more sustained fall in blood pressure. These animals had a somewhat higher average blood pressure prior to treatment than those which responded with a sustained reduction, but the significance of this factor is difficult to evaluate.

For comparative purposes the 17 hypertensive animals described above may be divided into two groups on the basis of duration of hypertension. Animals which had been hypertensive (blood pressure greater than 160 mm Hg) for over 2 months (5 rats) were considered as late hypertensives, and those hypertensive for less than 2 months (12 rats) were

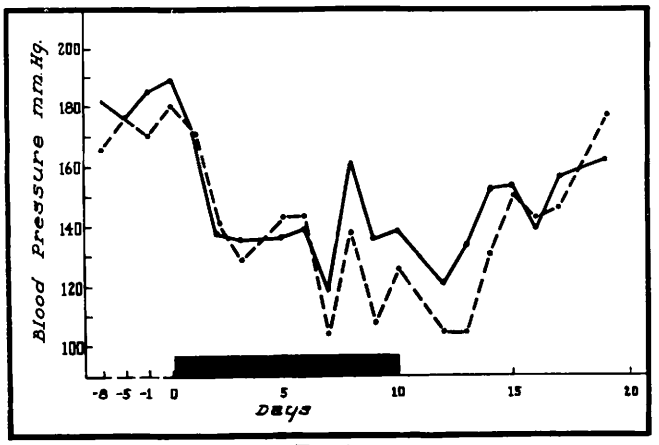

FIG. 2.

Blood pressure responses of renal hypertensive rats to daily oral administration of 100 mg/kg Dibenamine; (•————•) average of 5 animals and (•– – – –•) a representative individual. Period of Dibenamine administration indicated by black bar.

considered as early hypertensives. Two (40%) of the former and 9 (75%) of the latter developed a sustained lowering of the blood pressure with Dibenamine.

More prolonged experiments were carried out on 5 hypertensive rats previously shown to respond well to a single dose of Dibenamine. Single 100 mg/kg oral doses of the drug were given daily for 10 days. The blood pressure was determined just before each administration and for 10 days following the end of treatment. Averaged data from the 5 animals and the record of a representative individual are presented in Fig. 2. Although considerable fluctuation of the blood pressure occurred during the period of Dibenamine administration, the average pressure was lowered into the normal range for most of the period of treatment and for 3 to 4 days after the end of medication.

It is of interest that the blood pressures of all rats in this group tended to fluctuate together, indicating that the pressure in these animals was still capable of responding to environmental factors to at least a limited extent. The effect of external stimuli was confirmed by tests performed on the fifth day of Dibenamine administration. Two blood pressure readings were taken on this day—one the usual morning determination, the second while the animals were in a severely cramped position caused by moving the partitions in the round wire cages in which they were held during blood pressure determinations. The results of these readings are shown in Table I. In all cases the blood pressure was somewhat elevated by the factor of an environmental stress.

TABLE I.

Effect of Environmental Stress on Blood Pressure of Renal Hypertensive Rats Treated with Dibenamine.

Animal	Blood pressure	
	Normal reading	While cramped in cage
1	130	140
2	143	154
3	134	160
4	122	132
5	150	172
Avg	136	152

To determine whether the observed reductions in blood pressure were due to the adrenergic blocking action of Dibenamine, hypertensive rats were administered N,N-dibenzyl-ethanolamine, which differs from Dibenamine only in having the β-chlorine of the latter replaced by a hydroxyl group. Dibenzyl-ethanolamine is probably the compound to which Dibenamine is transformed in the

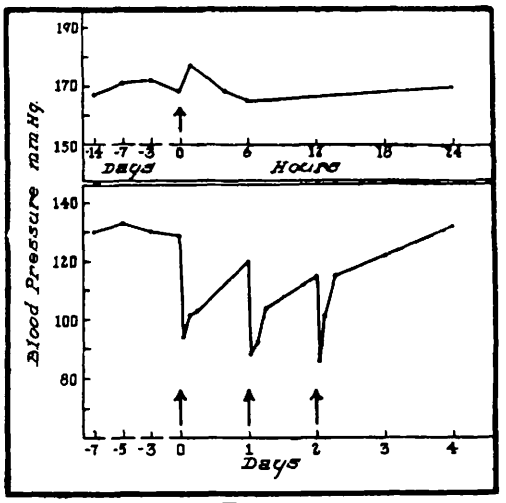

FIG. 3.

Upper record: Average blood pressure response of 5 renal hyptertensive rats to a single oral administration of 300 mg/kg N,N-dibenzyl-ethanolamine given at the arrow.

Lower record: Average blood pressure response of 6 normotensive rats to Dibenamine. Arrows indicate oral administration of 100 mg/kg Dibenamine.

body;[13] it has many pharmacological properties in common with Dibenamine, but has no demonstrable adrenergic blocking action. A single 300 mg/kg oral dose of this compound caused no reduction in blood pressure in 5 hypertensive rats (Fig. 3, upper).

Six rats which had been operated upon for compression of the kidneys, but which had not become hypertensive, were also administered 100 mg/kg doses of Dibenamine orally, with the results presented in Fig. 3 (bottom). Each administration of Dibenamine caused a transient lowering of the blood pressure, but no significant sustained reduction was induced.

In experiments on anesthetized cats the action of Dibenamine was found to be limited almost entirely to blocking excitatory effects of sympathetic nerve stimulation or sympathomimetic amines.[14] It does not significantly alter the pressor response to angiotonin† (Fig. 4).

[13] Nickerson, M., and Gump, W., to be published.

[14] Nickerson, M., and Nomaguchi, G. M., *J. Pharmacol. and Exp. Therap.*, 1948, **93**, 40.

† Kindly supplied by Drs. Helmer and Koehlstaedt of the Lilly Laboratory for Clinical Research, Indianapolis, Ind.

No significant side-effects of Dibenamine administration were noted in either the normotensive or hypertensive animals.

Discussion. The data presented above indicate that Dibenamine causes a decrease in the systemic arterial pressure of both normal and renal hypertensive rats. A hypostatic factor is probably involved in this hypotension inasmuch as the reductions obtained are considerably greater than are observed when Dibenamine is administered to anesthetized animals or normal recumbent humans.[9,15] The depressor effect is apparently dependent upon the adrenergic blocking action of the drug.

The Dibenamine-induced blood pressure reduction was greater and more sustained in most of the renal hypertensive animals than in the normotensives, but this does not necessarily imply that the difference represents increased sympathetic pressor activity. In the absence of stress, the blood pressure can be

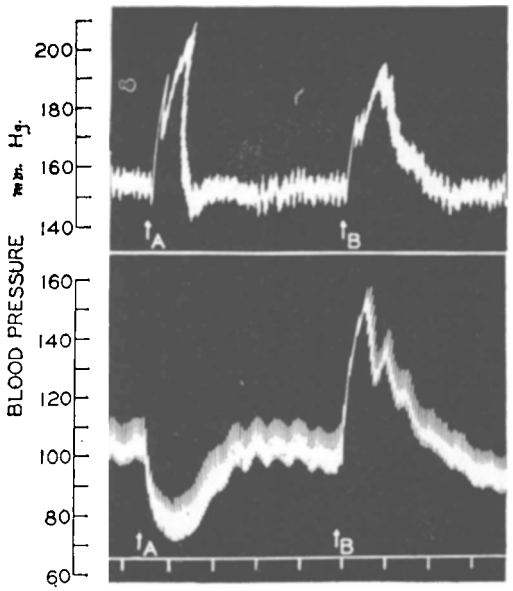

FIG. 4.

Blood pressure responses of cat to 1.5 μg/kg epinephrine (A) and 2 cat units angiotonin (B). Upper records before and lower records 45 minutes after the intravenous administration of 10 mg/kg Dibenamine. The apparent potentiation of angiotonin is due to the lowered initial pressure. Pentobarbital anesthesia. Time in minutes.

[15] Haimovici, H., and Medinets, H. E., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 163.

maintained within normal limits even in the complete absence of sympathetic nerve impulses. After complete sympathectomy in normal animals the blood pressure returns to essentially normotensive levels,[16-17] and a similar but less conspicuous adjustment appears to occur after sympathetic blockade with Dibenamine.[9] The possibility must therefore be considered that the ability of the vascular system to compensate for the absence of sympathetic impulses rather than the level of sympathetic activity may be of primary importance in determining the extent of the depressor response to sympathetic blockade. This possibility gains added weight when it is considered that the major difference between the responses of normal and renal hypertensive animals to Dibenamine is the much slower rate at which the blood pressure of the latter returns to pretreatment levels.

It is somewhat difficult to compare the results of this study with reports covering the use of other sympathetic blocking agents in experimental renal hypertension. Unfortunately, the work of Reed and coworkers[4] and Sapirstein and Reed[5] on the effects of yohimbine and 883F, respectively, on renal hypertension in rats involved only acute blood pressure changes following single injections of the drugs and the extent to which the recorded blood pressure changes were due to specific adrenergic blockade is difficult to evaluate. Bing and Thomas[18] observed no depressor action of single injections of 883F in dogs with chronic renal hypertension.

Jacobs and Yonkman,[3] and Wilburn *et al.*[19] studied the ability of yohimbine and Dibenamine, respectively, to produce a sustained lowering of the blood pressure of renal hypertensive dogs. In the latter report, the results were correlated with studies of the adrenergic blocking action of the drug. Both groups found some reduction in blood pressure, but were unable consistently to return the pressure to normotensive levels. In both studies the average reduction was somewhat less than observed in the present experiments on rats. As in the work reported above, they found wide variations in the response of different animals, and considerable fluctuations in blood pressure during the period of treatment.

None of the work reported to date allows a clear evaluation of the contention[4,5,20] that the sympatho-adrenal system is of importance in late but not in early renal hypertension. Although the number of animals in the experiments described above is not adequate to provide conclusive evidence on this point, the fact that a lower percentage of animals with hypertension of over 2 months' duration responded to Dibenamine with a sustained lowering of blood pressure does not support this view.

Summary. 1. Oral administration of 100 mg/kg Dibenamine causes some reduction of blood pressure in both normotensive and renal hypertensive rats. The fall is usually greater and more prolonged in hypertensive animals. 2. The fall in blood pressure is due to the adrenergic blocking action of Dibenamine and does not follow the administration of larger doses of the adrenergically inactive N,N-dibenzyl-ethanolamine. 3. In 65% of the hypertensive animals tested, repeated daily doses of Dibenamine produced a persistent, but somewhat variable lowering of the blood pressure. The pressure usually returned to pretreatment levels in 3 to 4 days after the last administration of Dibenamine. 4. The bearing of these data on postulated neurogenic factors in experimental renal hypertension is discussed, and it is concluded that they do not support the contention that a greater neurogenic factor is involved in late than in early renal hypertension.

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[17] Grimson, K. S., Wilson, H., and Phemister, D. B., *Ann. Surgery*, 1937. **106**, 801.

[18] Bing, R. J., and Thomas, C. B., *J. Pharmacol. and Exp. Therap.*, 1945, **83**, 21.

[19] Wilburne, M., Katz, L. N., Rodbard, S., and Surtshin, A., *J. Pharmacol. and Exp. Therap.*, 1947, **90**, 215.

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