Method for the Evaluation of Antihypertensive Agents, Including Thiazide-Type Compounds

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A system which allows continuous automatic measurement of systolic blood pressure in the unanesthetized rat, which appears to be useful in the assessment of antihypertensive agents of the thiazide type, is described. Results obtained with water and hydrochlorothiazide are presented, and the dissociation of diuretic and antihypertensive activity is discussed.

WITH THE introduction of the thiazides as clinical antihypertensive agents, it became evident that traditional antihypertensive screening procedures (*e.g.*, acute screens in anesthetized and unanesthetized normotensive and hypertensive rats and dogs) were inadequate. With the exception of a class of nondiuretic benzothiadiazines, these compounds do not produce an acute fall in the blood pressure of anesthetized normotensive dogs (1). In addition, it is generally accepted that similar results are obtained in hypertensive animals, although in at least one screening procedure presently employed (2) such compounds are evaluated on the basis of the generally statistically insignificant acute reduction in blood pressure of unanesthetized hypertensive rats. Maxwell and McLusky (3) report that hydrochlorothiazide does elicit acute hypotension in conscious normotensive dogs; however, the most desirable compounds may well be those which do not possess acute hypotensive activity in unanesthetized normotensive animals. On the other hand, it has been demonstrated (4) that the antihypertensive effect of chlorothiazide can be demonstrated upon prolonged administration to hypertensive rats. For these reasons, it appeared that a screening procedure for the detection of this type of antihypertensive activity was both possible and desirable.

METHODS

Hypertension was produced in male Sprague-Dawley rats, from 200 to 250 Gm. body weight, by the bilateral figure-of-eight method of Grollman (5). The rats were considered to be hypertensive when systolic blood pressure was in excess of 150 mm. Hg, at which time they were from 300 to 400 Gm. body weight. Unoperated rats of the same size had blood pressures of about 123 mm. Hg. Hydrochlorothiazide[1] was administered orally at doses of 1, 3.1, 10, and 31 mg./Kg. as an aqueous suspension, the concentration of which was adjusted such that each rat received 10 ml./Kg. The rats were allowed food and water *ad libitum* until 1.5 hr. prior to drug administration.

During those days when blood pressures were determined, the rats were individually housed in plastic holders (Fig. 1). Water at a constant temperature of 38° was circulated through the hollow base of the holders. A unit of 12 holders was employed. Rats were placed in the holders 1.5 hr. prior to drug administration and remained there for a total of 7.5 hr. The tail of each rat was passed through an inflatable Gaertner type cuff attached to a manifold, and a microphonic pick-up[2] was firmly attached to the tail distal to the cuff with an adhesive tape sleeve.

A system which permitted automatic measurement and recording of systolic blood pressure, incorporating the Infraton,[3] was constructed; a simplified diagram of this system is illustrated in Fig. 2. The control apparatus consists of a synchronous clock timer,[4] two of the rotating cams of which were rewired to activate a 115 v. a.c. pulse once per minute. These cams were spaced 30° of arc apart. The notch closing the relay on one cam was filed down to give an impulse lasting approximately 2.5 sec. This cam activates a 24-point stepper and a solenoid[5] which controls a 300 mm. Hg air supply. The second cam activates a double-pole double-throw relay which controls the chart paper and exhaust solenoid.[5]

One complete cycle of this system can be described as follows. (*a*) The first cam activates a 2.5-sec. impulse which switches the stepper to the next position (every other one of which is connected to a probe) and turns on the air supply, providing 300 mm. Hg pressure to the cuffs and the Infraton. This pressure is bled off through the Infraton; the duration of the operation is 55 sec., providing ample time for the pressure to slowly drop from 300 to 100 mm. Hg. During this operation pulse information provided by a probe and pressure information provided by the Infraton are superimposed on a single recorded[6] trace. Systolic blood pressure is that point at which pulses begin to appear. (*b*) At the end of this 55-sec. period, the second cam activates an impulse to the double-pole double-throw relay, the switching of which closes the circuit of the exhaust relay and opens the circuit to the chart motor. The relay re-

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[1] Kindly supplied by Dr. Frederick F. Yonkman, Ciba Pharmaceutical Co., Summit, N. J.

[2] Model D2 Miniature Arterial Pickup, Beckman Instruments, Spinco Division, Palo Alto, Calif.

[3] Electrical Manometer and Signal Divider, Beckman Instruments, Spinco Division, Palo Alto, Calif.

[4] Phipps and Bird, Richmond, Va.

[5] Model 94280-1, Central Scientific Co., Chicago, Ill.

[6] Model 5 Grass polygraph, Grass Instrument Co., Quincy, Mass.

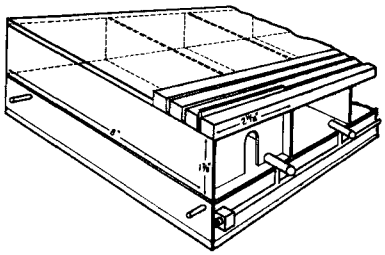

Fig. 1.—Plastic rat holder.

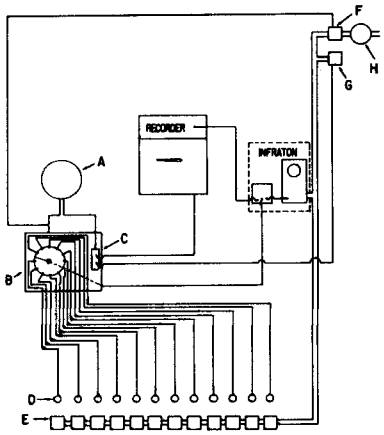

Fig. 2.—A system for automatic continuous measurement of systolic blood pressure in unanesthetized rats. Key: A, timer; B, stepping relay; C, double-throw double-pole relay; D, pickups; E, cuffs; F, air solenoid; G, exhaust solenoid; H, air regulator.

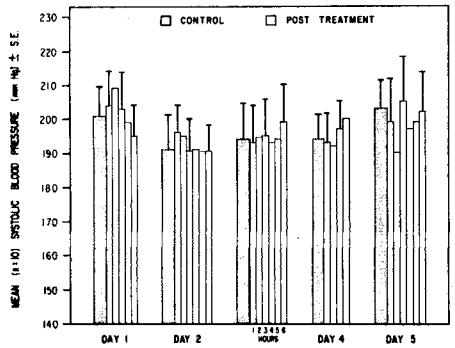

Fig. 3.—Influence of water (10 ml./Kg., p.o. once daily) on the systolic blood pressure of hypertensive rats.

mains in this position for 1 full min. to allow complete exhaustion of the system. (*c*) Five seconds after the initiation of operation (*b*), the first cam again provides an impulse which switches the stepper to a blank position and opens the air supply solenoid for 2.5 sec. However, the exhaust solenoid remains open, and the system is not pressurized. (*d*) One minute after the initiation of operation (*b*), the second cam again activates an impulse which closes the chart paper circuit and opens the exhaust solenoid circuit. Five seconds later operation (*a*) is initiated. Control blood pressure is an average of three blood pressure determinations made during a 1.5-hr. period prior to drug administration; thereafter, blood pressure was determined at hourly intervals. In this manner, it is possible to evaluate both the acute and chronic (control measurement 24 hr. after previous dose) effects of the agent of interest.

The diuretic activity of hydrochlorothiazide was assessed at various doses in normotensive rats by method II of Wiebelhaus *et al.* (6).

Standard deviations were calculated from the following data: (*a*) between rats, from the mean control blood pressure values obtained in each rat in each group each day prior to drug administration, (*b*) within rats, from the individual blood pressures determined before and after administration of water to the control rats on day 1, and (*c*) from day to day, from the mean control rat blood pressures obtained prior to administration of water on days 1 through 5.

RESULTS

The influence of water, 10 ml./Kg., or hydrochlorothiazide 1.0, 3.1, 10, and 31 mg./Kg. on the systolic blood pressure of hypertensive rats is illustrated in Figs. 3, 4, 5, 6, and 7, respectively. None of the blood pressure values obtained after the administration of water were significantly ($p < 0.05$) different from the day 1 control pressure; the same pattern was seen following the administration of 1.0 mg./Kg. of hydrochlorothiazide. Following the administration of larger doses of hydrochlorothi-

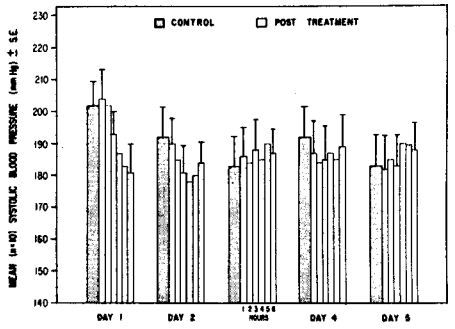

Fig. 4.—Influence of hydrochlorothiazide (1 mg./Kg., p.o. once daily) on the systolic blood pressure of hypertensive rats.

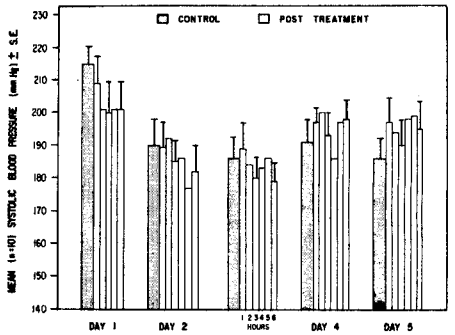

Fig. 5.—Influence of hydrochlorothiazide (3.1 mg./Kg., p.o. once daily) on the systolic blood pressure of hypertensive rats.

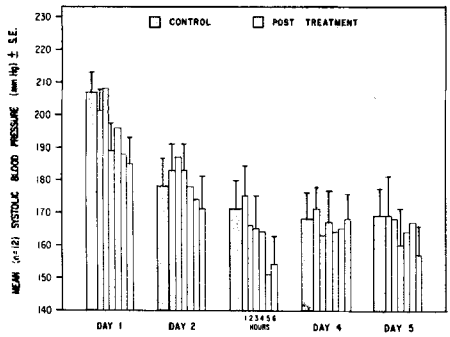

Fig. 6.—Influence of hydrochlorothiazide (10 mg./Kg., p.o. once daily) on the systolic blood pressure of hypertensive rats.

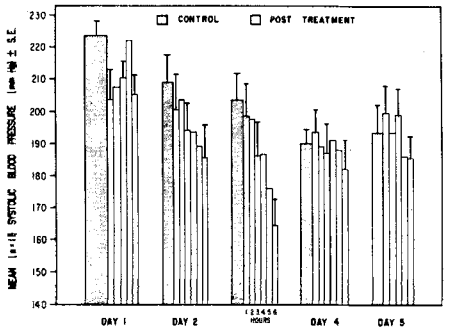

Fig. 7.—Influence of hydrochlorothiazide (31 mg./Kg., p.o. once daily) on the systolic blood pressure of hypertensive rats.

TABLE I.—STANDARD DEVIATIONS[a]

Variation	S.D. For Single Measurements	S.D. As Measured[b]
Between rats	18.2	8.2
Within rats	10.6	10.6
Between days	32.9	19.0

[a] mm. Hg. [b] Control blood pressures were averages of three determinations rather than single measurements.

TABLE II.—INFLUENCE OF ORALLY ADMINISTERED HYDROCHLOROTHIAZIDE[a] ON URINE VOLUME IN NORMOTENSIVE MALE RATS

Dose, mg./Kg.	n	ml. Urine/ Kg./5 hr.	% Increase in Urine Vol. over Controls
Control	6	14.4	
0.1	6	27.5	91.0
1.0	6	40.3	179.9
5	6	40.2	179.2
10	6	41.4	187.5
50	6	40.9	184.0

[a] Contained in 0.9% saline, 2.5 ml./100 Gm. body weight.

azide, control blood pressures on days 2, 3, 4, and 5 were always significantly ($p < 0.05$) lower than on day 1 (with the exception of the day 2 control pressure at 31 mg./Kg.). Although significant reductions in blood pressure were obtained, normotensive levels were not restored at any dose. Significant reductions in systolic blood pressure were observed following the first dose of hydrochlorothiazide at the 10 and 31 mg./Kg. dose levels. In addition, a significant acute reduction was observed on day 3 at the 31 mg./Kg. dose level. Hence, while significant acute responses to hydrochlorothiazide were observed at the two highest dose levels, the acute effects were not consistent.

The various standard deviations calculated from the data obtained in these experiments are shown in Table I. Using these figures it was calculated that it would be necessary to use seven rats to detect an acute 10% change in blood pressure on any given day with 95% confidence ($\alpha = 0.05$), while 16 rats would be required to detect a change of the same magnitude from the day 1 control value on any subsequent day during a 5-day period with the same certainty.

Results of the diuretic studies are summarized in Table II. The data indicate that 1 mg./Kg. of hydrochlorothiazide is the maximally effective dose; higher doses do not result in significantly greater urine volumes.

DISCUSSION

The system described can be employed in the continuous determination of systolic blood pressure in the rat, and the procedures employed do not themselves influence this blood pressure significantly.

The results indicate that at doses of 3.1 mg./Kg. and above, the antihypertensive effect of hydrochlorothiazide is clearly demonstrated. Hence, the system is suitable for the screening of antihypertensive agents having a similar pattern of activity as well as those eliciting an acute response. None of the dose levels employed resulted in blood pressure reduction to normotensive levels; however, in view of the findings of Weller and Haight (4), it appears that such levels might be obtained upon more prolonged drug administration.

It is of interest that the results obtained at the 1 mg./Kg. dose level suggest separation of diuretic and antihypertensive activity, at least over the time interval investigated. Urine volume studies indicated that the maximum diuretic effect of hydrochlorothiazide is seen at 1 mg./Kg.; yet, although there is a consistent trend toward reduced blood pressure, the same dose did not decrease blood pressure significantly. This observation may offer an explanation for the findings of Tobian and Coffee (7), who reported that no blood pressure reduction was observed in hypertensive (narrowed renal artery) rats following administration of methyclothiazide at a dose level sufficient to elicit a diuretic response during a 5-week period of drug administration.

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