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THE EFFECTS OF LOADING RATS WITH INTRAVENOUS ISOTONIC SODIUM CHLORIDE ON THE RENAL RESPONSE TO DIURETICS

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The effect of a number of diuretic agents was investigated on rats with different degrees of expansion of extracellular fluid volume. With moderate saline loading aminoisometradine and chlorthiazide were the most efficient diuretics. However, with a more severe saline load aminoisometradine and mersalyl had the greatest effect while chlorthiazide had no significant diuretic activity. The action of acetazolamide seemed independent of the saline load. Potassium chloride showed no diuretic activity. Injecting the drugs in pairs resulted in probable potentiation only with acetazolamide plus chlorthiazide. Since all the diuretics were used in doses which gave maximal effects, the summation of the effects of the other pairs of diuretics suggested different sites of action.

This investigation was to see whether certain diuretics acted as efficiently in animals loaded with saline as in normal animals. Saline loading expands the extracellular fluid space and, since diuretics are commonly used on subjects whose extracellular fluid compartment is expanded by the pathological retention of salt and water, these experiments should be of interest.

METHODS

All experiments were performed on unanaesthetized adult albino rats weighing between 175 g. and 210 g. Solutions of 5% dextrose or of 0.9% NaCl were infused continuously at a rate of either 0.075 or 0.75 ml./min. through cannulae inserted into the external jugular vein. This technique and the analytical procedures used have been described previously by Keeler and Schnieden (1958).

During the infusion at 0.075 ml./min. (slow infusion) urine was collected for 15 to 20 min. starting after about 100 min. from the beginning of the infusion. During the infusion at 0.75 ml./min. (fast infusion) urine was collected for 10 min. starting after 20 min.

Diuretic Agents.—Acetazolamide, aminoisometradine, and chlorthiazide were injected intravenously 20 min. before commencing the collection of urine. These drugs were given as 2.5% solutions in doses of 5 mg./100 g. body weight. Mersalyl was injected intravenously as a 0.5% solution (1 mg./100 g. body weight) 1 hr. before starting the collection period. Potassium was usually infused continuously as a 20 m.equiv./l. solution of KCl, but in some experiments KCl was injected as a 150 m.equiv./l. solution (75 μ.equiv./100 g. body weight) 2 min. before starting the collection period.

The fluid load retained by the animals was estimated by noting the change in weight during the infusion.

RESULTS

Table I shows the effects of various diuretics on the excretion of sodium, potassium, and water by normal rats during an infusion of 5% dextrose solution at 0.075 ml./min. or of 0.9% NaCl solution at a rate of either 0.075 or 0.75 ml./min. Whereas the infusion of 5% dextrose solution was not accompanied by retention of fluid (as indicated by mean % body weight change, Δ wt.), the infusion of 0.9% saline led to considerable fluid retention. The output of sodium by control animals receiving fast infusions of saline was nearly 40 times greater than the output of sodium by control animals receiving 5% dextrose solution. The excretion of potassium was 5 times greater and the inulin clearance (glomerular filtration rate) 1.9 times greater.

The Effect of Diuretics

Potassium.—The infusion of 20 m.equiv./l. KCl under the three conditions of fluid loading resulted in changes in sodium output ($U_{Na}V$) and urine flow (V) which were not statistically significant. The expected rise in potassium output (U_KV) was present (Table I, Fig. 1).

In view of this result, the effect of an acute loading with 75 μ.equiv. KCl/100 g. body weight was investigated during a fast saline infusion. Although the plasma potassium level was suddenly raised from a mean of 4.85 to 7.5 m.equiv./l., there was no statistically significant change in the excretion of either sodium or water, sodium output being 22.9 μ.equiv./min. and urine flow 159 μl./min.

Acetazolamide.—Fig. 1 and Table I show that the injection of acetazolamide during the infusion

TABLE I

THE EFFECTS OF DIURETICS UNDER DIFFERENT CONDITIONS OF FLUID LOADING

The table shows effects on urine flow (V, μl./min.), sodium and potassium excretion ($U_{Na}V$, U_KV, μ.equiv./min. respectively), inulin clearance (G.F.R., ml./min.) and mean % body weight change (Δ wt.). All results are expressed in terms of 100 g. body weight, and are mean values ± standard error.

	(a) 5% Dextrose (0·075 ml./min.) Δ wt.= −0·31±0·15					(b) Saline (0·075 ml./min.) Δ wt.=2·1±0·53					(c) Saline (0·75 ml./min.) Δ wt.=7·3±0·41				
	No. of Rats	V	$U_{Na}V$	U_KV	GFR	No. of Rats	V	$U_{Na}V$	U_KV	GFR	No. of Rats	V	$U_{Na}V$	U_KV	GFR
Controls	16	45·0 ± 2·6	0·60 ± 0·07	0·36 ± 0·05	0·92 ± 0·03	16	29·5 ± 2·3	4·44 ± 0·30	0·70 ± 0·045	1·00 ± 0·050	8	182 ± 13·5	22·8 ± 1·2	1·82 ± 0·04	1·65 ± 0·10
KCl	16	40·6 ± 1·6	0·57 ± 0·07	0·96 ± 0·03	0·96 ± 0·04	9	27·3 ± 1·5	4·45 ± 0·31	1·26 ± 0·086	0·97 ± 0·025	8	171 ± 19·3	21·8 ± 1·3	5·34 ± 0·3	1·58 ± 0·05
Acetazolamide	14	62·3 ± 3·8	4·30 ± 0·45	2·21 ± 0·07	0·71 ± 0·02	13	62·7 ± 2·7	9·25 ± 0·30	1·74 ± 0·104	0·82 ± 0·04	9	232 ± 11·7	26·3 ± 0·8	2·50 ± 0·10	1·21 ± 0·07
Aminoisometradine	8	68·5 ± 5·8	4·39 ± 0·51	1·10 ± 0·08	0·96 ± 0·10	8	81·5 ± 6·1	12·14 ± 0·76	1·21 ± 0·103	0·78 ± 0·051	8	272 ± 14·3	30·1 ± 1·4	2·08 ± 0·26	1·55 ± 0·09
Mersalyl	8	38·9 ± 4·5	1·84 ± 0·26	0·72 ± 0·08	0·76 ± 0·03	8	30·3 ± 2·7	5·21 ± 0·68	0·84 ± 0·085	0·81 ± 0·036	8	265 ± 17·6	28·7 ± 1·9	1·58 ± 0·17	1·51 ± 0·04
Chlorthiazide	8	87·0 ± 6·7	4·91 ± 0·52	1·81 ± 0·12	0·87 ± 0·03	8	77·5 ± 3·4	11·4 ± 0·63	1·46 ± 0·065	1·10 ± 0·062	8	165 ± 5·04	25·8 ± 1·6	2·07 ± 0·10	1·51 ± 0·07

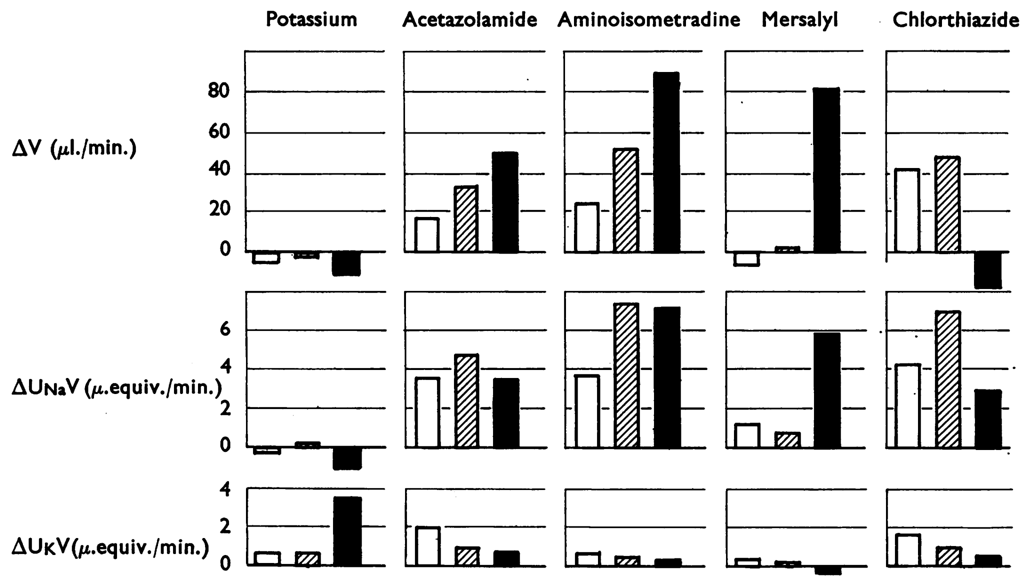

FIG. 1.—The effects of some diuretics on urine volume (ΔV) and sodium and potassium excretion ($\Delta U_{Na}V$ and ΔU_KV respectively) under the three conditions of fluid loading. Results shown as diuretic output minus control output. Mean values have been taken from the groups shown in Table I. Blank columns, 5% dextrose; cross-hatched columns, saline (0.075 ml./min.); and solid columns, saline (0.75 ml./min.).

of 5% dextrose solution increased the mean sodium output by 3.7 μ.equiv./min. above control values. Similar increments were noted after acetazolamide during both slow and fast saline infusions. The size of the increase was apparently independent of the control value. The size of the rise in urine flow following acetazolamide was independent of the control levels but roughly proportional to the mean % body weight change (Δ wt.).

Aminoisometradine.—In animals receiving 5% dextrose solution, aminoisometradine increased sodium output by 3.8 μ.equiv./min. However, when injected during the slow and fast saline infusions aminoisometradine increased the mean sodium output by 7.7 and 7.3 μ.equiv./min. respectively. The difference between the increments in sodium output caused by aminoisometradine during 5% dextrose solution and slow or fast saline infusions is significant ($P<0.05$). The change in urine flow was independent of control levels but proportional to Δ wt.

Mersalyl.—In these experiments mersalyl had little or no effect on urine flow or sodium output during the infusion of 5% dextrose solution or of 0.9% NaCl at 0.075 ml./min. During fast saline infusions, however, mersalyl increased urine flow by 83 μl./min. and sodium excretion by 5.9 μ.equiv./min.

Chlorthiazide.—Chlorthiazide given during the infusion of 5% dextrose solution increased the mean sodium output by 4.3 μ.equiv./min. and the mean urine flow by 42 μl./min. The corresponding values during slow saline infusions were 7.0 μ.equiv./min. and 48 μl./min.

These values were similar to those obtained after acetazolamide or aminoisometradine. However, when chlorthiazide was injected during fast saline infusions there was no significant change in either sodium or water output ($P>0.1$ in each instance).

Three observations were common to experiments with all the diuretics. Firstly, the control values (Table I) show that as the fluid load was increased so did the potassium output. Secondly, the increments in potassium excretion produced by acetazolamide, aminoisometradine, mersalyl, or chlorthiazide in every experiment decreased as the fluid load was raised (Fig. 1). Thirdly, the concentration of sodium in the urine ($U_{Na}V/V$, derived from Table I) of animals receiving 0.75 ml. of saline/min. was very much lower than that of animals receiving saline at only 0.075 ml./min., although the sodium concentration in the infusate was 154 m.equiv./l. in each experiment. For example, the mean urinary sodium concentration of control rats during slow saline infusions was 150 m.equiv./l.; during fast saline infusions it was 125 m.equiv./l.

Diuretic Combinations.—To get further information on the mechanism of action of the diuretics, they were injected in pairs during fast saline infusions. Each diuretic was used in the same dosage as in the previous experiments. The results of these combined injections on urine flow and on sodium and potassium output are shown in Table

TABLE II

(a) THE EFFECTS OF COMBINED DOSES OF DIURETICS ON THE EXCRETION OF SODIUM, POTASSIUM AND WATER (MEAN±S.E.) DURING LOADING WITH SALINE AT 0.75 ML./MIN.					(b) THE DIFFERENCES BETWEEN THE EXCESS SODIUM EXCRETION ABOVE CONTROL VALUES FOR COMBINED DIURETICS ($\Delta U_{Na}V_{(a+b)}$) AND THE SUMS OF THE EXCESS OUTPUTS FOR EACH DIURETIC SEPARATELY ($\Delta U_{Na}V_{(a)}+\Delta U_{Na}V_{(b)}$)	
Diuretic Combination	No. of Rats	V	$U_{Na}V$	U_KV	$\Delta U_{Na}V_{(a+b)} - (\Delta U_{Na}V_{(a)}+\Delta U_{Na}V_{(b)})$	*P*
Chlorthiazide + Acetazolamide	6	201±13·1	32·7±2·45	2·10±0·102	+3·4	<0·05
Chlorthiazide + Aminoisometradine	6	211±16·2	35·2±2·45	2·14±0·128	+2·1	≃0·1
Chlorthiazide + Mersalyl	6	221±17·5	35·8±1·67	1·60±0·092	+4·1	>0·05
Acetazolamide + Aminoisometradine	9	224±22·4	30·4±2·54	2·00±0·083	−3·2	>0·1
Acetazolamide + Mersalyl	8	223±14·0	30·8±2·08	1·93±0·129	−1·4	≃0·2
Aminoisometradine + Mersalyl	6	238±21·9	33·7±2·69	1·85±0·157	−2·3	>0·1

II*a*, and should be compared witn the results of the individual drugs given under the same conditions and shown in Table I*c*.

Preliminary experiments had shown that the doses of the drugs given individually produced maximal diuretic effects in rats with fast saline infusions as twice the dose produced no significant changes in diuresis; it seemed therefore of interest to calculate for sodium output whether the net increase above control levels for each combination of drugs was statistically different from the sum of the net increases in sodium output for each of the drugs given alone. The results are shown in Table II*b*. *P* values were obtained by analysis of variance. With the possible exception of acetazolamide given with chlorthiazide ($P<0.05$) no significant potentiation of action was observed. This suggests that these two drugs had different sites of action.

Discussion

The interpretation of the results involves two assumptions, that weight changes indicate the amount of retained fluid and that isotonic saline is distributed throughout the extracellular fluid compartment (taken as 20% of the body weight). If these assumptions are true, it will be seen from the mean % changes in body weight (Δ wt. in Table I) that rats receiving 5% dextrose solution had practically normal extracellular fluid volumes, whilst the mean expansion of the extracellular fluid compartment by slow infusions of saline was 10% and that by fast infusions was 36%. It is clear therefore that the diuretics are acting under three very different conditions.

Effects of Diuretics on Urine Flow and Sodium Excretion.—The effects of acetazolamide on sodium output seemed to be relatively independent both of the degree of fluid loading and of the sodium load presented to the tubules (Table I). Since Maren, Mayer, and Wadsworth (1954) have shown that acetazolamide is a powerful inhibitor of carbonic anhydrase, the present results suggest that the carbonic anhydrase mechanism in the kidney may be responsible for the reabsorption of a relatively constant amount of sodium.

Although the salts of potassium are frequently said to be diuretic, the infusion of as much as 7.5 μ.equiv. KCl/min./100 g. body weight failed to change significantly the rate of sodium and water output. Fuller, Macleod, and Pitts (1955) noted that potassium loading at a similar rate in dogs had relatively little effect on sodium output.

The most surprising observation arose from a comparison of the effects of mersalyl and chlorthiazide under the three conditions of fluid loading. During the slow infusion of 5% dextrose solution or of saline, mersalyl had practically no diuretic action whatever (Fig. 1). However, when the fluid load was raised by fast saline infusion, the diuretic activity of mersalyl became apparent. Pitts, Duggan, and Miner (1950) have argued that, if mersalyl acted on the distal tubules, then failure of mersalyl to cause diuresis may be related to a low glomerular filtration rate. It will be noted, however, that acetazolamide, which almost certainly acts upon the distal tubules (Pitts, 1958), caused a diuresis even though the fall in inulin clearance (glomerular filtration rate) was similar in size to that after mersalyl.

In contrast to mersalyl, the action of chlorthiazide was similar to that of aminoisometradine during slow dextrose solution or saline infusion. However, during the rapid infusion of saline chlorthiazide failed to change significantly either urine flow or sodium output.

It was pointed out in the results that the sodium concentration in the urine of rats infused with saline at 0.75 ml./min. was considerably less than its concentration in the infusate (154 m.equiv./l.). This accords with the theoretical predictions of Cole and Meredith (1957).

Effects of Diuretics on Urinary Potassium Excretion.—Koch, Brazeau, and Gilman (1956) have observed that, when sodium excretion was increased by isotonic saline infusion, the excretion of potassium was also raised. Similar results were noted in the present experiments when the saline load was increased in control animals (Table I).

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