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Comparative Antihypertensive Effects of a Diuretic, Reserpine, and Hydralazine in the Spontaneously Hypertensive Rat (41075)

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Abstract. In order to determine which components of a multiple drug mixture of antihypertensive agents were responsible for controlling the hypertension of SHR, the animals received graded doses of each component separately. Almost all of the antihypertensive activity was contained in hydralazine, whereas methyclothiazide or furosemide and reserpine exhibited essentially no antihypertensive activity even in high doses. This response of the SHR to the various antihypertensive agents contrasts with that of the human whose blood pressure responds well to all these drugs including diuretics and reserpine.

Previous studies in this laboratory (1, 2) have shown that continuous antihypertensive drug treatment of the Kyoto strain of the spontaneously hypertensive rat (SHR) will prevent the development of hypertensive target organ disease. In these studies a multidrug therapeutic regimen containing chlorothiazide, reserpine, and hydralazine was dispensed in the drinking water. A subsequent report from this laboratory (3) presented preliminary data on the relative antihypertensive effectiveness of the individual drugs used in the multidrug mixture. Hydralazine appeared to be substantially more effective in lowering blood pressure in the SHR than either chlorothiazide or reserpine. However, interpretation of these data was difficult because dose–response curves for each drug were not obtained and, in addition, the poor solubility of chlorothiazide in water made it impossible to determine the actual dose of the drug the rats had received. The present study is intended to determine in the SHR separate dose–response curves to three drugs as follows: a thiazide diuretic, reserpine, and hydralazine. Methyclothiazide was used in place of chlorothiazide because of its greater solubility in water. Also, a few animals received furosemide intraperitoneally.

Methods. In order to acclimate the rats to the recording technique their blood pressures were taken weekly for a period of 3 weeks. Recordings began when they were 3 months of age. The rats were males and females of the Charles River Strain of SHR. Systolic blood pressure only was determined by the tail cuff method in the restrained, unanesthetized state. Daily fluid intake was monitored in some of the experiments as described below.

The experiments with hydralazine and reserpine were repeated in part in order to define dosage more precisely. In the initial experiment hydralazine was made up in five strengths ranging from 5 to 80 mg/liter in tap water. Each strength was dispensed in the drinking water to groups of five SHR each for a period of 4 weeks. However, because the volume of fluid ingested was not monitored in the initial experiments it was not possible to calculate the 24-hr dosage consumed by the rats. Therefore, a second study was carried out which included monitoring of the volume of water ingested. In this second experiment two groups of three male SHR were given 20 or 80 mg/liter of hydralazine in the drinking water for a period of 4 weeks. The average daily dose of drug ingested over this period was 2.2 mg/kg/day for the 20 mg/liter concentration and 11.8 mg/kg/day for the 80 mg/liter strength. These daily doses correspond on a weight basis to 250 and 825 mg per day of hydralazine in a 70-kg man.

The initial study with reserpine utilized four strengths varying from 0.4 to 3.2 mg/liter. A second study with reserpine was carried out in which the volume of fluid in-

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gested was measured. The two dilutions of reserpine in drinking water used in the second study were 0.4 and 3.2 mg/liter, respectively. These dilutions provided average doses of 0.05 and 0.45 mg/kg/day, respectively. These doses correspond to daily doses of reserpine in a 70-kg man of 3.5 and 31.5 mg per day, respectively. In the second study both hydralazine and reserpine were made up from commercially available parenteral supplies which were already in solution.

Four dilutions of methyclothiazide in drinking water were used with three to four SHR receiving each dilution. Volume of fluid ingested was monitored daily so that a second study was not needed. The calculated average daily doses ingested ranged from 0.18 to 1.48 mg/kg/day for the four dilutions of methyclothiazide. These doses correspond to amounts ranging between 12.6 and 104 mg/day in a 70-kg man which are 1 and 10 times greater, respectively than the highest recommended daily dose in man. Three additional SHR were given single-dose intraperitioneal injections of 3 mg/kg furosemide daily for 2 weeks followed by twice daily injections of the same dose for an additional week. The same dosage schedule of furosemide was administered to six other SHR who were housed in metabolic cages. Twenty-four-hour urinary excretion of sodium was determined in these animals before and after each dose of furosemide.

Student's two-tailed t test for independent observations was used to analyze the significance of the differences in systolic blood pressure at the second and fourth post-treatment weeks for each drug and dose regimen. The percentage differences for each rat from his own control was determined and the group averages of the percentage change of each control group lar calculations were made for each of the untreated control groups. The mean percentage change of each control group was compared to the mean percentage change of each of the corresponding treatment groups to determine the significance of the differences.

Results. *Hydralazine.* The fall in systolic blood pressure was correlated with the strength of hydralazine in the drinking water (Table I). Minimal falls in blood pressure which were not significant as compared to control occurred with the 10 and 20 mg/liter strengths. However, 40 and 80 mg/liter concentrations of hydralazine in the drinking water resulted in falls of blood pressure of 24 and 31% respectively. The latter changes were highly significant ($P < 0.001$). In the second experiment where dosage ingested was determined the 2.2 mg/kg/day (20 mg/liter strength) dose resulted in a small rise of blood pressure while the 11.8 mg/kg/day (80 mg/liter strength) dose lowered the blood pressure by an average of 20% ($P < 0.01$).

Reserpine. By contrast, there was little or no reduction in systolic blood pressure following reserpine in any dose (Table I). In the first experiment the greatest fall was only 4% which occurred with the highest strength of reserpine of 3.2 mg/liter. In the second study the same concentration of reserpine in the drinking water raised the blood pressure slightly but insignificantly. This concentration of reserpine corresponds to a dose of 0.45 mg/kg/day which is equivalent of 31.5 mg per day in a 70-kg man.

Methyclothiazide. The reduction of systolic blood pressure following methyclothiazide was slight or absent and the changes observed were not dose related. For example, the blood pressure changes at the fourth week of treatment indicated essentially no change in blood pressure for the animals taking 0.18 and 0.4 mg/kg/day of the drug. A slightly greater reduction in blood pressure of 7.5% was observed in the animals who received 0.80 mg/kg/day of methyclothiazide ($P < 0.05$). However, the animals receiving the highest dose of the diuretic of 1.48 mg/kg/day exhibited a slight rise in blood pressure. Thus, no consistent or dose-related changes were observed after the diuretic and none of the reductions were comparable to those seen with hydralazine.

Furosemide. Because of the possibility that the negative results observed following methyclothiazide might have been due to poor absorption from the gastrointestinal tract three additional SHR were given

TABLE I. CHANGES IN BLOOD PRESSURE DURING TREATMENT WITH HYDRALAZINE, RESERPINE, OR METHYCLOTHIAZIDE

Drug	mg/liter	mg/kg/day	No. of SHR	Pretreatment systolic blood pressure (mm Hg)	Change in blood pressure percentage	
					at 2 wks.	at 4 wks.
Hydralazine	0"		5	183	1.2	5.0
	5		5	177	3.2	3.6
	10		5	174	−6.2	2.0
	20		5	176	−8.2	−4.0
	40		5	176	−24.4*	−20.2*
	80		5	175	−31.0**	−29.2**
Hydralazine	0"		6	175	5.3	6.3
Second study	20	2.2	3	186	6.3	8.7
	80	11.8	3	171	−20.0*	−20.7*
Reserpine	0"		5	181	10.0	12.2
	0.4		5	186	−0.4	2.2
	0.8		5	179	−0.8	−1.8
	1.6		5	179	2.6	1.0
	3.2		5	173	−4.0	−4.4
Reserpine	0"		4	180	1.8	4.0
Second study	0.4	0.05	3	175	1.7	0.3
	3.2	0.45	3	174	7.0	3.0
Methyclothiazide		0"	6	175	5.3	6.3
		0.18	4	178	−0.8	1.8
		0.40	3	168	−3.0	−1.7
		0.80	4	185	−5.0	−7.5
		1.48	3	163	4.3	5.7

Note. Note that above P values do not refer to the significance of the difference between pre- and post-treatment blood pressure but rather to the difference between mean percentage change of the untreated control group and the corresponding treated group (values shown in table).

" Indicates control group.

* $P < 0.01$ using two-tailed t test.

** $P < 0.001$ using two-tailed t test.

furosemide intraperitoneally for 3 weeks as described under Methods. As compared to the untreated controls there were no significant differences in blood pressure or body weight during the 3-week treatment periods. The mean change in blood pressure in the furosemide-treated animals at 3 weeks was an increase of 2.9% over the pretreatment control.

The 24-hr urinary excretion of sodium was determined in six SHR before and after the administration of furosemide given intraperitoneally. By the one-tail Student's paired t test analysis, sodium excretion increased significantly ($P = 0.02$) after the 1-mg twice daily dose of furosemide. The mean 24-hr excretions of sodium were 2.56 mEq/kg/24 hr prior to treatment, 2.78 mEq/kg/24 hr during treatment with furosemide 1 mg daily, and 4.63 mEq/kg/24 hr after treatment with 2 mg.

Weight changes during treatment varied depending on the drug administered. Hydralazine was associated with a gain of weight. For example, hydralazine in a dose of 11.8 mg/kg/day was associated with a weight gain 13% greater than the untreated controls after 1 week of treatment and 10% greater after 2 weeks of treatment. Reserpine, on the other hand, resulted in essentially no change in weight. All doses of methyclothiazide, however, were associated with a relative loss of weight as compared to the untreated controls. Weight gain averaged 5% greater in the control group than in the treated animals during

the first 2 weeks of treatment. After furosemide, body weight increased 5.2% after 3 weeks as compared to an 8.5% increase in the untreated controls.

Discussion. Previous studies in this laboratory demonstrated that the various types of cardiovascular complications occurring in the untreated SHR are preventable by antihypertensive drug treatment (1). These complications included mesenteric arteritis, nephrosclerosis, cerebral hemorrhage, and left ventricular hypertrophy, dilatation, and failure. Treatment consisted of a combination of reserpine, chlorothiazide, and hydralazine in the drinking water. The readings taken indirectly in the tail of the warmed rat during recovery from light ether anesthesia indicated marked reduction of blood pressure. Treatment also was associated with a considerable increase in longevity; the average life span of the treated SHR increased 36% as compared to the untreated SHR. Also, 11% of the treated SHR as compared to none of the untreated animals survived to 3 years of age (2).

In a later experiment an attempt was made to determine the relative antihypertensive effects of the various drugs that made up the components of the antihypertensive mixture. The drugs were given individually in the drinking water (3). In these experiments the blood pressure of the hydralazine-treated SHR was reduced over twice as much as that of the reserpine- or chlorothiazide-treated animals. However, the results were not conclusive because only one dose of each drug was tested. The dose chosen was the one that had been used in the original drug mixture; it was possible that a more effective dose of one of the drugs than of the others was contained in the mixture. For this reason the experiment was repeated utilizing a range of dosage which would preclude the possibility that differences observed between drugs could be due to choice of dose rather than to differences in the effectiveness of different drugs.

Methyclothiazide rather than chlorothiazide was used in the present experiments because of the former's greater solubility in water at therapeutic dose levels. However, despite its greater solubility methyclothiazide was as ineffective in lowering the blood pressure of the SHR as had been chlorothiazide in the prior experiments (3). Furthermore, furosemide given intraperitoneally also failed to lower blood pressure. The lack of antihypertensive effectiveness did not appear to be due to a failure of diuresis because there was a loss of weight relative to the controls and a significant saluresis following the 2 mg per day dose of furosemide. It would appear that the blood pressure of the SHR is resistant to sodium and volume depletion. Ueda and associates (4) found that the blood pressure of the SHR remained elevated despite daily intraperitoneal injections of trichlomethiazide. This study has shown that SHR also are resistant to the antihypertensive effects of reserpine. In addition several other investigators have reported that the hypertensive rat is nonresponsive to α-methyldopa (5, 6).

The enhanced antihypertensive effectiveness of hydralazine and relatively minor effectiveness or ineffectiveness of reserpine and methyclothiazide are the opposite of those seen in man where both thiazides and reserpine are considered to be at least as effective if not more effective antihypertensive agents than is hydralazine. The effective antihypertensive dosages of hydralazine in the rat were on a weight basis only slightly above the therapeutic range in the human being equivalent to 400 to 825 mg per day in a 70-kg human. Masson and Corcoran (7) also controlled the hypertension of rats with experimental renovascular hypertension using hydralazine 40 mg/liter in the drinking water. The greater antihypertensive effectiveness of hydralazine cannot be ascribed to choice of dosage which, in fact, was much higher in terms of equivalent human doses in the case of reserpine and of methyclothiazide than in the case of hydralazine. The highest dose of methyclothiazide considerably exceeded the customary human dosage of 5 to 10 mg per day being equivalent to 104 mg per day for a 70-kg man. Also, the equivalent doses of reserpine were 10 to 100 times higher than those used in the human although still

greater doses of 1–10 mg/kg/day have been reported to produce a dose-related decrease in blood pressure in the SHR (8).

While the SHR provides a valuable model for the study of hypertension, experimental results obtained in the SHR should be interpreted with caution with respect to man. In the present study differences were observed between the SHR and man in responsiveness to various antihypertensive agents. The differences could be due to species variation in responsiveness to the drugs, per se, or they might represent marked intraspecies differences in effective dosage on an equivalent weight basis. These species differences should be kept in mind when working with the SHR as an experimental animal in the drug treatment of hypertension.

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