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A New Class of Diuretic-Saluretic Agents, The α,β-Unsaturated Ketone Derivatives of Aryloxyacetic Acids. (28838)

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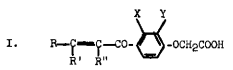
The chemistry of a new class of diuretic agents has recently been described by Schultz *et al*(1). We have studied these compounds in the dog and find that their activity differs in several ways from that of the thiazides, the organomercurials or other previously described saluretic or diuretic agents with which we have worked. In this paper are reported briefly the biological properties that are characteristic of these α,β-unsaturated ketone derivatives of aryloxyacetic acids (I). Their biological activity is illustrated by data from experiments in which the following specific examples were used:

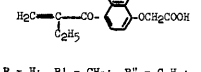


I.

II. R,R',Y = H; R" = C_2H_5; X = Cl — 3-Chloro-4-(2-methylenebutyryl)-phenoxyacetic acid

III. R,R' = H; R" = C_2H_5; X,Y = Cl — 2,3-Dichloro-4-(2-methylenebutyryl)-phenoxyacetic acid

IV. R,R' = H; R" = C_2H_5; X,Y = CH_3 — 2,3-Dimethyl-4-(2-methylenebutyryl)-phenoxyacetic acid

V.  — 4-(2-Methylenebutyryl)-naphthyloxy-acetic acid

VI. R = H; R' = CH_3; R" = C_2H_5; X,Y = Cl — 2,3-Dichloro-4-(2-ethylidenebutyryl)-phenoxyacetic acid

Methods. Trained unanesthetized fasted female dogs were used in the experiments. In the renal clearance studies, glomerular filtration rate (GFR) was estimated using creatinine, injected subcutaneously. A pH 7.4 phosphate buffer containing 4% mannitol was administered intravenously at 3.0 ml/min to provide a urine flow of 1.0-4.0 ml/min. The dogs received no other salt supplementation prior to or during these experiments. Replicate clearance periods were of 7.5-15 minutes' duration, the bladder being emptied through an indwelling catheter with washout. Each experiment described was substantiated by one or more replicate experiments in different dogs. In addition, the results cited for one compound are typical of those obtained with one or more of the other compounds I-VI, these data in turn being substantiated for each compound by 2 or more experiments in different dogs. The analytical methods were similar to those previously described (2,3), except that sodium and potassium were determined using the Autoanalyzer flame photometer.

Results and discussion. Acutely, the compounds produced a prompt and dramatic saluretic-diuretic effect when given intravenously or orally to dogs. Thus, a single intravenous dose of 1.0 mg/kg of III caused a rapid increase in excretion of sodium, chloride and water. The maximal natriuretic effect was much greater than was obtained with the same dose of hydrochlorothiazide (Fig. 1). The peak effect diminished rapidly, but, even at the end of an hour, the rate of sodium excretion exceeded the highest rate obtained with the thiazide. A total of 63.3 meq of sodium was excreted in the first hour. Although potassium excretion was also enhanced by III, the increase was less marked

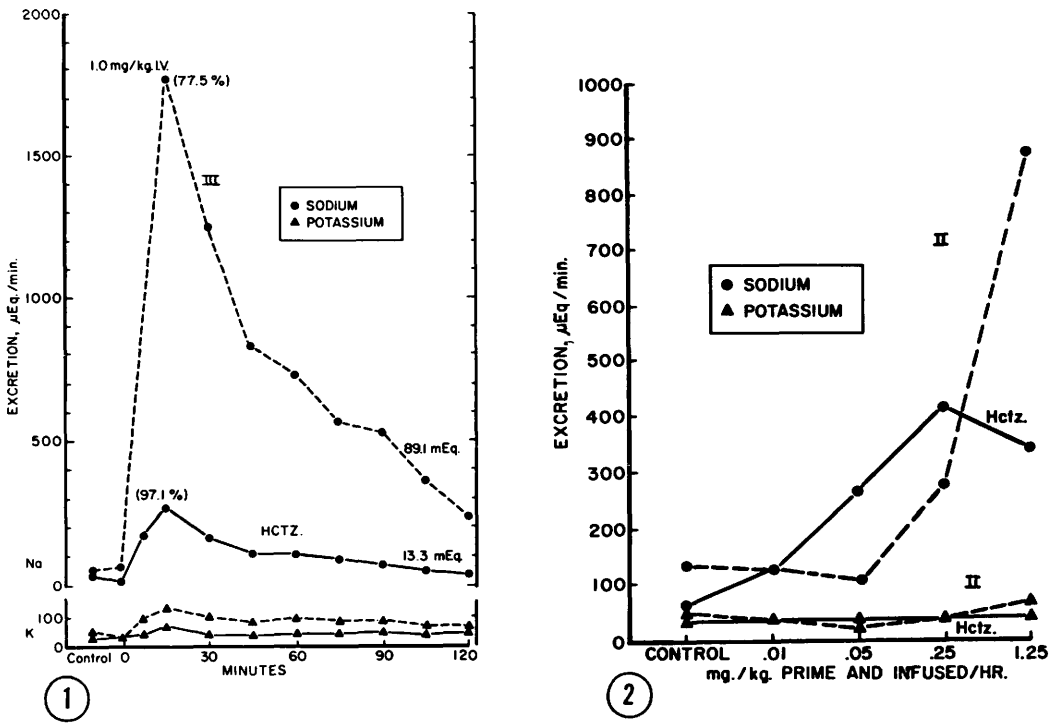


FIG. 1. Effect of single intravenous dose of 1.0 mg/kg on cation excretion rate in unanesthetized dogs. At 0 min one dog received 2,3-dichloro-4-(2-methylenebutyryl)-phenoxyacetic acid (III) ·····, and the other dog received hydrochlorothiazide ———. Phosphate buffer was given at 3.0 ml/min, intravenously, to both dogs throughout experiment (see Methods). Sodium rate (μeq/min) ●, top pair of curves; in parentheses, % of filtered load reabsorbed, as calculated for the clearance period of greatest sodium excretion rate; at right, total mEq sodium excreted in 120 min. Potassium rate (μeq/min) ▲, bottom pair of curves.

FIG. 2. Cation excretion pattern as hydrochlorothiazide ——— or 3-chloro-4-(2-methylenebutyryl)-phenoxyacetic acid (II) ····· was given intravenously in stepwise increments to Dog 834. Dog received drug as prime and 20-min venoclysis as indicated, two 10-min clearances were measured, then next dose level was given. Each point is average for the pair of clearance periods.

than that of sodium.

In renal clearance experiments, the diuretic-saluretic effect exceeded the maximal natriuretic and chloruretic effect obtainable with a maximally effective dose of hydrochlorothiazide. In Table I are shown data from typical renal clearance experiments using IV, V and hydrochlorothiazide at a dose sufficient to produce a maximal effect with the latter(4). Sodium was excreted at rates of 1539 and 1019 μeq/min following IV and V, compared to 361 μeq/min after hydrochlorothiazide. Thus, these compounds produced excretion rates 3 to 5 times that obtainable with the thiazides, of which hydrochlorothiazide is a prototype. Whereas hydrochlorothiazide depressed tubular sodium reabsorption to 95% of the filtered load from a control of 99.4%, the same dose of IV and V depressed sodium reabsorption to 85% of the filtered load. Chloride excretion was greatly increased. The chloruresis was nearly equivalent to that of sodium. The effect of IV and V on the potassium excretion rate was much less on a molar basis.

There were qualitative as well as quantitative differences between the action of the aryloxyacetic acids and the thiazides. Diuresis induced by the aryloxyacetic acids IV and V was striking (Table I). Urine flow rates exceeding 10 ml/min were obtained, representing in the former case a 12-fold increase in urine flow over the control rate. Hydrochlorothiazide had only a modest diuretic effect, and the rate, 2.5 ml/min, was less than the rate at which the sustaining veno-

TABLE I. Typical Renal Clearance Experiments in Dogs Given Aryloxyacetic Acids or Hydrochlorothiazide.*

	Excretion, μeq/min			Urine			
	Na^+	K^+	Cl^-	ml/min	pH	GFR	% Na reabsorbed
Dog 843							
Control phase	37	17	1	.8	6.5	45	99.4
Hydrochlorothiazide	361	27	237	2.5	7.5	48	94.9
Dog 914							
Control phase	17	29	19	1.0	8.3	62	99.8
IV†	1539	130	1521	12.3	7.1	72	85.9
Dog 893							
Control phase	13	13	4	4.3	5.8	52	99.8
V†	1019	89	1131	10.6	6.7	50	85.7

* Each figure is average of 2 or more successive 10-min clearance periods. Following the control phase, a priming dose of drug (2.5 mg/kg) was given, and drug was infused at 3 mg/kg/hr for a 20-min equilibration period and continued during the collection of clearance urines. This dose of hydrochlorothiazide had been shown [Baer *et al.*(3)] to produce a maximal saluretic effect.

† IV = 2,3-Dimethyl-4-(2-methylenebutyryl)-phenoxyacetic acid; V = 4-(2-Methylenebutyryl)-naphthyloxyacetic acid.

clysis was given. Qualitatively, also, hydrochlorothiazide produced an increase in the Na/Cl ratio (361/237 = 1.5) and in urinary pH that is typically seen at high doses of thiazides. In contrast, the Na/Cl ratio was nearly unity following IV and V, and urine pH was not consistently affected. In numerous other studies with high doses of the aryloxyacetic acids, we have noted a tendency for urinary pH to drop slightly and for the Na/Cl ratio to be slightly less than one.

The aryloxyacetic acids had a steeper dose-response curve than the thiazides. The relative activity was examined in clearance experiments in which the dosage was increased in 5-fold increments (Fig. 2). Hydrochlorothiazide was minimally active at 0.01 mg/kg and maximally active at the 0.25 mg/kg level, where a ceiling rate of 400 μeq/min was attained. In contrast, II was inactive at the lowest dose level, 0.01 mg/kg, but the sodium excretion rate increased sharply with increasing dosage to a rate of nearly 900 μeq/min at the top level (1.25 mg/kg); this was nearly twice the maximum rate attained with hydrochlorothiazide. Potassium excretion was not similarly increased, however.

After oral administration to dogs, the aryloxyacetic acids caused greater loss of sodium and chloride than did the thiazides. Thus, an increasing effect of VI was seen on sodium chloride excretion as the oral dose was increased from a low effective dose of 0.5 mg/kg (Table II). At 10 mg/kg, salt excretion exceeded that obtained with a maximal effective dose (3.0 mg/kg) of chlorothiazide (4).

Both in acidotic and alkalotic dogs a pronounced excretion of sodium chloride and water was obtained with the aryloxyacetic acids (Table III). In the ammonium chloride-treated dog, the pattern of electrolyte response was similar to that obtained with the thiazides, but markedly greater in magnitude. In addition, there was a more profound diuresis. Thus, sodium reabsorption was depressed from 99.5% to 87% of the filtered load; the Na/Cl ratio became < 1.0.

More surprising, the chloruresis and acidi-

TABLE II. Comparison of Electrolyte Excretion in Dogs, as Measured During a 6-Hour Period Following Oral Administration of Various Dose Levels of 2,3-Dichloro-4-(2-ethylidenebutyryl)-phenoxyacetic Acid (VI) or After a Maximally Effective Dose of Chlorothiazide(4). Data are averages for groups of animals.

Compound	Dose, mg/kg	No. of dogs	Excretion/6 hr, meq Na^+	K^+	Cl^-
Control	—	6	5.3	2.3	6.2
VI	.5	6	12.0	4.9	18.9
	2.0	8	11.0	2.0	17.5
	5.0	4	29.6	5.6	39.7
	10.0	2	41.3	6.0	50.7
Chlorothiazide	3.0	12	26.2	7.9	26.0

TABLE III. Effect of Acidosis or Alkalosis on Response to 3-Chloro-4-(2-methylenebutyryl)-phenoxyacetic Acid (II) in Renal Clearance Experiments in Dogs.*

		Excretion, μeq/min				Urine		
		Na^+	K^+	Cl^-	HCO_3^-	ml/min	pH	GFR
Dog 796; NH_4Cl: 100 meq/day × 4 days (B_{CO_2} = 17 mmol/l)	Control	44	20	14	2	1.0	6.4	63
	II	1288	109	1473	2	12.6	6.3	63
Dog 800; $NaHCO_3$: 100 meq/day × 4 days (B_{CO_2} = 24 mmol/l)	Control	348	128	25	357	2.2	8.1	60
	II	1182	142	1128	207	10.2	7.3	54

* Data are averages of triplicate successive clearance periods. Following the control phase, a priming dose of II (1 mg/kg) was given, and drug was infused at 3 mg/kg/hr for a 20-min equilibration period and continued during the collection of clearance urines.

fication of the urine were seen, even in the sodium bicarbonate-pretreated animal. Bicarbonate excretion was decreased in the presence of II, as chloride, sodium, hydrogen and potassium excretions were increased. The significance of this observation to the mode of action of the aryloxyacetic acids is not clear, but the pattern is quite unlike that obtained with the thiazides, acetazolamide or the organomercurials.

A unique pattern of diuretic activity for this structurally novel class of compounds emerges from the several types of experiments presented above. The magnitude of the natriuretic effect exceeds that attainable with even maximal doses of thiazides. The increase in potassium excretion is less impressive, whether on an absolute basis or relative to the kaliuresis induced by thiazides. The profound increase in urine flow produced by the present series is in contrast to the unimpressive effect of thiazides in acute experiments such as these. Furthermore, the thiazides are likely to increase the Na^+/Cl^- ratio and the urine pH, especially at maximally effective doses or under conditions of bicarbonate loading.

In contrast to the organomercurials, which also cause a profound increase in salt and water excretion, the phenoxyacetic acids show a very rapid onset of action when given intravenously to dogs and are effective orally as well. Furthermore, the aryloxyacetic acids appear remarkably effective as saluretic agents whether the dogs are acidotic, alkalotic or in acid-base balance.

Present attempts to demonstrate saluretic-diuretic activity of these agents in the rat have been unsuccessful, but preliminary studies of one of the compounds (III, ethacrynic acid) in man are encouraging(5,6).

Summary. 1. The diuretic-saluretic properties of a new series of compounds, the α,β-unsaturated ketone derivatives of aryloxyacetic acids, have been described. 2. These compounds cause a prompt and profound water diuresis and the excretion of nearly equivalent amounts of sodium and chloride when administered parenterally or orally. Some increase in potassium excretion is produced, especially following intravenous administration. 3. These compounds have a steeper dose-response curve and a substantially greater maximal saluretic effect than the thiazides. 4. The magnitude of the diuretic effect is similar to that seen with organomercurials. In distinction to the organomercurials, these compounds have a very rapid onset of action and are effective even in sodium bicarbonate-treated dogs.

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