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Renal Tubular Secretion of Pralidoxime in Man (38118)

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Pralidoxime is a quaternary ammonium compound used to reactivate organophosphate-inhibited cholinesterase *in vivo*. It has been established that pralidoxime is rapidly excreted by the kidney with a clearance rate approaching that of *p*-aminohippurate (PAH) (1, 2). Therefore, it appears that active tubular secretion is the major mechanism for elimination of the drug from the body (3).

Several studies have attempted to characterize the renal secretory mechanism for pralidoxime elimination, but these studies have been inconclusive. It has been shown, for example, that organic acids such as probenecid (3–5) and salicylates (4) do not interfere with pralidoxime excretion. The effect of organic bases (e.g., nicotinamide) on pralidoxime excretion has been studied (4), but no conclusive results have been forthcoming.

The frequent description of pralidoxime as a "weak acid" (3, 4, 6) (on the basis of the dissociation of the aldoxime group to an anion and H+) has only confused the discussion of the ultimate fate of the drug. Since the pK_a of pralidoxime is reported to be 7.8–8.0 (7), this compound exists largely as a dissociated anion (base) at physiologic pH.

The specific mechanism by which the renal tubule handles this drug is of considerable importance, not only in the clinical use of pralidoxime as an adjunct to atropine therapy in organophosphate poisoning, but also in describing the disposition of drugs with similar chemical structure and pharmacological properties.

Methods. Subjects. The subjects for these experiments were US Army enlisted personnel who volunteered for testing.[1] Subjects were given a thorough physical examination and routine laboratory screening.[2] All testing procedures and possible drug effects were explained to the volunteers.

Materials and Procedures. Drugs used in these studies included: pralidoxime chloride,[3] *p*-aminohippurate,[4] thiamine hydrochloride,[5] ammonium chloride,[6] and sodium bicarbonate.[7]

A total of 22 subjects were studied, each subject being tested once per week under a series of different metabolic conditions. Each of the 22 subjects did not participate in every condition; however, each subject served as his own control for purposes of statistical comparison.

The conditions and number of subjects involved were as follows: (a) all 22 subjects received pralidoxime (5 mg/kg as an intravenous injection over a 2-min interval) under conditions of forced hydration and bed rest, control; (b) 8 subjects received pralidoxime (same dose and route) under conditions of forced hydration and bed rest, one time after 36 hr of ammonium chloride (1 g every 6 hr,

[1] These tests were governed by the principles, policies, and rules for medical volunteers as established in Army Regulation 70-25 and the Declaration of Helsinki.

[2] Chest X-ray, electrocardiogram, complete blood count, routine urinalysis, blood urea nitrogen, serum creatinine, 24 hr creatinine clearance, and liver function tests (SGOT, alkaline phosphatase, total serum bilirubin).

[3] Protopam; Ayerst Laboratories; New York, N. Y.

[4] Sodium Aminohippurate; Merck, Sharp and Dohme; West Point, Pa.

[5] Thiamine hydrochloride, U. S. P.

[6] Ammonium hydrochloride, U. S. P.; Mallinckrodt Chemical Works; New York, N. Y.

[7] Sodium bicarbonate, U. S. P.; Allied Chemical Corp.; Morristown, N. J.

orally, until urine pH was less than 5.0), acidification, and another time after 24 hr of sodium bicarbonate (1 g every 4 hr, orally, until urine pH exceeded 7.5), alkalinization; (c) 9 subjects received pralidoxime[8] (same dose and route) under conditions of forced hydration and bed rest, 20–30 min after thiamine (200 mg total, intramuscular), organic base; (d) 8 subjects received pralidoxime[9] (same dose and route) under conditions of forced hydration and bed rest, simultaneously with *p*-aminohippurate (PAH) (900 mg total, intravenous), organic acid; and (e) 4 subjects received pralidoxime (same dose and route) under conditions of bed rest, after 8–12 hr of fasting, NPO.

Forced hydration consisted of a light breakfast and 1000 ml of fluids (containing no caffeine) in the 2 hr prior to pralidoxime administration, 250 ml of fluids every 20–30 min in the first 3 hr after pralidoxime administration, and fluids and meals *ad lib* thereafter.

Blood samples for the estimation of pralidoxime (and PAH where indicated) content were drawn at 5, 10, 20, 30, 45, 60, 90, 120, and 180 min after administration, through an indwelling "butterfly" intravenous apparatus, cleared with saline and dilute heparin after each sampling.

Urine samples for the estimation of pralidoxime (and PAH) content were collected by spontaneous voiding, and the collection for the first 3 hr was separated from that for the subsequent 21 hr. Individual specimens were measured for specific gravity (less than 1.010 in all hydrated subjects, greater than 1.020 in fasting subjects) and for pH by pH meter (pH was 6.0–7.0 in all control subjects).

Pralidoxime content of blood and urine samples was determined by the method of Groff and Ellin (8). PAH content was determined by the method of Harvey and Brothers (9).

Calculations. Plasma concentration values for pralidoxime (as well as for PAH) were fit to a bi-exponential equation using the computer program NONLIN.[10] This equation describes the drug disposition in the two-compartment model, described in detail by Wagner (10), and discussed with regard to pralidoxime elsewhere (1, 2).

The kinetic parameters reported here include: (a) the half-life values for both the rapid (initial) exponential phase ($t_{½,\alpha}$) and the slow (post-equilibrium) exponential phase ($t_{½,\beta}$) of the plasma disappearance curve, which describe the rates of drug distribution during the approach to equilibrium and drug elimination after reaching equilibrium, respectively; (b) the volume of distribution, both the central (plasma and plasma-like) compartment (V_1) and the peripheral (plasma-unlike) compartment (V_2); (c) the renal clearance using the area under the plasma disappearance curve and the urinary excretion rate (2, 11); (d) the urinary drug recovery, in the first 3 hr and in the subsequent 21 hr.

Statistical analysis consisted of comparing kinetic parameters for each subject under a given metabolic condition to that subject's own control values, using the paired *t* test.

Results. Physiologic measures (blood pressure and heart rate) did not change with pralidoxime administration, even though high doses (20 or more mg/kg) have been reported to elevate the systolic and diastolic blood pressure as well as the heart rate (1, 4, 6, 13). Observed side effects of the drugs given were limited to those previously reported for pralidoxime (2, 6, 12), including "blurred" vision, diplopia, "heaviness" in the eyes, and occasional nausea. These effects occurred immediately after injection and lasted less than 1 min.

(i) *Control.* The overall control values for all 22 subjects are listed in Table I. The percent change from control for any of the other metabolic conditions was calculated only for those subjects tested under those conditions; the control mean for each of these subgroups was not statistically different from the overall control group, however. The values reported here are consistent with those previously reported for pralidoxime and PAH under control conditions.

(ii) *Alkalinization* (Table I and Fig. 1). Raising the urine pH over 7.5 caused a decrease in the overall excretion of pralidoxime, both in the first 3 hr ($P < 0.05$) and in the

[8] 5 of these subjects also received PAH alone after thiamine administration.

[9] These 8 subjects also received PAH alone under the same experimental conditions.

[10] NONLIN was developed and supplied by C. Metzler at Upjohn Co. of Kalamazoo, Michigan.

TABLE I. Kinetic parameters for Pralidoxime Under Control and Experimental Conditions.

		Alkalinization	Acidification	Thiamine	PAH	NPO	Control
Urinary Recovery	3 hr	**66.9 ± 2.8	**69.8 ± 3.7	*68 ± 2.8	84.7 ± 1.6	82.9 ± 2.6	77.1 ± 1.1
(% of Dose)	3–24 hr	**6.1 ± 1.6	5.3 ± 1.2	***12.0 ± 1.1	8.7 ± 2.0	8.5 ± 2.1	7.4 ± 0.9
Renal Clearance	(ml/min)	**520 ± 30	**532 ± 29	****477 ± 50	570 ± 28	*522 ± 33	612 ± 19
Half-life	$t_{\frac{1}{2}}, \alpha$	**4.1 ± 0.7	5.5 ± 0.7	**7.2 ± 1.3	5.4 ± 0.4	*3.8 ± 0.8	5.4 ± 0.3
(min)	$t_{\frac{1}{2}}, \beta$	75.0 ± 6.0	81.8 ± 6.9	*94.9 ± 14	74.4 ± 4.0	72.8 ± 5.5	75.9 ± 3.6
Volume of Distribution	V_1	198 ± 28	287 ± 27	235 ± 25	233 ± 26	*156 ± 33	232 ± 24
(ml/kg)	V_2	522 ± 42	548 ± 45	54 ± 43	427 ± 58	452 ± 34	502 ± 33
Number of Subjects		8	8	9	8	4	22

* $P < 0.10$.
** $P < 0.05$.
*** $P < 0.02$.
**** $P < 0.001$ by paired t test.

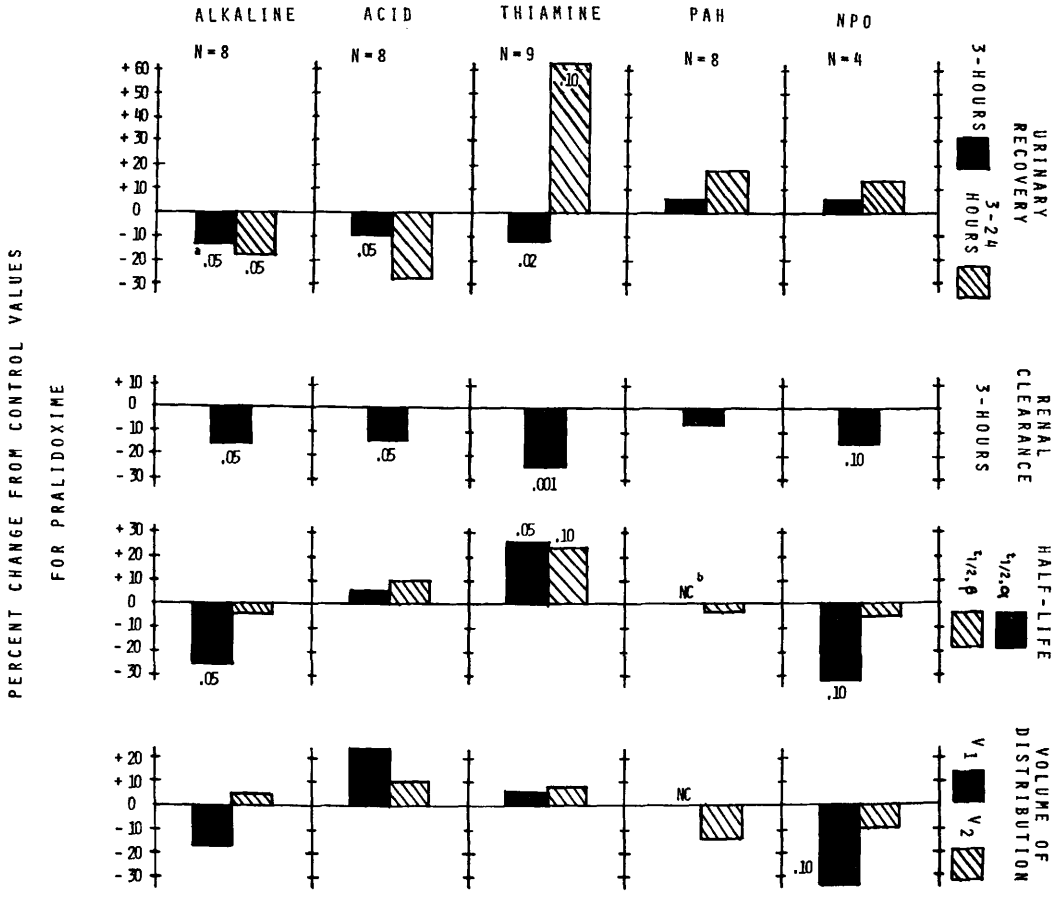

FIG. 1. Kinetic parameters for pralidoxime; percent change from control values under various metabolic conditions (see text). Change from control is expressed as a percentage of the control for each subgroup; the mean for each subgroup was not significantly different from the total group. (a) P values for the paired t test; (b) NC = no change.

total 24 hr ($P < 0.05$). There was a corresponding decrease of about 15% in the apparent renal clearance rate ($P < 0.05$). The slight contraction of the central volume (V_1) ($P > 0.10$) and the shortening of the initial phase half-life ($t_{\frac{1}{2}}$, α) ($P < 0.05$) suggest that distribution of drug occurred more rapidly than under control conditions. Even though the urinary excretion was reduced, the overall half-life for elimination $t_{\frac{1}{2}}$, β was not significantly altered.

(iii) *Acidification* (Table I and Fig. 1). Lowering the urine pH below 5.0 also caused a decrease in the total amount of pralidoxime excreted especially in the first three hours ($P < 0.05$). Again, the apparent renal drug clearance rate was reduced (13%) ($P < 0.05$). There were no significant changes in the other kinetic parameters, even though the mean central volume (V_1) did increase by over 20% ($P > 0.10$), in contrast to the decrease seen with *alkalinization*.

(iv) *Organic base* (Table I and Fig. 1). Thiamine administration caused a significantly lower urinary excretion of pralidoxime in the first 3 hr ($P < 0.02$), although a much larger amount of drug "reappeared" in the urine in the latter collection than was observed under any other condition ($P < 0.10$). The renal drug clearance was strikingly reduced in the first 3 hr, by 22% ($P < 0.001$), and was accompanied by a 25% prolongation in the half-life for the elimination of the drug ($t_{\frac{1}{2}}$, β) ($P < 0.05$). The volume of distribution of

pralidoxime was not altered significantly, suggesting that reduction of renal clearance was not a result of drug redistribution.

(v) *Organic acid* (Table I and Fig. 1). Simultaneous administration of PAH and pralidoxime did not alter the disposition of pralidoxime to any significant degree. There was a slight decrease in the drug disposition to the peripheral compartment (V_2) ($P > 0.10$), but half-life and renal clearance values were essentially unchanged. Overall urinary excretion was not changed, although the mean recovery was slightly higher after PAH administration.

(vi) *NPO* (Table I and Fig. 1). Fluid restriction resulted in striking changes in pralidoxime disposition, similar to those previously reported for other conditions of physical stress (2). It is noteworthy that the overall urinary recovery of pralidoxime did not decrease under these conditions, and that the reduction of the renal clearance was due to higher plasma concentration values caused by the contracted central volume (V_1) ($P < 0.10$), especially at zero time (i.e., the extrapolated intercept of the bi-exponential curve).

Discussion. To date, the study reported by Berglund *et al.* (3) remains the most detailed investigation into the specific mechanism of the renal tubular handling of pralidoxime; however, that investigation fell short of fully explaining the mechanism. Berglund *et al.* (3) demonstrated first, that pralidoxime excretion was pH-dependent between urine pH values of 5.7–8.0, with excretion rate decreasing as the pH increased; and second, that pralidoxime excretion was not changed by administration of a weak organic acid (probenecid).

The present studies confirmed that urine alkalinization (pH > 7.5) reduced pralidoxime excretion; but these studies demonstrated that more extreme acidification (pH < 5.0) also reduced overall pralidoxime excretion. It seems likely that in alkaline urine (where the hybrid resonant form of pralidoxime, the dissociated base, is more prevalent) (7) nonionic passive diffusion accounts for a considerable amount of reabsorption of the drug, and a decrease in the apparent clearance rates. In acid urine, however, the situation appears to be more complicated. It may well be that at very low pH values, the quaternary ammonium cation of pralidoxime which is more prevalent is actively reabsorbed. Alternatively, the hyperchloremic acidosis of ammonium chloride administration necessitates the active reabsorption of all cations other than H+, reducing the effective urinary excretion of the drug and the apparent clearance rates.

Active tubular reabsorption of pralidoxime has not been specifically studied, although it may be an important factor affecting measured clearance rates under metabolic conditions such as those in the present study and the study of Berglund *et al.* (3). This mechanism could be studied using a reabsorption inhibitor, such as phloridzin, although we were unable to use this potentially toxic compound on the human volunteers in these experiments.

Active tubular secretion can be characterized in a similar fashion, by inhibiting the known mechanisms for secretion. In the present studies we chose to use non-toxic competitive inhibitors of organic acid and of organic base secretion. The results strongly suggest that pralidoxime may indeed be handled by the *organic base* secretory mechanism, since clearance rates were markedly reduced by thiamine and only very slightly affected by PAH.

It is well-established that thiamine is secreted as an organic base, and that it competes with other "weak base" compounds for the secretory mechanism (14). Studies using nicotinamide and pralidoxime were reported by Calesnick and DiPalma (4), but were inconclusive. In the present studies, it is clear that pretreatment with thiamine strikingly altered the renal clearance of pralidoxime without altering the apparent distribution of the drug. Furthermore, the urinary recovery of pralidoxime demonstrates that drug which was not excreted in the first 3 hr was ultimately excreted, once the inhibitory compound (thiamine) was itself eliminated (i.e., about 2–3 hr) (15).

By contrast, the administration of PAH did not alter the excretion of pralidoxime to any significant degree. The slight change in the volume of distribution (peripheral compartment, V_2) suggests that PAH may displace a small amount of pralidoxime from its usual tissue reservoirs; however, this minor alteration did not affect the apparent renal clearance. Previous studies with organic acids, in particular probenecid and salicylates, have

shown no alteration in renal pralidoxime clearance. Moreover, other data which we have not included in this report shows that PAH clearance is, in turn, not affected to any significant degree by the simultaneous administration of pralidoxime. Therefore, the secretory mechanisms for these two drugs are probably distinct.

For drugs that are rapidly cleared, such as pralidoxime, PAH, etc., it has been demonstrated that renal blood flow changes will alter clearance rates (2). In the present studies, fluid restriction alone was found to alter clearance rates and volume of distribution; these changes were most likely attributable to decreased renal blood flow. It is not clear what effect the acid urine in fluid restricted patients, or the altered renal blood flow in patients given ammonium chloride, had on pralidoxime clearance. It is clear, however, that thiamine does not alter renal blood flow (as estimated by the PAH clearance), and we presume that the reduction which thiamine does cause in pralidoxime clearance is a result of direct inhibition.

Summary. Pralidoxime chloride is a quaternary ammonium used to reactivate organophosphate—inhibited cholinesterase. The drug is rapidly cleared from the plasma by renal tubular secretion, though the mechanism has not been specifically identified. Reduction of pralidoxime clearance rates and prolongation of the biologic half-life after thiamine administration as compared to those after PAH administration suggest that pralidoxime is secreted as an organic base. Furthermore, reduced excretion of pralidoxime under conditions of both urine alkalinization and urine acidification implicate an active reabsorption of pralidoxime not heretofore described.

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