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Research Article

THE DIURETIC EFFECTS OF LARGE DOSES OF ACETAZOLAMIDE AND AN ANALOG LACKING CARBONIC ANHYDRASE INHIBITING ACTIVITY *

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According to current concepts, acidification of the urine in the distal tubule and final removal of bicarbonate from the glomerular filtrate occur by a process of ion exchange in which hydrogen ions within tubular cells are exchanged for sodium in the filtrate (1). It is thought that carbonic anhydrase catalyzes an essential reaction which either directly supplies hydrogen ions to the exchange site or removes the base generated by the secretion of hydrogen ions. In support of this hypothesis is the fact that unsubstituted sulfonamides which inhibit carbonic anhydrase prevent acidification of the urine and produce a diuresis of sodium and bicarbonate. Using sulfanilamide, Pitts and Alexander found that approximately 20 per cent of the filtered bicarbonate could be diverted into the urine (2), and suggested that the reabsorption of bicarbonate in the distal tubule was dependent on carbonic anhydrase activity.

Subsequently, however, it was pointed out by Berliner (3) and by Schwartz, Danzig and Relman (4) that acetazolamide, a more potent sulfonamide inhibitor of carbonic anhydrase, is capable of producing much greater effects on bicarbonate reabsorption. A dose of 20 to 30 mg per kg of acetazolamide resulted in the loss of up to 50 per cent of the filtered bicarbonate, thus suggesting that a sodium-hydrogen exchange catalyzed by carbonic anhydrase might be responsible for the reabsorption of bicarbonate in the proximal as well as distal tubules (3, 4). With intravenous doses of 500 mg per kg, even greater effects have been observed, with more than 75 per cent of the filtered bicarbonate appearing in the urine for a brief period after injection (5). Simultaneously, there was an enormous but transient diuresis, in which more than half of the filtered sodium and water was excreted.

These experiments were at first interpreted (4) as showing that the mechanism by which most, if not all, sodium reabsorption is accomplished involves the carbonic anhydrase-dependent hydrogen exchange described above. However, a more recent examination of this problem, using an N^2-methyl analog (CL 8490) of acetazolamide which has little or no enzyme-inhibiting activity, indicates that this drug also is a potent diuretic when given in comparably large doses (5). The purpose of the present report is to present in detail the experiments with high doses of acetazolamide as well as to report comparative data with the analog. The results are compatible with the view that a significant part of the diuretic action of large doses of acetazolamide resides in an additional property of the drug not related to carbonic anhydrase inhibition. It is probable, however, that further inhibition of enzyme also plays a role.

METHODS AND MATERIALS

Anesthetized dogs were used in all experiments. For the most part sodium pentobarbital was employed, but in some experiments morphine was administered. In some experiments the animals were curarized and artificially ventilated with 100 per cent oxygen using an intermittent

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positive pressure device (Mine Safety Pneophore) and in other experiments the animals were allowed to breathe room air spontaneously. In most experiments arterial P_{CO_2} ranged between 30 and 50 mm Hg, but in a few it was between 50 and 60 mm Hg. Continuous intravenous infusions of normal saline or saline and sodium bicarbonate were administered at rates necessary to maintain high urine flows and steady blood bicarbonate levels. Blood samples were collected from a catheter in the femoral artery, and infusions and all injections of drugs were made through a catheter in the femoral vein. The clearance of inulin or exogenous creatinine was used as a measure of glomerular filtration rate. Urine was collected under neutral mineral oil through an inlying bladder catheter. Inulin, pH, creatinine, sodium, potassium, CO_2 content and chloride were measured in blood and urine by standard methods, and P_{CO_2} and bicarbonate were calculated from the Henderson-Hasselbalch equation. A Donnan factor of 1.05 was used in the calculation of the filtered load of bicarbonate.

The usual experimental plan was as follows. After stable blood levels and urine flow were established, two or more 10-minute control periods were obtained. The desired dose of sulfonamide was then injected intravenously over a period of 2 to 3 minutes. The dosage levels of acetazolamide were 10 and 500 mg per kg, and equimolar quantities of the analog (CL 8490) were employed (10.6 and 536 mg per kg). Drugs were administered in volumes of 10 to 90 ml. The free compounds were put into solution by the addition of 1.8 mmoles sodium hydroxide per mmole of drug to make a final pH of approximately 8.5 to 9.0. In control experiments, sodium bicarbonate or sodium hydroxide was administered in amounts roughly equivalent to the alkali released into the blood by the larger doses of sulfonamide. Three minutes after the completion of each injection a blood sample was drawn, the bladder was emptied, the urine discarded and urine collections were begun anew. Because of the evanescent nature of the diuresis following the large doses, postinjection clearance periods were usually only 6 minutes in length.

The two sulfonamide compounds employed were: acetazolamide (Diamox, 5-acetamido-1,3,4-thiadiazole-2-sulfonamide), and CL 8490 (N^2-methyl-5-acetamido-1,3,4-thiadiazole-2-sulfonamide.)[1] The latter substance has a titration curve which closely resembles that of acetazolamide. (Acetazolamide pKa'_1 7.4; pKa'_2 9.1; CL 8490 pKa'_1 7.7; pKa'_2 10.0). However, CL 8490 does not demonstrate significant carbonic anhydrase inhibitory activity either *in vitro* or *in vivo* (6). In several experiments blood and urine samples obtained just before and after injection of the sulfonamide were assayed by Dr. Thomas Maren for their carbonic anhydrase inhibitory activity by the enzymatic method (6, 7).

RESULTS

I. Comparative effects of equimolar small doses of CL 8490 (10.6 mg per kg) and acetazolamide (10 mg per kg). In five experiments 10.6 mg per kg of CL 8490 was directly compared with 10 mg per kg of acetazolamide. In all but one instance CL 8490 was injected first, followed in 1.5 to 2 hours by acetazolamide. In addition, CL 8490 was given alone on one occasion, and acetazolamide alone on two occasions. Table I summarizes the data from a typical experiment in which both drugs were given. Injection of CL 8490 produced little or no change in the renal excretion of water and electrolytes, and blood and urine contained no measurable carbonic anhydrase inhibiting activity. Subsequent injection of acetazolamide, on the other hand, was followed by a sharp rise in bicarbonate excretion and a reduction of approximately 40 per cent in the reabsorption of this ion. There was no

[1] This analog, synthesized by the American Cyanamid Company, was made available through the kindness of Dr. Thomas H. Maren of the Research Division.

TABLE I

*Comparative effects of equimolar small doses of CL 8490 (10.6 mg/kg) and acetazolamide (10 mg/kg) **

Total elapsed time	Plasma					Urine						HCO_3 reabsorbed
	pH	P_{CO_2}	HCO_3	CAI activity†	GFR	Flow	Na	K	Cl	HCO_3	CAI activity†	
min		*mm Hg*	*mEq/L*	*μg/ml*	*ml/min*	*ml/min*		*μEq/min*			*μg/min*	*mEq/100 ml GFR*
60–70	7.32	38	20.0		70	5.1	955	52	975	26		1.96
70–80	7.32	39	20.2		70	5.1	992	49	995	27		1.98
80–90	7.31	41	20.3		72	4.7	949	47	951	27		1.99
90–92	CL 8490, 10.6 mg/kg i.v.											
95–105	7.31	42	21.6	0	70	4.5	932	41	926	37	0	2.11
105–115	7.32	40	21.3	0	75	4.6	984	44	960	56	0	2.06
115–190	Discard											
190–200	7.31	40	20.8	0	70	5.1	991	56	982	52	0	2.01
200–210	7.33	39	21.1		76	4.9	969	55	957	51		2.04
210–212	Acetazolamide, 10 mg/kg i.v.											
215–225	7.35	37	20.2	25	56	7.7	1,350	142	875	504	1,540	1.12
225–235	7.35	34	19.2	12	62	6.7	1,200	115	819	418	791	1.25

* Dog 131: weight 13.2 kg; sustaining infusion, 0.14 M NaCl at 8.5 ml/min beginning at 0 time. Control plasma, Na 140, K 2.6, Cl 112 mEq/L with no significant change throughout.

† Carbonic anhydrase inhibiting activity, expressed in terms of micrograms of acetazolamide.

TABLE II

Effect of 500 mg/kg of acetazolamide

Total elapsed time	Plasma							Urine						Excreted/filtered					HCO_3 reabsorbed
	pH	P_{CO_2}	HCO_3	Na	K	Cl	GFR	Flow	Na	K	Cl	HCO_3	H_2O	Na	K	Cl	HCO_3	
min		*mm Hg*		*mEq/L*			*ml/min*	*ml/min*	*μEq/min*						%				*mEq/100 ml GFR*
A. Dog Hazel: weight 22 kg. Sustaining infusion, NaCl 0.125 M, $NaHCO_3$ 0.025 M, at 9.6 ml/min at 0 time																			
120–130	7.43	37	23.6				81	8.0	1,140	67	800	292	9.9				15.2	2.00
130–140							80	8.0	1,140	63	800	280	10.1				14.9	2.01
140–150	7.41	38	23.4	157	2.6	118	83	8.4	1,190	71	848	286	10.1	9.6	32.9	8.7	15.0	1.99
150–158 Acetazolamide, 500 mg/kg i.v.																			
161–167	7.60	30	28.1	159	2.4	119	66	26.2	3,790	178	1,886	1,520	40.0	38.2	113.4	24.2	82.6	0.49
167–173	7.39	43	25.5	159	2.4	119	71	22.3	3,250	175	1,606	1,330	31.6	30.4	103.0	19.1	70.0	0.81
B. Dog 138: weight 12.7 kg. Sustaining infusion, NaCl 0.11 M, $NaHCO_3$ 0.035 M at 9.6 ml/min at 0 time																			
120–130	7.30	52	26.1	156	1.6	119	45	6.8	952	53	514	371	15.1	13.6	73.6	9.6	31.6	1.79
130–140	7.29	54	26.1			121	38	6.5	953	54	495	363	17.1	16.1	67.6	10.8	36.6	1.66
140–150	7.30	51	26.1	156	2.5	124	43	6.7	966	60	507	388	15.6	14.4	55.8	9.5	34.6	1.71
150–152 Acetazolamide, 500 mg/kg i.v.																			
153	7.68	29	34.9																
155–161	7.49	43	33.3	164	2.5	117	38	23.8	3,550	121	1,990	1,180	62.6	57.0	127.4	44.8	93.3	0.22
161–167	7.45	43	30.2			119	28	12.2	1,930	89	922	719	43.6	42.0	127.1	27.7	85.1	0.45
167–173	7.38	46	29.1	161	2.6	124	33	8.5	1,420	86	588	607	25.8	26.7	100.2	14.4	63.2	1.07

increase in chloride excretion and, although potassium excretion increased, most of the increment in bicarbonate was associated with a nearly equivalent rise in sodium excretion. Significant levels of carbonic anhydrase inhibiting activity were demonstrable in both plasma and urine. Urine flow increased slightly despite a drop in glomerular filtration rate.

Injection of 10.6 mg per kg of CL 8490 on five other occasions was similarly without effect on water and electrolyte excretion. Acetazolamide in this dose never failed to produce a significant reduction in bicarbonate reabsorption, but in no case increased chloride excretion.

II. The effects of 500 mg per kg of acetazolamide. In 15 experiments acetazolamide was administered intravenously at a dose of 500 mg per kg. On five occasions the drug was injected 2 to 3 hours after a comparable dose of analog, and in the remaining 10 experiments acetazolamide alone was injected. In Table II are summarized the results of two such experiments. In the first experiment (A), the dog had not received any other agent prior to injection of acetazolamide; in the other (B), a large dose of CL 8490 had been given approximately 90 minutes before, but its effect had almost entirely disappeared prior to injection of acetazolamide.

In both instances, injection of 500 mg per kg of acetazolamide resulted in a significant rise in blood pH and plasma bicarbonate concentration. There was also a very large diuresis of water and electrolytes. At the height of the diuresis in Experiment A, approximately 40 per cent of the water, 38 per cent of the sodium and 83 per cent of the bicarbonate in the filtrate was diverted into the urine. In Experiment B, the effect was even greater, with excretion of about 60 per cent of the filtered water and sodium and 90 per cent of the bicarbonate. In both experiments the diuresis was quite evanescent, for it was clearly waning after the first 6-minute clearance period. In both experiments there was also a large chloride and potassium diuresis, net secretion of the latter becoming demonstrable. The reabsorption of bicarbonate was briefly reduced to 0.49 mEq per 100 ml glomerular filtrate in A, and to 0.22 mEq per 100 ml glomerular filtrate in B. There was a slight reduction in glomerular filtration rate in both experiments.

In all the other experiments in which similar doses of acetazolamide were used the response was similar. The maximal rate of excretion of bicarbonate ranged from 70 to 95 per cent of the filtered load and the reduction in the rate of reabsorption ranged from approximately 45 per cent to 85 per cent of the control value, with an average of 65 per cent.

The maximal excretion of sodium and water was 20 to 57 per cent, and of chloride 13 to 45 per cent of the filtered load. As in the experiments in Table II, the glomerular filtration rate usually dropped slightly after the injection, but in a few cases there was no change. Qualitative tests of the urine for sugar in three experiments were negative. Phosphorus excretion, measured in sev-

TABLE III

Effects of 2 mmoles/kg of NaOH and $NaHCO_3$

Total elapsed time	Plasma							Urine					Excreted/filtered					HCO_3 reabsorbed
	pH	P_{CO_2}	HCO_3	Na	K	Cl	GFR	Flow	Na	K	Cl	HCO_3	H_2O	Na	K	Cl	HCO_3	
min		*mm Hg*		*mEq/L*			*ml/min*	*ml/min*		*μEq/min*					%			*mEq/100 ml GFR*
A. Dog 177, 12.9 kg. Sustaining infusion, 0.114 M NaCl plus 0.036 M $NaHCO_3$ and creatinine at 14 ml/min at 0 time.																		
144–154	7.35	51	32.5	153	2.7	105	52	4.8	1,120	42	636	349	9.2	14.2	30.0	11.6	20.7	2.58
154–164	7.41	49	31.2	153	2.9	103	48	3.8	903	41	546	310	7.9	12.3	29.5	11.1	20.7	2.48
165–167	NaOH, 26 mmoles in 90 ml H_2O i.v.																	
168–	7.54	42																
170–176	7.42	52	35.2	153	2.7	100	52	7.0	1,470	68	825	571	13.4	18.5	48.6	15.9	31.2	2.42
176–182	7.41	53	34.8	152	2.8	100	37	3.6	784	38	417	323	9.7	14.0	36.5	11.3	25.1	2.61
182–188	7.37	56	33.3	153	2.9	102	45	2.8	668	48	277	327	6.2	9.7	36.6	6.0	21.8	2.60
B. Dog 50, 11.4 kg. Sustaining infusion, 0.114 M NaCl + 0.036 M $NaHCO_3$ and creatinine at 14 ml/min at 0 time.																		
0–10	7.44	35	24.0	148	2.4	114	53	7.1	1,100	50	895	226	13.4	14.0	39.3	14.8	17.8	1.97
10–20	7.44	33	23.0	145	2.4	116	57	6.9	1,110	50	918	233	12.1	13.4	36.5	13.9	17.8	1.89
21–25	$NaHCO_3$, 23 mmoles in 75 ml H_2O i.v.																	
26–32	7.51	41	33.2	151	2.1	114	51	13.3	2,110	70	1,440	722	26.1	27.4	65.4	24.8	42.6	1.90
32–38	7.50	37	29.1	147	2.1	114	60	13.5	2,030	60	1,470	629	22.5	23.0	47.6	21.5	36.0	1.86
83–93	7.46	36	25.9	146	2.1	115	55	7.9	1,220	50	885	347	14.4	15.2	43.3	14.0	24.4	1.96
93–103	7.48	34	25.4	145	2.1	115	57	7.7	1,170	50	862	318	13.5	14.2	41.8	13.2	22.0	1.98

eral experiments, did not change. Urine and plasma osmolality were measured (by depression of freezing point) in five experiments. Values were in the range of 320 to 380 mOsm and were essentially equal in plasma and urine. Roughly estimated, either indirectly from the difference between total osmolality of the urine and the sum of the measured electrolytes or directly from the measurements of carbonic anhydrase inhibiting activity in the urine at the height of the diuresis (cf. Tables V and VI), the excretion of acetazolamide appeared to be less than 10 per cent of the total solute.

III. Sodium bicarbonate and sodium hydroxide. In order to determine whether any of the effects of acetazolamide could be attributed to the alkalinity of the infusate, experiments were carried out under comparable conditions, in which 2 mmoles per kg of NaOH or $NaHCO_3$ was given—an amount estimated to be at least equal to the alkali given up to the body fluids by the quantities of alkaline acetazolamide (or CL 8490) injected in the previous experiments.[2] Three experiments were carried out with sodium bicarbonate and four with sodium hydroxide. A summary of one of each type of experiment is shown in Table III. It can be seen that, although the injection of sodium hydroxide or sodium bicarbonate produced a rise in plasma bicarbonate concentration comparable with that effected by acetazolamide, there was a much smaller rise in the urinary excretion of sodium, chloride and water and very little increase in potassium excretion. Bicarbonate reabsorption was essentially unaffected although, as would be expected, the absolute excretion and the per cent of the filtered load excreted rose moderately.

IV. Effects of large doses of analog (CL 8490). In six experiments, 536 mg per kg of CL 8490 (equimolar with 500 mg per kg of acetazolamide) was administered intravenously. Table IV presents the results of an experiment in which 536 mg per kg of CL 8490 was injected approximately 90 minutes after injection of 10.6 mg per kg. It is evident that the low dose produced only a very slight effect on electrolyte excretion and no effect on the reabsorption of bicarbonate, as already shown in the experiment of Table I. With the large dose of analog, approximately 40 per cent of the filtered water, sodium and chloride appeared in the urine in association with two-thirds of the filtered bicarbonate. Bicarbonate reabsorption was transiently reduced by 50 per cent to a minimum rate of 0.9 mEq per 100 ml glomerular filtrate.

Tables V and VI summarize two experiments in which a large dose of CL 8490 was compared

[2] This quantity was calculated in the following manner. Acetazolamide has a pKa'_1 of 7.4 and a pKa'_2 of 9.1; the drug was injected at a pH of between 8.5 and 9.0 and was titrated by the blood to a pH of approximately 7.4 to 7.6. Therefore, it can be estimated that each buffer group would be titrated by approximately 0.5 equivalent per mole, or a total of 1 mEq per mmole of drug administered. Since the total dose of drug was approximately 2 mmoles per kg, the same load of NaOH or $NaHCO_3$ was administered. Similar considerations apply to CL 8490 which has virtually the same pK′ values.

with an equimolar dose of acetazolamide. In the experiment of Table V, the injection of analog increased the rate of sodium excretion from 6 to 28 per cent of the filtered load and chloride excretion from 2 to 21 per cent of the filtered load. Urine flow rose from 6.1 to 19.7 ml per minute, and at the height of the diuresis 34 per cent of the volume of the filtrate was excreted. Bicarbonate excretion was 47 per cent of the filtered load, while the reabsorptive rate dropped 25 per cent. Subsequently, injection of acetazolamide resulted in a similar effect, with an increase in sodium excretion from 9 to 32 per cent of the load, in water from 8 to 33 per cent, and in chloride from 3 to 18 per cent. Bicarbonate excretion increased to a rate equal to 61 per cent of the filtered load and reabsorption was reduced by 37 per cent. Plasma and urine contained large quantities of carbonic anhydrase inhibiting activity following injection of acetazolamide, but never showed more than traces of activity after the analog.

The experiment in Table VI illustrates the more usual result, in that there was a distinctly greater effect on electrolyte excretion with acetazolamide than with CL 8490. With CL 8490 urine flow increased by 5 ml per minute, sodium excretion increased from 14 to 26 per cent of the filtered load, water from 13 to 28 per cent, chloride from 11 to 18 per cent, and bicarbonate from 30 to 55 per cent. Bicarbonate reabsorption was reduced by approximately 20 per cent. By contrast, after acetazolamide urine flow increased by 13 ml per minute and the maximal excretion of sodium rose from 13 to 40 per cent of the load, of bicarbonate from 34 to 75 per cent of the load, and of chloride from 8 to 27 per cent of the load. Bicarbonate reabsorption was reduced by approximately 55 per cent. As in the previous experiment, carbonic anhydrase inhibiting activity in urine was negligible immediately after administration of CL 8490, but very high after acetazolamide.

In Figure 1 is shown the maximal excretion of sodium following each of the experiments with acetazolamide and CL 8490. Sodium excretion, plotted as a per cent of the filtered load, is related to the simultaneous urine to plasma ratio for inulin or creatinine. Solid circles represent data obtained with acetazolamide and open circles, CL 8490. It is seen that maximal sodium excretion ranged from approximately 20 to 55 per cent of the filtered load with acetazolamide and from 20 to 40 per cent of the load with CL 8490. Although there was overlap, acetazolamide appeared to have a substantially greater effect. The heavy solid line in the figure represents the theoretical relationship between sodium and water excretion which would obtain if sodium were excreted isotonically. The close fit of the experimental points to this line indicates the virtually isotonic nature of the urine during diuresis. For comparison, the dotted lines in the lower part of the figure show the approximate range of the relationship between sodium and water observed during maximal osmotic diuresis with mannitol (8) and with urea (9). It is apparent that sodium excretion at any given rate of water excretion was considerably less with osmotic diuresis than with the drugs studied here. To the authors' knowledge, the maximal rates of sodium excretion observed in the

TABLE IV

Comparative effects of 10.6 mg/kg and 536 mg/kg of CL 8490 *

Total elapsed time	Plasma							Urine						Excreted/filtered				HCO$_3$ reabsorbed
	pH	P_{CO_2}	HCO_3	Na	K	Cl	GFR	Flow	Na	K	Cl	HCO_3	H_2O	Na	K	Cl	HCO_3	
min		*mm Hg*		*mEq/L*			*ml/min*	*ml/min*		*μEq/min*					%			*mEq/100 ml GFR*
50–60	7.42	29	19.0	149	3.3	121	65	5.9	1,070	76	1,110	60	9.1	11.0	35.4	14.1	4.9	1.81
60–70	7.38	32	19.4	149	3.4	123	63	5.0	1,110	81	1,140	63	9.4	11.8	37.8	14.7	5.2	1.84
70–80	7.38	32	19.6	148	3.5	122	64	6.1	1,120	89	1,180	66	9.5	11.8	39.7	15.1	5.3	1.86
80–81	CL 8490, 10.6 mg/kg i.v.																	
85–91	7.41	31	19.7	149	3.3	123	66	7.2	1,270	108	1,270	91	10.9	12.9	49.6	15.6	7.0	1.83
91–97	7.40	30	19.1	147	3.4	121	60	6.3	1,160	105	1,160	87	10.5	13.2	51.5	16.0	7.6	1.77
97–153	Discard																	
153–163	7.41	29	18.7	144	3.2	119	62	6.5	1,160	87	1,160	80	10.5	13.0	43.9	15.7	6.9	1.74
163–173	7.41	29	21.0	144	3.3	120	68	6.2	1,140	88	1,170	70	9.1	11.6	39.2	14.3	4.9	2.00
173–175	CL 8490, 536 mg/kg i.v.																	
175	8.00	11	28.5															
178–184	7.63	27	28.2	153	3.5	110	52	20.8	3,270	203	2,460	998	40.0	41.1	111.5	43.0	68.1	0.90
184–190	7.49	29	22.3	154	3.0	118	52	12.5	2,120	153	1,700	541	24.0	26.5	98.1	27.7	46.6	1.19

* Dog 127: weight 12.8 kg; sustaining infusion, 0.15 M NaCl at 8.5 ml/min at 0 time.

TABLE V

Comparative effects of equimolar large doses of CL 8490 (536 mg/kg) and acetazolamide (500 mg/kg) *

Total elapsed time	Plasma						CAI activity†	GFR	Urine					Excreted/filtered					HCO_3 reabsorbed	CAI activity†
	pH	P_{CO_2}	HCO_3	Na	K	Cl			Flow	Na	K	Cl	HCO_3	H_2O	Na	K	Cl	HCO_3		
min		*mm Hg*		*mEq/L*			*μg/ml*	*ml/min*	*ml/min*	*μEq/min*						%			*mEq/100 ml GFR*	*μg/min*
120–130	7.38	64	39.0	158	2.7	115		50	5.2	392	33	108	300	10.4	5.0	24.4	1.9	15.4	3.30	
130–140	7.40	61	38.6	158	2.7	115	0	56	6.1	512	39	149	354	10.9	5.8	25.8	2.3	16.4	3.23	0
140–142 CL 8490, 536 mg/kg i.v.																				
145–151	7.64	44	45.5	164	3.1	113	2	58	19.7	2,620	108	1,350	1,250	34.0	27.5	60.1	20.6	47.4	2.40	40
151–157	7.52	55	43.5	164	2.9	113	2	48	13.7	1,730	87	825	936	28.5	22.0	62.5	15.2	44.8	2.40	
157–298 Discard																				
298–308	7.49	47	36.1	158	2.8	119	4	44	3.6	583	54	111	473	8.2	8.4	43.8	2.1	27.5	2.62	61
308–318	7.43	53	38.1	158	2.8	119		49	4.1	689	67	146	520	8.4	8.9	48.8	2.5	27.9	2.75	
318–320 Acetazolamide, 500 mg/kg i.v.																				
323–329	7.65	39	43.3	160	3.0	118	1,700	54	18.0	2,750	150	1,160	1,430	33.3	31.8	92.6	18.2	61.2	1.68	40,500
329–335	7.49	52	40.2	162	2.6	116	950	42	13.3	2,060	80	959	988	31.7	30.3	81.5	19.7	58.5	1.67	20,615

* Dog 120: weight 16.4 kg; sustaining infusion, 0.06 M $NaHCO_3$ + 0.08 M NaCl at 8.5 ml/min starting at 0 time.
† Carbonic anhydrase inhibiting activity, expressed in terms of acetazolamide.

TABLE VI

Comparative effects of equimolar large doses of CL 8490 (536 mg/kg) and acetazolamide (500 mg/kg) *

Total elapsed time	Plasma						GFR	Urine						Excreted/filtered					HCO_3 reabsorbed	CAI activity†
	pH	P_{CO_2}	HCO_3	Na	K	Cl		Flow	Osmol.	Na	K	Cl	HCO_3	H_2O	Na	K	Cl	HCO_3		
min		*mm Hg*		*mEq/L*			*ml/min*	*ml/min*		*μEq/min*						%			*mEq/100 ml GFR*	*μg/min*
50–60	7.46	44	32.2	151	3.4	115	57	6.9	367	1,140	125	610	566	12.1	13.2	64.5	9.3	30.8	2.23	
60–70	7.46	42	30.7	151	3.0	116	45	6.1	379			573	528	13.6			11.0	38.2	1.90	
70–80	7.46	42	30.7	151	3.0	116	56	7.3	368	1,210	123	696	523	13.0	14.3	73.2	10.7	30.4	2.14	0
80–91 CL 8490, 536 mg/kg i.v.																				
91–97	7.61	36	38.0	172	2.6	111	44	12.2	327	1,930	172	864	911	27.7	25.5	150.3	17.7	54.5	1.73	22
97–103	7.55	37	33.3			112	45	9.2	347	1,540	118	800	662	20.4			15.9	44.2	1.86	23
103–109	7.53	37	32.4	159	2.2	112	45	10.5	337	1,710	118	966	691	23.3	23.9	119.2	19.2	47.4	1.70	38
109–192 Discard																				
192–202	7.42	42	27.7	158	2.9	122	47	7.3	297	986	106	451	495	15.5	13.3	77.8	7.9	38.0	1.72	104
202–212	7.42	42	27.7	158	2.9	122	47	7.0	294	952	96	475	440	14.9	12.8	70.4	8.3	33.8	1.83	106
212–214 Acetazolamide, 500 mg/kg i.v.																				
214–217 Discard																				
217–223	7.45	47	33.9	164	3.2	119	47	20.5	299	3,100	203	1,530	1,200	43.6	40.2	135.0	27.4	75.3	0.84	68,700
223–229	7.43	46	31.7			120	43	12.5	314	1,960	149	923	836	29.1			17.9	61.3	1.23	35,600
229–235	7.43	45	30.7	164	2.9	121	42	10.3	315	1,640	128	770	713	24.5	23.8	105.1	15.2	55.3	1.37	30,900

* Dog 140: weight 13 kg; sustaining infusion, 0.11 M NaCl and 0.03 M $NaHCO_3$ at 10 ml/min starting at 0 time.
† Carbonic anhydrase inhibiting activity, expressed in terms of acetazolamide.

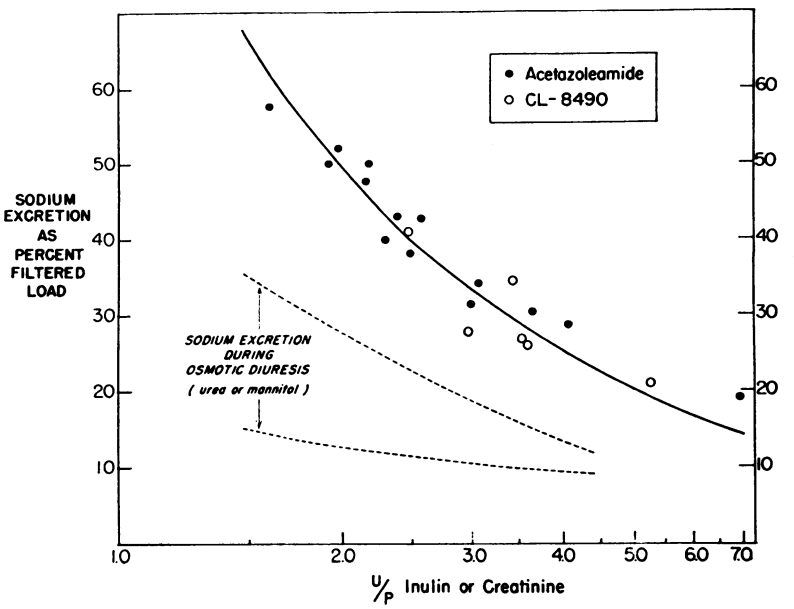

FIG. 1. MAXIMAL SODIUM EXCRETION FOLLOWING ACETAZOLAMIDE (500 MG PER KG) OR CL 8490 (536 MG PER KG). This is plotted as a per cent of the filtered load in relation to the simultaneous urine to plasma ratio for inulin or creatinine. Solid dots represent acetazolamide, open circles CL 8490. The heavy solid line represents the theoretical relationship between these parameters for the excretion of an isotonic solution of sodium. Note that the experimental points closely fit this theoretical line. For comparison, the dotted lines in the lower part of the figure indicate the range of the relationship between excretion of sodium and water during maximal osmotic diuresis with mannitol (8) and urea (9).

present study exceed any which have previously been reported.

DISCUSSION

These experiments show that rapid intravenous administration of 500 mg per kg of acetazolamide produces a large, though transient, diuresis which considerably exceeds that which has been produced by any other experimental technique. At the height of this effect as much as 40 to 60 per cent of the sodium and water in the glomerular filtrate was diverted into the urine, together with 25 to 50 per cent of the filtered chloride. Bicarbonate reabsorption was depressed by 40 to 85 per cent from control rates and the rate of excretion was as much as 70 to 95 per cent of the filtered load. Aside from its magnitude, the feature of this diuresis which distinguished it from the effects of the usual small doses of acetazolamide was the massive chloruresis. When 10 mg per kg was given, there was little or no effect on chloride, and almost all of the increment in anion was due to bicarbonate (Table I). By contrast, in the present experiments with 500 mg per kg, although there was a further enhancement of bicarbonate excretion, a large part of the increased excretion of anion was due to chloride.

The explanation for this phenomenon was at first thought to reside solely in the carbonic anhydrase inhibiting activity of the large dose (4). It would now appear that an additional mechanism must be invoked because equimolar large doses of an inactive analog (CL 8490) also produce a substantial diuresis of electrolyte and water. This diuresis is not usually as large as that resulting from acetazolamide, but closely resembles the latter in its duration and in its effect on urine composition.

It seems clear from the data at hand that neither the osmotic effect nor the acute systemic alkalinizing action of the large dose of the sodium salts of acetazolamide or CL 8490 can explain the results.

An osmotic diuresis would appear to be excluded by the fact that the drug constituted only a small fraction of total urine solute, probably less than 10 per cent. That neither the sodium load nor the extracellular alkalinizing effect of the sulfonamide base could account for the depression of chloride and bicarbonate reabsorption was demonstrated by control experiments in which loads of sodium bicarbonate and sodium hydroxide equivalent to the alkali yielded by the sulfonamides were found not to reduce the reabsorption of bicarbonate and to only slightly increase the excretion of sodium and chloride. Administration of the alkali produced a moderate bicarbonate diuresis, but this was due to the rise in plasma bicarbonate level and not to any significant reduction in reabsorption.

What then is the explanation for the diuretic action of CL 8490 and for that part of the action of the large dose of acetazolamide which presumably is not related to carbonic anhydrase inhibition? The available data do not permit a definite answer to this question. However, in view of the large doses required to produce diuresis and the transient nature of the effect, it is tentatively suggested that the mechanism does not involve direct inhibition of an enzymatic process required for electrolyte transport. Rather, it would appear more likely that the effect depends upon some temporary change in renal cell composition resulting from the sudden accumulation of large amounts of drug. There is evidence that acetazolamide is both secreted and reabsorbed by tubular cells (10) and it is reasonable to assume similar handling of the analog. Movement of the monobasic form of either drug from the relatively alkaline plasma or proximal tubular fluid (pH 7.5 to 7.7) into the relatively acid renal cells (estimated pH < 7.0) would result in binding of intracellular protons by the weaker of the two basic groups on each molecule (pK'_a 7.4 and 7.7, respectively) and as a consequence cell pH would rise. Since there is strong evidence to suggest that reabsorption of bicarbonate involves a sodium-hydrogen exchange (1), acute reduction in the availability of protons within the tubular cell might inhibit this process and produce a bicarbonate diuresis. Whether a similar sodium-hydrogen exchange is also involved in the reabsorption of chloride (3) is not known, but if such were the case, a rise in cellular pH would also be expected to reduce the reabsorption of chloride. If, on the other hand, a sodium-hydrogen exchange does not precede chloride reabsorption, it might be postulated that increased cell pH in some manner slows the metabolic processes upon which all active sodium transport depends. Since chloride reabsorption appears to be a passive result of active sodium transport (11), the final results would be the same. The transient nature of the diuretic effect, assuming the latter to be the result of abrupt changes in cell pH, could be explained on the basis of the brief time during which plasma and tissue concentrations of drug might remain at levels high enough to affect cell pH, and to rapid turnover of protons within the cell, which would be expected to restore the usual pH very quickly and thus nullify the diuretic effect.

While such speculation seems consistent with available data, it is obvious that alternative mechanisms cannot be excluded. Thus, for example, it is possible that the diuretic action shared by acetazolamide and CL 8490 resides in some other property of the thiadiazole-sulfonamide configuration which also belongs to the benzothiadiazole-sulfonamides of the chlorothiazide family. The latter are potent saluretics which in relatively low dosage produce changes in electrolyte excretion qualitatively similar to those reported here (12, 13). More information will be needed to settle this question.

Finally, it should be emphasized that, in three of five paired experiments, acetazolamide appeared to have a considerably greater effect on sodium and water than did CL 8490. The maximal diuresis achieved with CL 8490 never approached that observed in other experiments with acetazolamide (Figure 1). It is likely, therefore, that an appreciable part of the action of the 500 mg per kg dose of acetazolamide is in fact due to carbonic anhydrase inhibition. Limited supplies of CL 8490 unfortunately prevented a more definitive quantitative comparison of acetazolamide with CL 8490.

SUMMARY AND CONCLUSIONS

Intravenous injection of 500 mg per kg of sodium acetazolamide in dogs produced a massive but transient diuresis in which as much as 40 to 60 per cent of the filtered sodium and water and 25

to 50 per cent of the filtered chloride were diverted into the urine. Bicarbonate reabsorption was depressed by 40 to 85 per cent from control rates and the rate of excretion reached 70 to 95 per cent of the filtered load. A substantial part of this effect is probably not explained by inhibition of carbonic anhydrase, because the sodium salt of the N^2-methyl analog of acetazolamide (CL 8490), which has virtually no carbonic anhydrase inhibiting activity *in vivo* or *in vitro,* was found to produce a similar though somewhat smaller diuresis.

The data indicate that the diuretic action shared by the sodium salts of acetazolamide and CL 8490 cannot be explained on the basis either of the osmotic activity of the administered drugs or their extracellular alkalinizing effects. In view of the size of the dose required and of the transiency of the effect, it is proposed that the shared mechanism of action depends upon some temporary alteration in renal cell composition. It is tentatively suggested that depression of electrolyte reabsorption may result from transient intracellular alkalosis produced by cellular accumulation of the monobasic form of the drug.

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