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STUDIES ON THE RENAL ELIMINATION OF N-METHYLPYRIDINIUM-2-ALDOXIME*

FREDRIK BERGLUND†, CARL-ERIC ELWIN and ANDERS SUNDWALL

Department of Pharmacology, Karolinska Institutet, Stockholm 60,
and the Research Institute of National Defence, Dept. 1,
Sundbyberg 4, Sweden

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Abstract—The renal elimination of N-methylpyridinium-2-aldoxime has been studied in unanaesthetized dogs. The amount excreted in the urine always exceeds the amount filtered and a secretory Tm has been demonstrated. Although the drug is a weak acid the secretion is not depressed by probenecid.It is preferentially excreted into an acid urine. The excretion is markedly reduced by alkalosis produced by infusion of sodium bicarbonate. Alkalinization of the urine with acetazolamide has no such effect. On the contrary the excretion is increased. It is suggested that active transport is the principal transport mechanism but an element of non-ionic diffusion cannot be excluded.

N-METHYLPYRIDINIUM-2-ALDOXIME is a valuable antidote against nerve gases and certain insecticides.[1] The antidotal action is unfortunately of short duration, and is greatly reduced if the drug is injected more than one hour before the toxic agent[2, 3] or if the latter is applied on to the skin.[4] Studies of the half-life of oxime in plasma also indicate that the oxime concentration in the blood falls below effective levels within 1–2 hr.[5, 6, 7] As the clearance of the drug inman exceeds that of creatinine,[7] a study was undertaken of its mechanism of elimination by the kidneys in order to see whether the duration of action could be prolonged by various means.

MATERIALS AND METHODS

Substances used. N-methylpyridinium-2-aldoxime methane sulphonate (P2S) was synthetized according to Creasy and Green.[8] Other substances used were commercially available.

Urinary excretion. The experiments were performed in unanaesthetized female dogs. Constant plasma levels were maintained through intravenous injections and infusions. The glomerular filtration rate was measured by the clearance of exogenous creatinine. Venous blood samples were used throughout, as the concentration of P2S in them did not differ significantly from that in arterial blood during constant infusion. Urine was obtained through an in-dwelling urethral catheter, and residual urine was rinsed from the bladder with 20 ml of water, and with air.

Analytical methods. P2S was analysed by the method of Creasy and Green.[8] For plasma a blank sample was always taken before administration of P2S. Urine was diluted 1:25 or 1:50 before analysis. The blank value in the urine was negligible. Drugs or other substances given during the experiments did not interfere with the analysis of P2S. Creatinine was analysed according to Bonsnes and Taussky.[9]

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† Present address: Department of Pharmacology, University of Göteborg, Göteborg, Sweden.

RESULTS

Tubular secretion of P2S. The amount of P2S excreted in the urine always exceeded the amount filtered in the glomeruli. Thus P2S is secreted by the renal tubules. A secretion Tm for P2S was demonstrated in two dogs (Fig. 1). The Tm was reached at a plasma concentration of about 6–8 μg per ml.

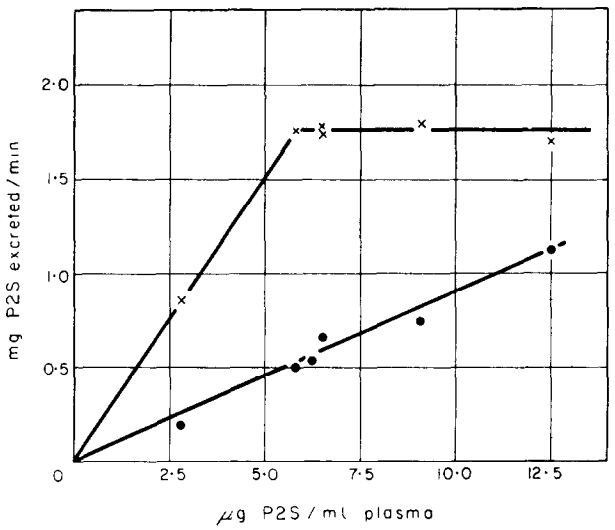


FIG. 1. Urinary excretion of P2S at different plasma levels. Dog 4, 19 kg Expt. nos. 23, 24 and 25. Pretreated with ammonium chloride. Urine pH 5·6. Each symbol corresponds to 3 clearance-periods.
× secreted amount of P2S.
● filtered amount of P2S.

TABLE 1.—EFFECT OF PROBENECID ON TUBULAR SECRETION OF P2S

Time	Urine pH	Urine flow	GFR	P2S			
				Plasma	Filtered	Excreted	Secreted
min		ml/min	ml/min	μg/ml	mg/min	mg/min	mg/min
	Expt. no. 22		Dog 4, 19 kg		Date: July 20, 1961		
−12	300 ml H_2O orally						
0	Prime: 0·25 g P2S. Infuse: 0·23% P2S, 0·9% NaCl, 1 ml/min						
29– 39	6·4	4·3	76	11·9	0·90	1·98	1·08
39– 49	6·6	5·7	69	11·9	0·82	1·75	0·93
49– 59	6·5	6·2	69	11·7	0·81	1·63	0·82
59– 69	6·7	5·6	59	10·3	0·61	1·69	1·08
81	Prime: 0·5 g probenecid. Infuse: as above + 1·3% probenecid, 1 ml/min						
93– 99	7·3	1·4	71	10·1	0·71	1·59	0·88
99–109	7·4	2·5	76	10·0	0·76	1·68	0·92
109–119	7·3	2·1	65	9·8	0·64	1·42	0·78
119–129	7·4	2·0	85	9·8	0·84	1·77	0·93

GFR = Glomerular Filtration Rate.
Filtered = GFR × Plasma P2S.
Secreted = Excreted − Filtered.

Effect of probenecid. The demonstration of a secretory Tm indicated that the P2S might be actively transported through the renal tubular epithelum. As P2S is a weak acid, we tried to depress its secretion with probenecid. However, large doses of probenecid (25 mg/kg + 40 mg/kg/hr intravenously) failed to depress the secretion of P2S significantly (Table 1).

Effect of metabolic acidosis and alkalosis. Four dogs were pretreated with 6 g ammonium chloride orally for three days preceding the experiments in order to get an acid urine. Alkalosis was then induced by intravenous infusion of 0·6 M sodium bicarbonate. With this technique urine pH's from 5·1 to 8·2 were obtained. It was found that the P2S was preferentially secreted into an acid urine. In alkaline urine the amount of P2S secreted was markedly reduced (Fig. 2 and Table 2). In some

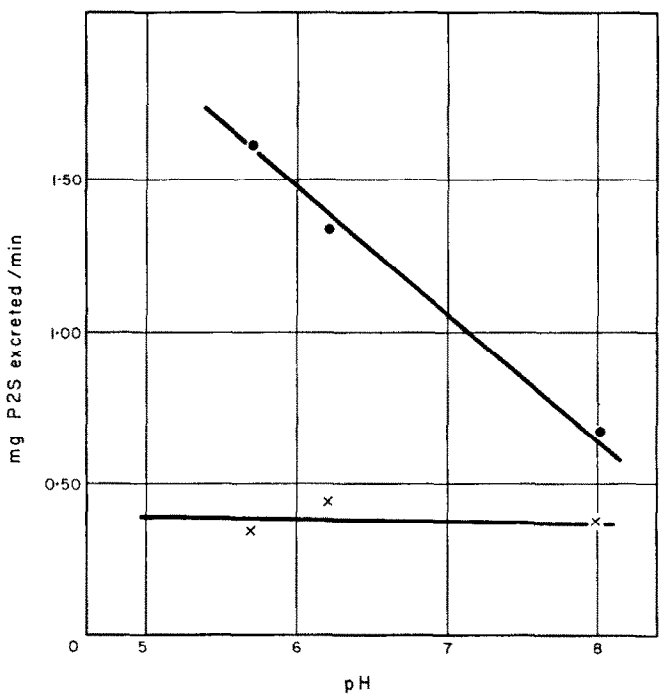


FIG. 2. Urinary excretion of P2S at different urine pH's. Expt. nos 13 and 16. Low pH (5·7 and 6·2) after NH_4Cl-loading, high pH (8·0) after infusion of $NaHCO_3$. Plasma level of P2S 9·5–9·9 μg/ml in all three instances.
● urinary excretion of P2S.
× filtered amount P2S.

experiments the clearance ratio ($C_{P2S}/C_{Creatine}$) became close to unity. On the other hand clearance ratios less than unity were never observed, i.e. net reabsorption did not occur.

Effect of acetazolamide. If acetazolamide was infused in dogs pretreated with ammonium chloride, the urine rapidly went alkaline. Surprisingly enough the urinary excretion of P2S was not depressed, but actually increased slightly (Table 3, expt. 16).

If the urine was first alkalinized by infusion of sodium bicarbonate, the subsequent administration of acetazolamide increased secretion of P2S markedly (Table 3, expt. 18).

Effect of urine flow. Increase in urine flow, due to hydration, from about 1 to about 10 per cent of GFR, with a constant urine pH, did not influence the amounts of P2S excreted (Expts 13 and 16, Tables 2 and 3).

TABLE 2.—EFFECT OF URINE pH ON TUBULAR SECRETION OF P2S. ALKALINIZATION WITH SODIUM BICARBONATE

The dogs were pretreated with NH_4Cl 6 g daily for three days.

Time	Urine pH	Urine flow	GFR	P2S			
				Plasma	Filtered	Excreted	Secreted
min		ml/min	ml/min	μg/ml	mg/min	mg/min	mg/min
	Expt. no. 14		Dog 4, 16 kg		Date: June 21, 1960		
−43	300 ml H_2O orally						
0	Prime: 0·15 g P2S. Infuse: 0·15% P2S, 0·9% NaCl, 1 ml/min						
31– 37		5·2	20	7·7	0·16	0·87	0·71
37– 43	6·5	5·7	21	7·3	0·15	0·80	0·65
43– 49	6·7	5·3	21	6·8	0·15	0·78	0·63
49– 55	6·7	5·2	20	6·5	0·13	0·76	0·62
55	Prime: 48 mmoles $NaHCO_3$. Infuse: as above + 0·6 M $NaHCO_3$, 1 ml/min						
78– 84	8·0	3·3	21	7·0	0·15	0·41	0·27
84– 90		3·2	26	6·5	0·17	0·39	0·22
90– 96	8·0	3·0	31	6·6	0·20	0·41	0·21
96–102		3·0	35	6·6	0·23	0·43	0·20
	Expt. no. 13		Dog 3, 14 kg		Date: June 13, 1960		
−182	300 ml H_2O orally						
0	Prime: 0·15 g P2S. Infuse: 0·15% P2S, 0·9% NaCl, 1 ml/min						
32– 42	5·8	0·2	29	10·6	0·31	1·08	0·76
42– 57	5·7	0·3	38	9·6	0·37	1·85	1·48
57– 72	5·5	0·8	40	10·0	0·40	2·14	1·75
72– 87	5·5	5·0	46	9·1	0·42	1·81	1·39
87– 97	5·8	5·7	28	9·2	0·22	1·19	0·97
97	Prime: 84 mmoles $NaHCO_3$. Infuse: as above + 0·6 M $NaHCO_3$, 1 ml/min						
121–131	8·0	5·8	41	9·1	0·37	0·66	0·33
131–141	8·1	4·1	32	10·0	0·32	0·70	0·42
141–151	8·0	4·6	51	10·0	0·51	0·76	0·28
151–159	8·0	4·5	31	10·3	0·32	0·55	0·24

DISCUSSION

The results obtained indicate that P2S might be actively secreted by the renal tubules. Different mechanisms of active transport were considered. Although P2S is a weak acid, its secretion was not blocked by probenecid. P2S is therefore probably not secreted by the same mechanism as *p*-aminohippurate, penicillin, phenolsulphonphtalein, etc.[10] The molecules secreted by this mechanism contain certain characteristic groups.[11] These include an unsaturated oxygen group (—C:O ; —S:O), able to form a hydrogen bond at the unsaturated oxygen, and an ionic function (—COOH; —SO_2OH; SO_2NH—), able to form both a hydrogen bond and an ionic bond. P2S lacks these typical groups.

Although P2S is a weak acid, it contains a quaternary nitrogen and structurally resembles N-methylnicotinamide, It might therefore be secreted by the base-secreting

mechanism (N-methylnicotinamide, etc.),[12] but this alternative has not been satisfactorily tested. The basic cyanine dye 1′-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2′-cyanine chloride (no. 863), which inhibits this system,[12] unfortunately interfered with the P2S analysis.

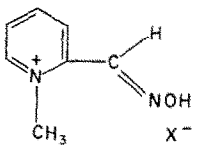
TABLE 3.—EFFECT OF URINE pH ON THE TUBULAR SECRETION OF P2S. ALKALINIZATION WITH ACETAZOLAMIDE

Time	Urine pH	Urine flow	GFR	P2S			
				Plasma	Filtered	Excreted	Secreted
min		ml/min	ml/min	μg/ml	mg/min	mg/min	mg/min
	Expt. no. 16		Dog 3, 16 kg		Date: Sept. 26, 1960		
-45	300 ml H_2O orally						
0	Prime: 0·2 g P2S. Infuse: 0·17% P2S, 0·9% NaCl, 1 ml/min						
62– 77	6·2	0·2	35	13·8	0·49	1·40	0·91
77– 92	6·1	0·3	—	12·6	—	1·40	—
92–107	6·0	2·2	51	9·1	0·46	1·31	0·85
107–117	6·1	4·5	46	9·6	0·45	1·29	0·84
117–127	6·4	3·4	43	9·7	0·42	1·43	1·01
127	Prime: 0·15 g *acetazolamide*. Infuse: as above + 0·5% *acetazolamide*, 1 ml/min						
137–143	8·0	3·3	44	8·6	0·38	1·73	1·35
143–149	8·1	3·4	41	7·6	0·31	1·55	1·24
149–155	8·1	2·2	37	9·1	0·34	1·45	1·11
155–161	8·2	1·2	32	8·8	0·28	1·26	0·97
	Expt. no. 18		Dog 4, 18 kg		Date: Jan. 12, 1961		
-46	300 ml H_2O orally						
0	Prime: 0·175 g P2S. Infuse: 0·17% P2S, 0·6 M $NaHCO_3$, 1 ml/min						
116–122	7·7	4·2	35	8·4	0·29	0·65	0·36
122–128	7·8	3·3	34	8·0	0·27	0·70	0·43
128–134	7·9	2·3	33	8·1	0·27	0·58	0·31
134–140	8·1	1·9	31	7·0	0·22	0·61	0·39
140	Prime: 0·15 g *acetazolamide*. Infuse: as above + 0·46% *acetazolamide*, 1 ml/min						
148–154	8·1	2·5	25	5·8	0·15	0·70	0·55
154–160	8·2	2·8	29	5·6	0·16	0·83	0·67
160–166	8·2	2·0	21	6·1	0·13	0·77	0·64
166–172	8·2	2·1	24	6·0	0·14	0·79	0·65

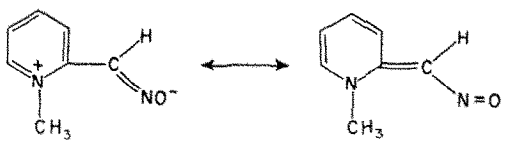
The possibility of non-ionic diffusion was also considered. P2S is an acid with pK_a of 7·82.[13] If "non-ionic diffusion" through the tubule cells played a rôle in its excretion, one might expect higher excretion into alkaline urine than into acid urine. However, the opposite occurred after infusion of sodium bicarbonate. Actually at pH 6, 98·5 per cent of the oxime is in the undissociated form (left in Fig. 3) which is highly polar and cannot be expected to penetrate lipoid membranes at any appreciable rate by simple diffusion.[14] At pH 7·8 about 50 per cent of the compound is in the dissociated form, which is a resonance hybrid between the two structures shown to the right in Fig. 3, and is soluble in chloroform to about 2 per cent.[15] This might explain how the un-ionized but polar P2S would be preferentially trapped in an acid urine, as found in the experiments with ammonium chloride and sodium bicarbonate. On the other hand, the excretion of P2S is increased by acetazolamide. Whether this effect is due to changes in intracellular hydrogen or potassium ion concentration

or to a direct stimulation of the secretory mechanism, is impossible to judge from the present experiments.

In summary, P2S is eliminated by the kidneys by glomerular filtration and tubular secretion. The exact mechanism of its tubular secretion is not known. Of practical interest is, however, the fact that systemic acidosis favors the urinary excretion of P2S.



Undissociated form insoluble in chloroform



Dissociated form 2% soluble in chloroform

FIG. 3. Structural formulas of P2S at low pH (above) and high pH (below).

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