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Received Sept. 14, 1967. P.S.E.B.M., 1968, Vol. 127.

Sex Specific Diuresis (32701)

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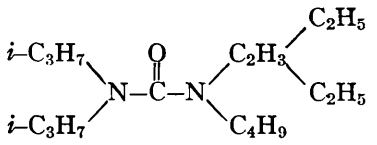
During a general biological investigation of a series of aminoalkylureas, certain of these compounds exhibited significant diuretic properties. The most potent of these, *N,N*-diisopropyl-*N'*-*n*-butyl-*N'*-diethylaminoethylurea (P-275),



was found to be an effective saluretic only in male animals. A close homologue *N,N*-diisopropyl-*N'*-isoamyl-*N'*-diethylaminoethylurea (P-286), differing from P-275 merely by a methyl group, has received extensive attention as a selective autonomic blocking agent (1–3). Whereas P-275 also has this blocking property, but is slightly less potent, laboratory study indicated that P-286 was devoid of diuretic activity. This was of interest in that the latter compound was found to be diuretic in the human and will be discussed in terms of the animal laboratory findings.

Materials and Methods. Evaluation of diuretic efficacy was determined mainly in conscious animals of two species. Male rats, weighing 50–100 gm, were subjected to the experimental procedure described by Lipschitz *et al.* (4) using a four-point biological assay. (5) Compounds were administered in the hydration fluid (isotonic NaCl, 25 ml/kg by stomach tube) and urine collected for 5 hours immediately thereafter.

Dogs of either sex were given test compound or placebo, orally in capsule form, and 60–90 min later were loosely restrained in a recumbent position. The bladder was emptied, employing an indwelling catheter. An iv infusion of 5% glucose was started and continued at a rate of 2 ml/min during the subsequent 2 hours of urine collection. All animals were without food for 16 hours prior to the experiment. Urinary Na^+ was determined in all experiments, and urinary K^+ in a few, using a Beckman DU flame spectrophotometer.

Certain surgical and hormonal treatments were completed, to investigate the sex-dependent diuretic effect of P-275. Female dogs were spayed under asceptic conditions and the animals were allowed a 30-day recuperative period before drug testing was continued. Male dogs treated with estrogen received 15 μgm/kg of estradiol benzoate daily im (9) for 8 days before drug testing was continued. Female dogs treated with testosterone received daily im injections of testosterone propionate in a dose of 0.5 mg/kg (10,11). Drug testing was continued beginning with the fourth week of such treatment. All injections of these hormones were made into the hind legs. The P-275 and P-286 were used as the HCl salts.

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TABLE I. Four-Point Diuretic Assay.[a]

	P-275		Urea		
Test	log 1.92169 25 mg/kg	2.22246 50 mg/kg	4.09656 750 mg/kg	4.39751 1500 mg/kg	Total
1	2.94	4.88	2.69	3.40	13.91
2	1.52	2.67	1.73	2.33	8.25
3	3.00	4.92	2.13	5.07	15.12
Total	7.46	12.47	6.55	10.80	37.28
Mean	2.49	4.16	2.18	3.60	
	log 0.39620	0.61909	0.33846	0.55630	

[a] Each value represents the ratio of urinary volume for eight rats receiving drug to eight control rats.

Para-aminohippurate (PAH) clearance for measuring effective renal plasma flow (RPF) (6) and creatinine clearance for determining glomerular filtration rate (GFR) (7) were performed in 6 dogs receiving test compound parenterally. Fifteen-minute urinary collection periods were used. Blood samples were obtained from a femoral vein midway through each period. Plasma protein was precipitated by an adaptation (6) of the cadmium sulfate-NaOH method of Fujita and Iwatake. Creatinine for both serum and urine was determined by the Jaffe reaction (8). The creatinine and PAH were placed in isotonic NaCl and infused at 1 ml/min to maintain the appropriate plasma concentrations. Priming infusions of these substances were given 45 min before the start of the collection periods.

Results. Rat bioassay. The data determining diuretic potency is presented in Table I. Each value represents the ratio of 8 animals with drug to 8 control animals. Although the parameter was urine volume, total urinary Na^+ was determined and with P-275 was proportional to volume. The logarithm of the doses (mM/kg) in Table I was increased by 10^3 to circumvent negative logarithms for P-275 in the calculations.

In Table II these data are subjected to variance analysis for determining the validity of the assay. Since the variance ratio (0.05: 0.38) indicated that the dose–response regression lines were parallel, relative potency was calculated according to the following formula,

$$M = x_s - x_t - \frac{Y_s - Y_t}{b}$$

Using the logarithmic dose scale,

$$\log R = \log \frac{12490}{83.5} + 0.56 \times 0.1505$$

$$R = 149.6 \times 1.2134 = 181.5$$

Thus P-275 was approximately 182 $\times$ more potent than urea on a mole basis.

Renal studies in dogs. Creatinine and PAH clearance studies were completed in anesthetized (pentobarbital Na, 30 mg/kg) female dogs receiving 2.5 mg/kg doses (2 expts.) and 5.0 mg/kg (3 expts.) doses of P-275 injected into the left renal artery. Drug administration was preceded by 1 control period and followed by 13 consecutive test periods. The drug (solution buffered to pH 6.9) was found to be without effect on GFR and RPF. At

TABLE II. Analysis of Variance for the Data of Table I.

(Adjustment for mean . . . 115.82)

Nature of variation	*df*	Sum of squares	Mean of square
Preparations	1	0.55	
Regression	1	7.15	
Parallelism	1	0.05	0.05
Between doses	3	7.75	
Between tests	2	6.72	
Error	6	2.27	0.38
Total	11	16.74	

the higher dose approximately a 6-fold increase in Na^+ excretion occurred on the injected side during the first 15-min test period. However, this was short lived, returning to control rates within the subsequent 15-min test period. A clearance experiment on an unanesthetized male dog demonstrated that P-275 in a dose of 5 mg/kg iv also was without effect on GFR and RPF. This experiment was composed of 2 collection periods before and 6 following drug administration. Slightly more than a 2-fold increase in Na^+ excretion was recorded, beginning 15 min after treatment and with a duration about 45 min. From these experiments it was assumed that P-275 was excreted or transformed too rapidly after a single intravascular injection to exert any potent or prolonged diuretic effect.

Listed in Table III are the Na^+ excretions

TABLE III. Average Na^+ Excretion (meq/2 hours) Relative to Sex and Hormonal Treatment.

Dogs	Control (placebo)	P-275 (10 mg/kg)
Female	0.97 (12)[a]	1.70 (11)
Male	3.31 (9)	15.52 (9)
Female, spayed	1.36 (6)	8.98 (6)
Male, estrogen	2.72 (3)	2.09 (3)
Female, testosterone	1.53 (6)	2.45 (6)

[a] No. of expts. in parentheses.

in unanesthetized dogs having received a placebo or P-275 in an oral dose of 10 mg/kg. The male animals responded to the drug with approximately a 5-fold increase in Na^+ excretion whereas the females had less than a 2-fold increase. When one of the males, that consistently responded well to the renal effect of P-275, was treated with estrogen a refractoriness to the diuretic action developed. Responsiveness to the drug was present again when tested 4 weeks after cessation of estrogen treatment. Removing the ovaries of one of the female dogs established diuretic efficacy in this animal while two females treated with testosterone but not spayed remained refractory.

Discussion. It is interesting to speculate that this compound is not unique as a sex specific diuretic. So far as we are aware, all initial diuretic testing is accomplished on the male rat. This probably derives from the classic antidiuretic assay of Burn (12) in which females are not used because of their variable (urinary) behavior. When such testing is carried over to the dog, the female of the species is used, especially if the animal is not anesthetized. Although the sex related tolerance to the saluretic effect may be considered, most reasonably, a direct antagonism by estrogen, an indirect effect through adrenal cortical hormone cannot be ignored, since estrogen treatment increases the concentration of unbound plasma cortisol (13).

Drug–corticosteroid antagonism has been demonstrated with certain diuretic agents (14). In the present study urinary K^+ was measured only in the female dogs treated with testosterone (Table III). The P-275 was found to decrease the total excretion of this ion from an average of 3 meq to 1.6 meq, thus giving evidence of a property compatible with antiadrenal steroidal activity. The diuretic potency of P-275 in the dog, however, was of such a magnitude (about 5-fold increase in Na^+ excretion) that estrogen treatment could mask its effect by causing a nearly equivalent increase in unbound plasma cortisol. (In the studies of Plager *et al.* estrogen increased the unbound plasma cortisol about 4 fold.)

Allied to these considerations were the results of a single trial with the isoamyl homologue, P-286, in the human. Because selective adrenal medullary blockade had been demonstrated by this compound, its therapeutic use in patients with pheochromocytoma had been contemplated. For this purpose the drug was administered to a 51-year-old female with a diagnosed malignant pheochromocytoma, who was undergoing menopause and whose condition was further complicated by hyperadrenocorticism. Within 72 hours of the onset of therapy (250 mg qid. orally) the patient had a 5.8 kg weight loss due to an enhanced urinary output. Reinstituting therapy following a 3-week respite again produced an increased urine flow during the first 72 hours, a 3 kg weight loss and a 3-fold increase in urinary Na^+ excretion. On both occasions the greatest urinary response occurred on the first day of treatment although diuresis was

still apparent on the fourth day of continued drug administration. During these treatment periods there was no significant improvement in the cardiovascular symptoms arising from the tumor. Weight gain resulted only after cessation of treatment.

The difference between laboratory and clinical findings regarding diuretic activity for P-286 cannot be explained presently. However, the close chemical similarity between P-286 and P-275 and the results obtained make it tempting to speculate that these compounds act in the kidney through an antiadrenal steroid mechanism.

Summary. A renal evaluation of a series of aminoalkylureas administered orally to male rats disclosed several compounds with diuretic activity, the most potent of which (P-275) was studied in some detail. When administered intravascularly to dogs, P-275 was without effect on glomerular filtration and renal plasma flow while sodium and water excretion was either only transiently or moderately elevated. Oral administration to dogs produced Na^+ and water diuresis in males. Removing the ovaries induced responsiveness in the female while treatment of the male with estrogen produced a reversible refractoriness to the saluretic effect of this compound. Testosterone treatment failed to overcome the refractory state to P-275 in the intact female. These findings plus the results from an isolated clinical case with the chemical congener, P-286, are discussed in terms of a possible antagonism of adrenal cortical hormone.

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Received Sept. 14, 1967. P.S.E.B.M., 1968, Vol. 127.

An Additional Effect of Yohimbine-HCl (32702)

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That yohimbine-HCl affects the endocrine organs and the reproductive cycle of the female rat has been amply demonstrated. Twenty mg of yohimbine-HCl per kg of body weight induced pseudopregnancy, caused decidual reaction of traumatized uteri, increased the weight of the ovaries and adrenal glands, enlarged corpora lutea, and produced lobuloalveolar development of the mammary glands. These effects were postulated to be due to a release of luteotropin by the action of yohimbine on the hypothalamo–hypophyseal axis (1,2).

In the present study several new aspects of yohimbine action were studied. An attempt was made to determine to what extent the endocrine effects were mediated by stress. In addition, the growth rate of young rats