Action of Acetazolamide-Reserpine Co-treatment on Metacorticoid Hypertension.* (26372)

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The clinical effectiveness of chlorothiazide and its congeners in treatment of hypertension has been demonstrated by a majority of the investigators in this field. The history, modes of action and efficacy of these drugs as antihypertensives have been reviewed extensively at two recent Hahnemann symposia(1,2). In contrast, the role of other types of diuretic drugs in treatment of hypertension has not been thoroughly explored. Although some reports indicate that acetazolamide does not share antihypertensive activity(3,4), other studies, such as the finding that acetazolamide augments antihypertensive action of mecamylamine(5), suggest that this potent carbonic anhydrase inhibitor may deserve a place in hypertension therapy.

The purpose of the present investigation was to study the effect of acetazolamide alone and in combination with reserpine on blood pressure of metacorticoid hypertensive rats. Chlorothiazide alone and in combination with reserpine was included for comparative purposes. The action of acetazolamide-reserpine co-treatment on development of left ventricular hyperthrophy was also assessed in this disease model.

Methods and materials. Metacorticoid hypertension was produced in male albino Sherman rats by a microcrystalline aqueous suspension of desoxycorticosterone acetate (DCA), 10 mg/0.2 ml, injected subcutaneously 5 days a week for 3 weeks. During this time, the animals were given 0.85% sodium chloride drinking fluid *ad libitum* and were maintained on a basal diet of Purina Laboratory Chow. In the metacorticoid period (the interval of time following corticoid treatment), the rats drank tap water. Body weights of the animals at start of the DCA-saline regimen ranged from 60-89 g. Indirect systolic blood pressure readings were taken at weekly intervals using the foot densitometer method(6). All indirect readings were taken approximately 2 hours prior to dosing. The compounds employed, routes of administration and dosage schedules are shown in Fig. 1 and 2. Gavage volumes were kept constant at 2.5 ml/kg body weight. Gavage treatments were administered once/day, 5 days/week. In comparing the effects of gavage and drug diet treatments, it should be noted that the rats on a 0.001% drug diet ingested approximately 1 mg drug/kg body weight/day. Eighty-four rats, 12/group, were employed in the first experiment (Fig. 1) and 4/group were autopsied at weekly intervals after 1, 2 and 3 weeks of treatment. In the second experiment (Fig. 2), 30 rats were employed, 6/group, and all animals were terminated after 3 weeks of treatment. Cardiac hypertrophy in metacorticoid hypertensive rats was characterized by determining ventricular ratios; *i.e.*, weight of left ventricle and whole septum/weight of the remaining free part of right ventricle.

Results. The average antihypertensive effects of acetazolamide, reserpine, and different combinations of the 2 drugs are shown in Fig. 1. Evaluation of these data by analysis of variance revealed that the effect of acetazolamide on systolic blood pressure of hypertensive rats was significant ($P<.01$). The regression of blood pressure on the dosage of reserpine employed (0-0.001% in diet) was likewise significant at the 1% probability level. The regression coefficient equalled −2.18 mm Hg/mg reserpine/kg of food. Combined administration of acetazolamide and reserpine resulted in algebraic summation of

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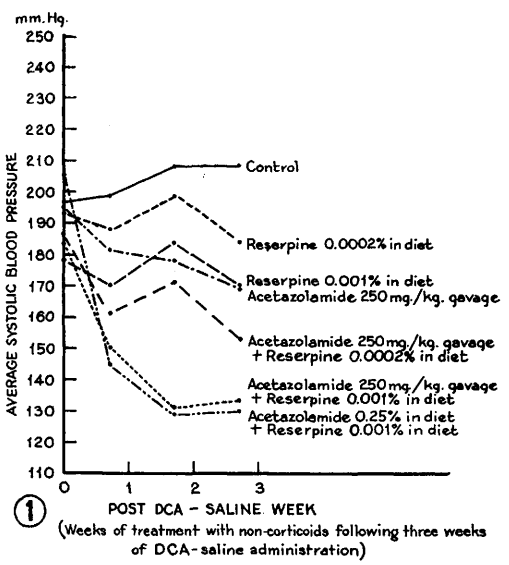

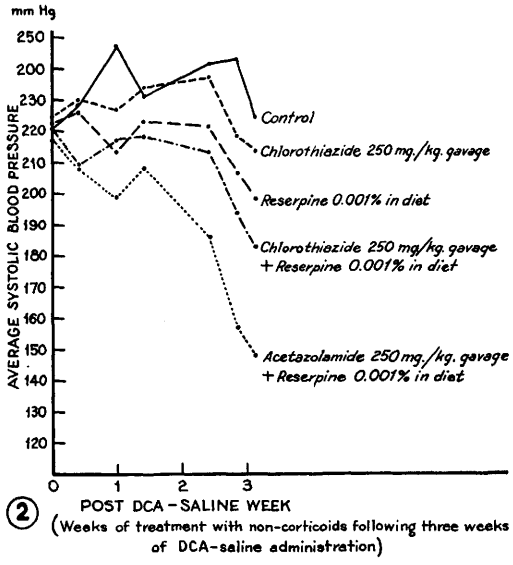

FIG. 1. Effect of different combinations of acetazolamide and reserpine on blood pressure of hypertensive metacorticoid rats. At 5% probability level, all blood pressures of treated animals were significantly less than control.

FIG. 2. Effect of combinations of chlorothiazide, acetazolamide and reserpine on blood pressure of hypertensive rats. At 5% probability level, all blood pressures of treated animals except those receiving chlorothiazide alone were significantly less than control.

the effects of the 2 drugs on systolic blood pressure. The difference between acetazolamide-reserpine co-treatments and either reserpine or acetazolamide alone was significant ($P<.05$). The interaction between number of weeks and treatments was not significant with any of the drug regimens.

Fig. 2 compares the effect of acetazolamide-reserpine and chlorothiazide-reserpine co-treatments on blood pressure of metacorticoid hypertensive rats. The effect of chlorothiazide alone on systolic blood pressure was not significant ($P>.05$) whereas reserpine alone showed a significant action. The greater reduction in blood pressure of the hypertensive rats which received a combination of chlorothiazide and reserpine as compared to the animals which received only reserpine is attributed to an additive effect since the interaction between the 2 drugs was not significant. Acetazolamide-reserpine co-treatment was associated with a significant reduction in blood pressure from control and from the means of all other treatments ($P<.05$).

The typical unilateral left cardiac hypertrophy associated with metacorticoid hypertension can be characterized by means of a ventricular ratio; *i.e.*, weight of left ventricle and whole septum/weight of right ventricle. In examining the data of the present study by analysis of variance, no statistical evidence was found for concluding that ventricular ratios changed in relation to number of weeks on drug treatment. Average ventricular ratios of treated and untreated hypertensive rats are shown in Table I. For comparative purposes, mean ventricular ratios of 18 normotensive control rats are also pre-

TABLE I. Ventricular Ratios (Weight of Left Ventricle and Whole Septum/Weight of Right Ventricle) of Untreated and Treated Hypertensive Rats and Untreated Normotensive Rats.

Treatment	Mean	95% confidence limits
Hypertensive control	4.23	4.00-4.46
Acetazolamide (A), 250 mg/kg	3.86	3.63-4.09
Reserpine (R), .001% diet	3.77*	3.54-4.00
A, 250 mg/kg + R, .001% diet	3.67*	3.44-3.90
Reserpine, .0002% diet	3.85	3.62-4.08
A, 250 mg/kg + R, .0002% diet	3.78*	3.55-4.01
A, .25% diet + R, .001% diet	3.78*	3.55-4.01
Normotensive controls	3.38*	3.19-3.57

* Significantly less than hypertensive control at 5% probability level.

sented. As may be noted, ventricular ratios of the metacorticoid rats receiving acetazolamide-reserpine co-treatment were significantly less than ratios of the control hypertensive rats. Analysis of the data by means of Dunnett's multiple comparison procedure (7) revealed that all of the mean ventricular ratios of the treated rats less than 3.84 are significantly smaller than the mean of 4.23 for the untreated hypertensive control rats.

Discussion. The absence of a significant reduction in arterial blood pressure following repetitive administrations of chlorothiazide to hypertensive rats attests to the difficulty encountered experimentally in demonstrating an antihypertensive action for this type of compound. The summary table of Sturtevant(8) indicating that chlorothiazide lowers the blood pressure of metacorticoid rats cannot be assessed since supporting data were not presented. The augmented antihypertensive action of reserpine-chlorothiazide co-treatment over that produced by either drug alone is in accord with the usual clinical finding(1,2). The pronounced antihypertensive action of acetazolamide-reserpine combination treatment was striking. The sustained hypotension encountered following repetitive co-treatments was of a greater magnitude than that previously recorded under identical experimental conditions after repetitive administrations of ganglioplegic and vasodilator agents. The interesting finding that acetazolamide-reserpine co-treatment tended to obviate development of left ventricular cardiac hypertrophy indicates that the combination therapy exerted a desirable effect on both the hemodynamic and the morphologic disturbances associated with metacorticoid hypertension.

Summary. An increased antihypertensive action of a combination of reserpine and acetazolamide over that produced singly by either drug was demonstrated in metacorticoid hypertensive rats. Acetazolamide alone had a moderate antihypertensive action, and the enhanced reduction in blood pressure following co-treatment was an additive rather than a potentiated effect. The typical hypertensive left ventricular cardiac hypertrophy was significantly reduced in the metacorticoid rats receiving acetazolamide-reserpine.

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Tolerance of Skin Homografts Induced in Adult Mice by Multiple Injections of Homologous Spleen Cells.* (26373)

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It seems now well established that acquired immunological tolerance of tissue homografts can be induced in adult mice of certain strains closely related genetically. For example, tolerance of male skin isografts was obtained in adult female mice of C57Bl (Subline 1) and A strains by a single intravenous injection of

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