complement, the immune aggregates are washed and then resuspended in a standard dose of complement. The relative merits of the standard and modified procedures are discussed.

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N-Amidino-3-Amino-6-Chloropyrazinecarboxamide: A New Diuretic Which Antagonizes the Renal Actions of Aldosterone. (30538)

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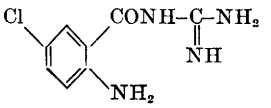
Evidence has been accumulated within the past decade which indicates that aldosterone plays an important contributory role in the salt and water retention associated with nephrosis, liver cirrhosis, toxemia of pregnancy and congestive heart failure(1,2,3,4). In view of the participation of aldosterone in these edematous states, efforts were undertaken which resulted in the discovery of both steroidal(5,6) and non-steroidal(7) antagonists of aldosterone. We wish to report the aldosterone-blocking and other actions of a new series of non-steroidal diuretics. The structure of the compound to be reported upon is shown below.



N-amidino-3-amino-6-chloropyrazinecarboxamide (ACP)

Methods. Male Holtzman rats (130 ± 3 g) were bilaterally adrenalectomized and maintained on sugar cubes and distilled water for 18 hours. The animals were randomized into groups and all except the control group were injected subcutaneously with 12 μg of desoxycorticosterone acetate (DCA) in 0.2 cc of Mazola oil. The test and reference compounds were then administered either subcutaneously or orally in 0.2 cc of dimethyl formamide (DMF). All animals were then given 5.0 cc of 0.85% sodium chloride solution by stomach gavage. The animals' bladders were voided of urine by slight suprapubital pressure and the animals placed in individual metabolism cages equipped with paraffin-coated funnels. The urine was collected in graduated centrifuge tubes with flared tops. At the end of the 7-hour collection period, the voiding of urine was again induced and the volume of the urine recorded. The floors of the cages and the funnels were repeatedly washed with distilled water and the sample and washings made up to a volume of 50 cc. Individual samples were analyzed for sodium and potassium content using a Baird Atomic Clinical Flame Photometer, Model KY. The sodium and potassium concentrations thus obtained were utilized in computing the log (Na × 10)/K ratio.* As both DCA and aldosterone are known to promote Na retention and enhance K loss, the test and reference compounds were evaluated for their ability to reverse the effects of DCA. Either or both of the following compounds were used as reference materials in these studies: 1) spironolactone (Aldactone: 3-[3-oxo-7-α-acetylthio-17β-hydroxy-4-androsten-17α yl] propionic acid α-lactone)† and 2) triamterene (2,4,7-triamino-6-phenyl-pteridine).‡

The ability of ACP to reverse the electrolyte effects of other steroids was also studied. A series of adrenaledtomized rats was given d-aldosterone monoacetate (0.5 μg/0.2 cc of a 20% ethanol solution) subcutaneously and

* Multiplication of Na values by 10 eliminated negative log (Na/K) values.

† G. D. Searle & Co., Aldactone.

‡ Smith, Kline and French Laboratories, Triamterene.

TABLE I. Effect of Orally Administered Spironolactone, Triamterene and ACP in DCA-Treated Adrenalectomized Rats.

Treatment/rat				Avg total electrolyte excretion in meq/7 hr		Avg log (Na × 10)/K
Compound	Dose in μg	DCA, μg	Animals per group	Na^+	K^+	± S.E.
—	—	—	20	.60	.13	1.63 ± .03
—	—	12	18	.22	.21	1.00 ± .02
Spironolactone	200	"	20	.32	.19	1.22 ± .03
"	400	"	8	.39	.17	1.36 ± .04
"	800	"	20	.44	.16	1.45 ± .02
"	1600	"	21	.49	.14	1.55 ± .02
"	3200	"	15	.50	.14	1.54 ± .03
"	6400	"	7	.43	.10	1.61 ± .07
Triamterene	10	"	7	.37	.19	1.29 ± .03
"	100	"	7	.50	.15	1.53 ± .03
"	800	"	6	.69	.10	1.74 ± .04
"	3200	"	7	.47	.09	1.71 ± .06
N-amidino-3-amino-6-chloropyrazinecarboxamide	10	"	15	.29	.18	1.22 ± .03
	100	"	15	.47	.16	1.48 ± .03
"	200	"	8	.46	.13	1.55 ± .02
"	800	"	15	.77	.12	1.80 ± .02
"	1600	"	8	.75	.05	2.14 ± .07
"	3200	"	13	.85	.07	2.03 ± .05

ACP orally. In another study, hydrocortisone (500 μg and 1500 μg) and spironolactone, triamterene and ACP were given orally to adrenalectomized rats. ACP and spironolactone were also administered to non-DCA loaded adrenalectomized rats to determine whether these compounds had a direct effect upon excretion of sodium and potassium. Another study was also performed wherein spironolactone and ACP were given to intact animals to determine whether they were active against endogenous aldosterone.

The chemistry of ACP has been described (8).

Results. The ability of spironolactone, triamterene and the chloropyrazinecarboxamide (ACP) administered orally to block the action of DCA in adrenalectomized rats is summarized in Table I. It is of interest to note that the responses are monotonic, or, in other words, the responses, in all cases, increase with increasing dose. As had been reported previously(9), the spironolactone was capable of reversing the electrolyte effects of the DCA dose. Both triamterene and ACP, in addition to reversing the DCA effect, proved capable of promoting excretion of greater amounts of sodium and retention of potassium. When the data were analyzed statistically, it was determined that ACP was 12.5 times (95% confidence limits 9.1-17.3) as active as the spironolactone, while the relative potencies of the triamterene and ACP were not statistically different(10). Essentially identical potencies were obtained when the 3 materials were administered subcutaneously.

The ability of ACP to block the effects of d-aldosterone-21-monoacetate is presented in Table II. Aldosterone (0.5 μg) appreciably depressed the log (Na × 10)/K ratio. The 100 μg dose of ACP significantly reversed the effect of aldosterone, while the

TABLE II. Effect of Orally Administered ACP in Aldosterone-Treated Adrenalectomized Rats.

Treatment/rat					Avg total electrolyte excretion in meq/7 hr		Avg log (Na × 10)/K
Compound	Dose in μg	Mineralocorticoid	Dose in μg	Animals per group	Na^+	K^+	± S.E.
—	—	—	—	8	.67	.15	1.67 ± .04
—	—	Aldosterone	.5	8	.19	.18	.99 ± .05
ACP	100	"	.5	7	.41	.15	1.43 ± .05
"	900	"	.5	8	.79	.09	1.94 ± .02

TABLE III. Effect of Orally Administered Spironolactone and ACP in Non-DCA Treated Adrenalectomized Rats.

Treatment/rat			Avg total electrolyte excretion in meq/7 hr		
Compound	Dose in μg	Animals/group	Na^+	K^+	Avg log (Na × 10)/K ± S.E.
—	—	11	.72	.12	1.78 ± .03
Spironolactone	1000	9	.52	.13	1.61 ± .03
ACP	1000	10	1.00	.07	2.18 ± .04

TABLE IV. Effect of Subcutaneously Administered Spironolactone and ACP in Intact Rats.

Treatment/rat			Avg total electrolyte excretion in meq/7 hr		
Compound	Dose in μg	Animals/group	Na^+	K^+	Avg log (Na × 10)/K ± S.E.
—	—	7	.43	.21	1.32 ± .03
Spironolactone	800	7	.51	.18	1.46 ± .03
"	1600	7	.62	.18	1.55 ± .05
ACP	100	7	.49	.16	1.50 ± .07
"	800	7	.74	.11	1.83 ± .07

TABLE V. Effect of Orally Administered Spironolactone, Triamterene and ACP in Hydrocortisone Treated Adrenalectomized Rats.

Treatment/rat				Animals per group	Avg total electrolyte excretion in meq/7 hr		Avg log (Na × 10)/K ± S.E.
Compound	Dose in μg	Aldosterone antagonist	Dose in μg		Na^+	K^+	
—	—	—	—	7	.80	.15	1.72 ± .02
Hydrocortisone	500	—	—	8	.43	.31	1.14 ± .05
"	500	Spironolactone	800	7	.86	.34	1.39 ± .03
"	500	Triamterene	500	8	1.22	.21	1.79 ± .06
"	500	ACP	500	8	1.07	.15	1.88 ± .05
"	1500	—	—	8	.37	.41	.93 ± .04
"	1500	Spironolactone	800	8	.59	.43	1.13 ± .07
"	1500	Triamterene	500	8	1.05	.27	1.62 ± .04
"	1500	ACP	500	8	1.04	.24	1.63 ± .02

900 μg dose resulted in greater sodium excretion and potassium retention than that observed for the control group.

The electrolyte effects of spironolactone and ACP, when given to non-DCA loaded adrenalectomized rats, are summarized in Table III. As previously reported(9), an effect on excretion of sodium and potassium was not observed with spironolactone. However, ACP markedly enhanced the loss of sodium and retention of potassium.

Intact normal animals (Table IV) were given either spironolactone or ACP. The 1600 μg dose of spironolactone produced a statistically significant rise in the log (Na × 10)/K ratio, due to enhanced excretion of sodium and retention of potassium. The 2 doses of ACP markedly altered urinary excretion of sodium and potassium by the intact rat.

In the final study, spironolactone, triamterene and ACP were administered orally to hydrocortisone-treated adrenalectomized rats (Table V). While spironolactone significantly altered the log ratio (Na × 10)/K of the rats receiving either 500 or 1500 μg of hydrocortisone, its effect was primarily upon sodium excretion with no effect observed on the kaluresis. Both triamterene and ACP proved more potent in their action on electrolytes and altered the pattern of sodium and potassium excretion.

Discussion. Liddle(11) described the parameters defining specific and non-specific inhibition of mineralocorticoid activity. Of importance to this discussion is one stated

condition, "The antagonist should block all of the effects of the agonist (DCA or aldosterone) at a particular receptor site, and should have this effect only in the presence of the agonist. Furthermore, if the antagonist is effective in the absence of the agonist, it cannot be acting through the mechanism of competition." Adequate information has been presented indicating the mechanism of action of the spironolactones to be one of competitive inhibition of mineralocorticoid(11). All of the data we have obtained with spironolactone would support this view. The only results obtained in our laboratories which, in any way, differ from those previously presented(9), is the effectiveness of spironolactone against the endogenously produced aldosterone of the intact rat (Table IV). This difference is undoubtedly due to procedural differences and experimental design.

Although it had been suggested, on the basis of preliminary data, that triamterene (2,4,7-triamino-6-phenylpteridine) was an aldosterone antagonist(7,12,13), subsequent studies(11) demonstrated that triamterene not only reversed all of the renal effects of aldosterone, but was also effective in the absence of mineralocorticoid action. All of the data presented in the results would indicate that ACP is a nonspecific inhibitor of mineralocorticoid action and, furthermore, that ACP, like spironolactone and triamterene, is of particular importance because of its action upon potassium excretion. These 3 compounds display the capacity to decrease or prevent the loss of potassium, whereas most of the known diuretics show a tendency to enhance the excretion of potassium(4). The halopyrazinecarboxamides appear to be a new class of potent non-specific diuretics some 12.5 times as active as the spironolactones.

Summary. N-amidino-3-amino-6-chloropyrazinecarboxamide (ACP) was found to: 1) antagonize the renal actions of exogenously administered aldosterone, DCA and hydrocortisone in the adrenalectomized rat, 2) reverse the action of endogenously produced aldosterone in the intact rat, and 3) produce a marked natriuresis and anti-kaluresis in the non-DCA treated rat. ACP was found to be 12.5 times as potent as spironolactone.

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