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Ability of Isopregnenolone-21-Carboxylates to Block Renal Effects of Deoxycorticosterone and Aldosterone in Rats. (26205)

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Previous reports described the synthesis of a number of steroidal 17-spirolactones(1-3) with ability to block the acute renal electrolyte effects of aldosterone and aldosterone-like steroids in laboratory studies(4-7). The compounds studied clinically caused diuresis in edematous patients with associated hyperaldosteronism(8,9), demonstrating the therapeutic value of aldosterone-blocking agents (10). Our effort was recently directed toward synthesis of isopregnenolone-21-carboxylic acids, and the present report demonstrates that these compounds are potent deoxycorticosterone- and aldosterone-blocking agents in laboratory studies.

Materials and methods. The procedures for evaluating aldosterone-blocking activity have been described(5,6). Briefly, the method consisted of subcutaneously injecting groups of adrenalectomized rats (150 to 200 g) with isotonic saline and with an oil solution of 12 μg of deoxycorticosterone acetate (DCA). Test compounds were given additionally either as an injection or an oral dose in saline.* The animals were placed in metabolism cages after treatment for collection of a 4-hour sample of urine. No food or water was provided during collection period. In this assay, DCA alone reduces the urinary ratio of Na/K ions by causing simultaneously Na retention and K loss. Blocking activity was determined by reversal of the Na/K response and expressed in terms of proportion of the standard dose of DCA inhibited by the compound. Na and K analyses were done on a Beckman flame photometer.

Isopregnenolone-21-carboxylates were obtained by treatment of steroidal 17-spirolactones with a molar equivalent of methanolic Na or K hydroxide at reflux temperature for 30 minutes, and identified by elemental analysis and infrared spectral data. The following compounds were prepared for tests in animals: 3-oxo-17β-hydroxy-19-nor-17α-pregn-4-ene-21-carboxylic acid as the Na salt (I); 3-oxo-17β-hydroxy-17α-pregn-1,4-diene-21-carboxylic acid as the Na (IIa) and K (IIb) salts; and 3-oxo-9α-fluoro-11β, 17β-dihydroxy - 17α - pregn - 4 - ene - 21 - carboxylic acid as the K salt (III).

Results. Data demonstrating the DCA-blocking properties of the 4 compounds in adrenalectomized rats are shown in Table I, together with electrolyte data of untreated and DCA controls for reference. DCA alone reduced the Na/K ratio by inducing a strong retention of Na and some K loss. It can be noted that Compound I, IIb and III at the given doses blocked Na/K response primarily by changing Na output, with only slight and variable effects occurring on K excretion. At times, however, reversal of Na/K was obtained because of a greater effect on K than on Na excretion, as shown with a dose of 0.1 mg of Compound IIa. Over a wide range of effective doses, the maximal Na, K and Na/K responses achieved with the compounds approximated, but did not represent

* Compounds are soluble in water to extent of about 15% by weight.

TABLE I. Action of Na and K Salts of Various Isopregnenolone-21-Carboxylic Acids in Blocking Renal Electrolyte Effects of DCA.

Treatment/animal		No. of animals	Mean log response		
DCA	Compound		Na	K	(Na × 10)/K
—	—	96	1.17	.83	1.34
12 μg	—	96	.77	.99	.75
12 "	.3 mg I	8	.91*	.95	.96*
12 "	.1 " IIa	8	.84	.81*	1.03*
12 "	.15 " IIb	8	.94*	.95	.99*
12 "	5.0 μg III	8	.95*	1.05	.91*

* P <.05 relative to DCA alone; P of .05 response = +.12 for Na, −.11 for K and +.11 for Na/K; Na and K = mM/l after dilution of sample/4 rats/4 hr to 50 ml; all treatment was by subcut. inj.

reversal beyond, those obtained in the untreated controls.

The dose for 50% block of the standard dose of DCA (median effective dose or MED) was determined by interpolation using cumulated dose-response data. Table II demonstrates that a dose of 0.33 mg of I was required by injection for 50% inhibition; orally, dosage for the same degree of blockade is believed to be > 2.4 mg. While IIa or IIb appeared to be more potent than I by a comparison of MED values, high variability precluded statistical significance for differences of the MEDS. The Na (IIa) and K (IIb) salts were approximately equipotent as blocking agents by injections or orally. Compound III, in the form of a K salt, gave the strongest activity in the series, producing 50% block at doses of 0.4 μg parenterally and 22 μg orally. Comparisons showed highly significant differences in MED values (P<0.01) for III relative to I, IIa or IIb by either route of treatment. Absorption from the gastrointestinal tract appeared to be relatively incomplete for III, as indicated by MED ratios orally/parenterally of 55/1. IIa and IIb showed dosage ratios by the 2 routes of 5 to 7/1. There was no significant difference in slope of dose-response curves in the comparisons involving the compounds; thus, comments on relative potencies based on the MED values are expected to apply also at other levels than 50% block.

TABLE II. Relative DCA-Blocking Properties of Various Isopregnenolone-21-Carboxylates Given Subcutaneously and Orally.

Compound	Median effective dose (MED)* Subcut.	Orally
I	.33 (.08 -1.30)	>2.4
IIa	.07 (.01 - .38)	.52 (.13 -2.05)
IIb	.15 (.07 - .32)	.82 (.37 -1.80)
III	.0004(.0001- .0020)	.022 (.005- .095)

* Dose in mg/rat for 50% block (P = .02 approx., using an 8-animal assay), or for reduction of the log Na/K effect of 12 μg of DCA to that obtained with 6 μg; values in parentheses describe 95% confidence interval.

TABLE III. Ability of An Isopregnenolone-21-Carboxylate (Compound III) to Block Renal Electrolyte Effects of Aldosterone.

Treatment/animal		Mean log response
Aldosterone	III	(Na × 10)/K
.3 μg	—	.85
.3 "	10 μg	1.04*
.3 "	100 "	1.02*

* P <.05 relative to aldosterone alone; least significant difference = +.11; 16, 12 and 12 animals for 0, 10 and 100 μg of III, respectively; all treatment was by subcut. inj.

Compound III was tested against *dl*-aldosterone-21-monoacetate† in similar adrenalectomized animals (Table III). Aldosterone alone gave a low urinary ratio of Na/K, by promoting Na retention and K loss. The effect on Na/K by aldosterone, however, was significantly reversed with III as a result of changes in both Na and K output; increasing the dose by 10-fold did not give a stronger blocking response. The isopregnenolone carboxylate was also given to similar animals in the absence of aldosterone or DCA (Table IV). It was ineffective on urinary Na, K

† Kindly supplied by Dr. R. T. Hill, Nat. Inst. Health; the racemate showed approximately 40 times the activity of DCA as measured by Na/K response in adrenalectomized rats.

TABLE IV. Effects of An Isopregnenolone-21-Carboxylate (Compound III) Given Alone on Renal Electrolyte Excretion.

Treatment/animal	Mean log response Na	K	(Na × 10)/K
—	1.18	.81	1.37
10 μg III	1.30	.88	1.42
1000 " "	1.18	.75	1.43

Least significant change (P = .05) = ± .15 for Na, ± .14 for K and ± .13 for Na/K; 16, 12 and 12 animals for 0, 10 and 1000 μg of III, respectively; Na and K = mM/l after dilution of sample /4 rats/4 hr to 50 ml.

and Na/K over a wide range of doses.

Discussion. Chemically, the isopregnenolone-21-carboxylates may be considered as 17-spirolactones in which the lactone ring has been opened; the compounds may be expected to demonstrate aldosterone- and deoxycorticosterone-blocking properties. Our studies demonstrate that such an effect is present and that the compounds are pharmacologically equivalent to the 17-spirolactones. Available evidence indicates that the mechanism of electrolyte effects of isopregnenolone-21-carboxylates is by competition with aldosterone-like steroids for receptor sites in target tissues. Support for the concept is 2-fold: 1) compounds in the series block DCA and aldosterone without showing manifestations of physiological antagonism, and 2) basic structures of the compounds are close to those of steroidal spirolactones reported as competitive inhibitors(4-7). Other data also indicate that competitive interaction occurs in the kidneys, the generally accepted site of action of aldosterone-like steroids for effects on urinary electrolytes(11-14), and of steroidal spirolactones(15,16).

Compound III, the most potent in the series, is approximately 20 times more effective orally in laboratory tests than Aldactone‡ ($P<0.01$), a steroidal spirolactone(2,6) with diuretic properties in edematous states(10). However, it remains to be seen how these data will extrapolate to man.

‡ Trademark of G. D. Searle and Co. for brand of spironolactone, or for 3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone.

Summary. Studies demonstrate that isopregnenolone-21-carboxylates are potent inhibitors of the renal electrolyte effects of deoxycorticosterone in adrenalectomized rats. Compound III, the most active in the series, was also effective against aldosterone. Available evidence indicates that the compounds affect electrolyte excretion by competition with aldosterone-like steroids at receptor sites in target tissue.

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