Disposition of Prothionamide in Rats and Armadillos[1]

John H. Peters, G. Ross Gordon, and John F. Murray, Jr.[2]

The thioamides, ethionamide (ETH) and prothionamide (PTH), are well established as bactericidal drugs against *Mycobacterium leprae* in animals and man ([4, 20, 29, 31]). ETH and PTH are virtually interchangeable and give cross resistance with each other ([31]). Thus, the finding ([12]) that ETH was effective in mice infected with *M. leprae* resistant to dapsone (DDS) and rifampin (RFM) suggests that PTH would be equally active against organisms resistant to these primary antileprosy drugs. The minimal inhibitory concentration (MIC) of ETH and PTH was estimated to be about 0.05 μg/ml, and it has been estimated that in man an oral dose of 375 mg would yield serum concentrations in excess of the MIC during 24 hr ([31]). However, these estimates may be conservative because they do not consider the contribution of the circulatory S-oxide metabolites of ETH and PTH, which have been found previously to exhibit antimycobacterial activities equal to their parent drugs ETH ([2, 16]) and PTH ([17]). These considerations indicated the need for an analytic method for study of these drugs that would measure both the thioamide and its S-oxide metabolite at sensitivities adequate to detect levels in the range of the MICs of 0.05 μg/ml of plasma or serum. Because at the time these studies were initiated it had been concluded that ETH was being replaced by PTH due to the apparent lower systemic toxicity of the latter drug ([4]), we conducted all our studies on PTH.

Previously published methods of insufficient sensitivities ([4, 5, 6, 15]) did not provide the bases for development of a method capable of detecting levels as low as 0.05 μg/ml, and we chose to develop a high-performance liquid chromatographic (HPLC) technique because of our success with this approach for quantitating the other antileprosy drugs, DDS ([18]), RFM ([22]), and clofazimine ([24]), in biological fluids at sensitivities at or below their respective MICs. After completion of our work, Jenner and Ellard ([13]) reported an HPLC method for determining ETH and PTH with sensitivities of about 0.01 μg/ml of plasma, but these authors did not measure the S-oxide metabolites. Also, Rossi and Rubsamen ([27]) described a quantitative thin-layer chromatographic technique for measuring PTH and prothionamide-S-oxide (PTHSO) in biologic fluids, but the limit of sensitivity of their method was about 0.2 μg/ml.

In this report, we present our HPLC method for the measurement of PTH and PTHSO in biological fluids, and we summarize our studies on the metabolic disposition of PTH and PTHSO in rats and armadillos.

MATERIALS AND METHODS

Drugs and animals. The sources and characteristics of PTH, PTHSO, and 2-propylisonicotinamide (PINA) used in these studies were described previously ([17]) as were those of DDS and MADDS ([21]). ETH was obtained from the Pasteur Institut, Paris, France. Other reagents were of the highest purity available commercially.

Mature male and female Lewis rats weighing 200–300 g were obtained locally (Simonsen Laboratories, Gilroy, California, U.S.A.) and acclimated to our animal rooms for at least one week prior to any experiment. They were maintained on laboratory chow and water *ad libitum*. For the acute tests, the rats were fasted 24 hr prior to treatment and during the duration of the studies. In the chronic studies, the rats were not fasted. For both intravenous (IV) and oral administrations, PTH and PTHSO were dissolved in polyethylene glycol-200 (J. T. Baker Chemical Co., Phillipsburg, New Jer-

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[2] J. H. Peters, Ph.D., Director, Biochemical Pharmacology Program; G. R. Gordon, M.S., Senior Biochemist; J. F. Murray, Jr., B.S., Biochemist (deceased), Life Sciences Division, SRI International, Menlo Park, California 94025, U.S.A.

sey, U.S.A.) to obtain concentrations of 38 mg/ml and 42 mg/ml, respectively. All rats received 1.0 ml of the drug solution/kg of body weight. The dose of PTH (38 mg/kg) was equivalent to 500 mg PTH given to a 70 kg man when the doses are expressed as mg/m^2 of body surface area ([7]). The dose of PTHSO (42 mg/kg) was equimolar to the PTH dose.

Heparinized blood was collected by terminal heart puncture from four ether-anesthetized rats of each sex at 1, 2, 4, 6, and 8 hr after PTH treatment. Plasma was prepared immediately and stored frozen. Studies of the disposition of PTHSO given IV and orally followed the same pattern, except that only three animals were sacrificed at each time post treatment. Also, to examine the effects of chronic doses of PTH, we compared levels of PTH and PTHSO in unfasted rats that were given a single and eight oral doses of 38 mg PTH/kg. In this case, we also employed three rats of each sex per time period.

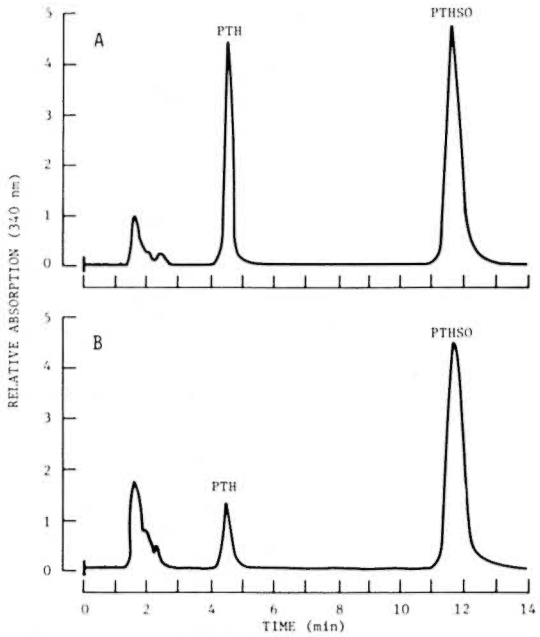
To examine the disposition of PTH and PTHSO in armadillos, we employed one male and one female animal from our colony. These animals had been subjects of studies on DDS disposition completed several months earlier ([19]) and were maintained on moistened laboratory chow and water *ad libitum*. They had been purchased from C. P. Chase Co., Inc., Miami, Florida, U.S.A., and on arrival were tested for infection in Dr. A. H. Fieldsteel's laboratories at SRI International, Menlo Park, California, U.S.A. No acid-fast bacteria were detected in ear snips of these animals by the Nucleopore filter technique ([28]) prior to the studies with DDS. In the studies of PTH and PTHSO in these armadillos, we administered the drugs IV (femoral vein) after the animals had been anesthetized with an intramuscular dose of 50 mg ketamine hydrochloride/kg (Parke, Davis & Co., Detroit, Michigan, U.S.A.). Heparinized blood samples were taken by heart puncture after administration of a second identical dose of ketamine. To minimize the trauma associated with ketamine, drug administration, and the blood collection, only one blood sample from the armadillos was taken on one day. Data sufficient for determining the half-times of disappearance of PTH and PTHSO were obtained after four or five separate administrations of drugs over a two-week period, during which time a single blood sample was obtained at 1, 1.4, 2, 3, or 4 hr after each administration. The armadillos were given a two- or three-day rest between each administration of drugs coupled with the single bleeding. For administration, we dissolved appropriate amounts of PTH or PTHSO in polyethylene glycol-200 to obtain doses of 16 mg PTH/kg and 18 mg PTHSO/kg when giving the vehicle at 0.5 ml/kg. These doses were equivalent to the doses of PTH and PTHSO used in the rats when calculated on the basis of mg/m^2 of surface area using surface area parameters for the rabbit ([7]). Heparinized plasma was obtained and stored frozen as in the rat studies.

Preliminary studies revealed that PTH and PTHSO were stable in rat plasma stored at −20°C for 42 days. Others ([27]) reported no loss of PTH and PTHSO in human plasma stored frozen for 12 months.

Extraction procedure and liquid chromatography. Aliquots (1.0 ml) of experimental plasma or control rat plasma containing 0, 0.05, 0.10, 0.50, 1.0, 5.0, and 10 μg of added PTH or PTHSO were mixed with 0.10 ml of 1 M phosphate buffer, pH 7.0, and then extracted by vigorous mechanical shaking with 5.0 ml of $CHCl_3$ for 20 min. The mixture was centrifuged to separate the phases, and the aqueous phase was aspirated off and discarded. An aliquot (4.0 ml) of the $CHCl_3$ phase was transferred to a tube containing 1.0 ml of 0.1 N HCl. PTH and PTHSO were extracted into the acid phase by vigorous agitation for 10 min. The phases were separated by centrifugation, and 0.90 ml of the acid phase was transferred to a tube containing 0.20 ml of 1 M phosphate buffer, pH 8.0. The mixture was vigorously extracted with 5.0 ml of $CHCl_3$ for 20 min. After phase separation by centrifugation, the aqueous phase was aspirated off and 4.5 ml of the $CHCl_3$ phase was transferred to a clean tube. Following evaporation of the solvent at 40°C under a stream of high purity nitrogen, we dissolved the residue obtained in 150 μl of the HPLC mobile phase, which was chloroform : methanol : water (187:14:1). Aliquots (100 μl) were injected onto a 4.6 × 250 mm column of 5-μm LiChrosorb SI-60 (Altex Scientific, Inc., Berkeley, California, U.S.A.) using a

100-μl fixed loop injection valve (Rheodyne, Inc., Cotati, California, U.S.A.). The mobile phase was pumped at a flow rate of 1.0 ml/min using a controlled volume minipump (Milton Roy Company, Riviera Beach, Florida, U.S.A.), and the column effluent was monitored at 340 nm using an 8-μl flow cell (Model 153 Detector, Altex Scientific, Inc.). Quantitation was by peak area using an electronic integrator (Model 3380A, Hewlett-Packard, Avondale, Pennsylvania, U.S.A.). We employed absorption at 340 nm for quantitation, even though PTH and PTHSO absorb maximally in chloroform at 295 nm and 360 nm, respectively ([17]), to obtain maximum sensitivities for both compounds and to reduce interference by endogenous materials which absorb less at 340 nm than at lower wavelengths.

Calculations. Standard statistical methods were employed to calculate the various parameters used to evaluate the results of these studies ([8]).



THE FIGURE. Elution profiles of (A) an extract of control rat plasma with 10 μg PTH and 10 μg PTHSO added per ml; and (B) an extract of rat plasma 2 hr after administration of PTH.

RESULTS

Chromatograms showing the elution times of PTH (4.2 min) and PTHSO (11.8 min) are shown in The Figure. The upper drawing shows the recovery of PTH and PTHSO added to control rat plasma; the lower one, the peaks of PTH and PTHSO from a rat plasma taken 2 hr after administration of PTH. Both chromatograms show that endogenous materials that absorb at 340 nm are eluted at about 2 min. No other peaks were noted in experimental extracts. Possible interference by other compounds was examined in separate experiments. Interference by 2-propyl isonicotinic acid (PINA) was eliminated because it does not absorb at 340 nm. Also, using absorption at 280 nm for monitoring column eluates, we established that PINA was well resolved from PTH and PTHSO, exhibiting a retention time of 8.2 min. ETH was completely resolved from PTH and elutes at about 6.0 min. Thus, as others have done ([13]), ETH could be used as an internal standard in our system also. Finally, using absorption at 254 nm for detection, we found that DDS (elution at 3.4 min) and MADDS (elution at 6.8 min) were resolved from PTH and PTHSO, even though they do not absorb strongly at 340 nm and are thus noncontributing to the assays for the thioamides.

The recoveries of added PTH and PTHSO from control rat plasma are shown in Table 1. We obtained an average of 97% of added PTH (range, 88% to 110%) and an average of 88% of added PTHSO (range, 76% to 98%). HPLC elution profiles of extracts of control plasma containing no added PTH or PTHSO yielded no extraneous peaks or

TABLE 1. *The recovery of PTH and PTHSO from rat plasma.*

Level of compound (μg/ml)	PTH		PTHSO	
	Found (μg/ml)	Recovery[a] (%)	Found (μg/ml)	Recovery[a] (%)
0	<0.01	—	<0.01	—
0.050	0.045	90	0.048	96
0.10	0.088	88	0.088	88
0.50	0.55	110	0.49	98
1.0	0.97	97	0.84	84
5.0	5.2	104	4.2	84
10	9.4	94	7.6	76
Mean recovery		97		88
Standard deviation		8.4		8.2
Coefficient of variation		8.7		9.4

[a] The recovery was calculated from directly injected standards of PTH and PTHSO.

TABLE 2. *Plasma levels of PTH and PTHSO in rats receiving PTH or PTHSO.*

Route of administration	Time after treatment (hr)	Mean (±S.D.) PTH level (μg/ml)		Mean (±S.D.) PTHSO level (μg/ml)	
		Male	Female	Male	Female
		38 mg PTH/kg administered (N = 4)			
IV	1	8.82 ± 2.78	17.8 ± 4.86	7.13 ± 2.82	6.37 ± 1.00
	2	1.55 ± 0.74	8.73 ± 5.14	3.74 ± 0.14	5.30 ± 1.56
	4	0.17 ± 0.12	0.43 ± 0.36	0.90 ± 0.36	1.33 ± 0.54
	6	0.03 ± 0.02	0.11 ± 0.08	0.25 ± 0.16	0.86 ± 0.28
	8	0.02 ± 0.02	0.02 ± 0.02	0.11 ± 0.10	0.14 ± 0.10
Oral	1	7.53 ± 2.40	10.1 ± 4.48	9.08 ± 0.54	3.90 ± 0.82
	2	2.81 ± 1.26	3.77 ± 2.12	5.86 ± 0.88	3.27 ± 0.74
	4	0.08 ± 0.06	0.45 ± 0.44	0.86 ± 0.48	1.52 ± 0.68
	6	<0.01	0.34 ± 0.14	0.06 ± 0.04	1.65 ± 0.28
	8	<0.01	0.02 ± 0.02	<0.01	0.29 ± 0.20
		42 mg PTHSO/kg administered (N = 3)			
IV	1	0.78 ± 0.43	4.79 ± 2.60	6.04 ± 1.37	7.78 ± 0.97
	2	0.22 ± 0.03	0.77 ± 1.04	2.30 ± 0.16	2.72 ± 1.38
	4	<0.01	0.04 ± 0.05	0.20 ± 0.21	0.63 ± 0.38
	6	0.04 ± 0.07	0.01 ± 0.02	0.39 ± 0.28	0.24 ± 0.17
	8	<0.01	<0.01	<0.01	0.12 ± 0.16
Oral	1	1.23 ± 0.88	2.06 ± 3.05	4.83 ± 1.11	2.84 ± 2.03
	2	0.22 ± 0.16	1.39 ± 1.23	2.06 ± 0.88	2.21 ± 1.75
	4	<0.01	0.05 ± 0.02	0.37 ± 0.07	0.63 ± 0.19
	6	<0.01	<0.01	<0.01	0.31 ± 0.19
	8	<0.01	<0.01	0.07 ± 0.04	0.07 ± 0.12

tailing in the region of elution of either drug. From the noise level of the HPLC traces, we estimate that the practical limit of sensitivity is 0.01 μg of either compound per ml of plasma. This was about five-fold lower than our target limit sensitivity of 0.05 μg/ml.

The results of all studies in fasted rats receiving single doses of 38 mg PTH or 42 mg PTHSO per kg are summarized in Table 2. The first five lines show the mean plasma values of PTH and PTHSO found in the male and female rats receiving PTH IV. The PTH disappeared very rapidly in both sexes, and we calculated t½ values of 0.74 hr and 0.69 hr, respectively, for the male and female rats (Table 4). As shown in Table 2, the corresponding plasma levels for PTHSO declined more slowly in both sexes, and this delayed disappearance was reflected in the t½ values of 1.2 hr found for both sexes as shown in Table 4. In both sexes, the slopes of the regression lines for PTHSO were significantly greater than the corresponding slopes for PTH ($p < 0.005$). Also, comparisons of the areas under the curves (AUCs) for PTH and PTHSO in the two sexes (first two lines of Table 5) show that the amount of PTH in the plasma during 8 hr was substantially less in the male than in the female. This was apparently a result of the more extensive conversion of PTH to PTHSO by the male compared to the female. Thus, of the total of PTH and PTHSO of 25.6 (μg-hr/ml) in the male rat shown in the last column of Table 5, 55% was due to PTHSO; whereas the corresponding fraction due to PTHSO in the female rat was 35%. Also, the larger amount of unchanged PTH in the female was reflected in the total of PTH and PTHSO being about twice that in the male rat.

The plasma levels of PTH and PTHSO observed in the two sexes after oral administration of the same dose of PTH are shown in the second entry of Table 2. Again PTH levels declined more rapidly than PTHSO levels in both sexes, and this was reflected in significantly shorter ($p < 0.001$) t½ values for PTH than for PTHSO (second entry of Table 4). Also, after oral dosing, the t½ values for both PTH and PTHSO were significantly shorter ($p < 0.001$) in the male rats than in the female rats. Finally, the AUCs of Table 5 indicate again that the male rat formed PTHSO to a greater extent

TABLE 3. *Plasma levels of PTH and PTHSO in unfasted rats receiving acute and chronic oral doses.*

Number of treatments	Time after last treatment (hr)	Mean (±S.D.) PTH level (μg/ml)		Mean (±S.D.) PTHSO level (μg/ml)	
		Male	Female	Male	Female
1	1	1.20 ± 1.04	5.96 ± 0.82	4.70 ± 1.77	5.31 ± 0.50
	2	0.83 ± 0.87	1.99 ± 1.11	3.20 ± 1.96	3.42 ± 0.19
	4	0.02 ± 0.02	0.08 ± 0.02	0.85 ± 0.03	0.95 ± 0.21
	6	0.02 ± 0.03	0.50 ± 0.54	0.60 ± 0.21	1.73 ± 1.20
	8	<0.01	0.05 ± 0.07	0.24 ± 0.24	0.62 ± 0.61
8	1	1.25 ± 0.68	4.06 ± 4.40	4.43 ± 0.81	2.95 ± 0.92
	2	0.34 ± 0.24	1.52 ± 2.58	2.51 ± 0.90	1.80 ± 2.36
	4	0.03 ± 0.05	0.50 ± 0.61	0.69 ± 0.45	1.11 ± 0.24
	6	0.11 ± 0.02	0.18 ± 0.22	1.21 ± 0.17	0.79 ± 0.61
	8	0.01 ± 0.02	0.09 ± 0.07	0.33 ± 0.26	0.65 ± 0.29

than did the female rat, although the totals of PTH and PTHSO were nearly identical in the two sexes receiving oral PTH.

Comparisons of the slopes of the regression lines from oral and IV treatments with PTH indicated that the t½ values for both PTH and PTHSO were significantly shorter following oral than IV treatments in the male rat. However, in the female rat, the opposite was observed with the oral route yielding a slightly but nonsignificantly longer t½ value for PTH after oral versus IV treatment; the t½ value for PTHSO was significantly ($p < 0.02$) longer following oral than IV treatment with PTH.

The lower half of Table 2 lists the mean values of PTH and PTHSO found in rats receiving a dose of PTHSO equimolar to the PTH given. It is immediately apparent that PTHSO was readily converted to PTH in both sexes receiving PTHSO by either route. The t½ values calculated from these data are shown in the fifth and sixth entries of Table 4. Following IV treatment with PTHSO, our comparisons of the slopes of the regression lines for PTH and PTHSO in both sexes indicated no significant differences in any of the four possible comparisons. Similarly, following oral treatment with PTHSO, the two sexes were not significantly different in the t½ values for PTH and PTHSO, respectively. However, in both sexes receiving oral PTHSO, we found that the t½ values for PTH were significantly shorter ($p < 0.05$) than those for PTHSO, again emphasizing that PTHSO was retained longer in the rat than was PTH. All four comparisons possible between IV and oral treatments with PTHSO in regard to sex and compound did not yield significant differences between the slopes of the regression lines.

The AUCs derived from these data (last two lines of Table 5) are revealing from several points of view. First, the values from both routes of administration of PTHSO indicate that the female rat reduced PTHSO to PTH more efficiently than did the male rat. Also, as in the results after IV dosing with PTH, we found that the total of PTH and PTHSO was higher in the female rat than in the male rat after IV dosing with PTHSO. Again, this sex difference was not found following oral treatment with PTHSO, as was the case with PTH. Perhaps the most important observation was that the total of the two compounds was substantially less when PTHSO was given as compared to administering PTH, even though the t½ values were not different. Thus, giving PTHSO would be less efficient therapeutically than giving PTH, even though PTHSO is retained in the body longer.

To test whether PTH would induce its own metabolism, we compared the plasma decay patterns of PTH and PTHSO in unfasted rats receiving a single and eight daily doses of 38 mg PTH/kg (Table 3). No striking differences were noted among the levels after these two regimens. The t½ values calculated from the data are presented in the third and fourth entries of Table 4. Tests for significant differences between the slopes for all eight possible combinations of sex, compound, and number of treatments did not yield any significant differences. Also,

TABLE 4. *Half-times of disappearance (t½) of PTH and PTHSO in the rat studies of PTH and PTHSO.*

Drug given and study conditions[a]	Sex	PTH			PTHSO		
		N	t½ (hr)	r	N	t½ (hr)	r
PTH, IV	Male	18	0.74	−0.930	20	1.2	−0.953
	Female	19	0.69	−0.954	20	1.2	−0.938
PTH, oral	Male	13	0.43	−0.979	15	0.70	−0.979
	Female	19	0.86	−0.928	20	1.9	−0.832
PTH, oral, unfasted	Male	10	0.78	−0.726[b]	15	1.4	−0.865
	Female	14	1.0	−0.780	15	2.0	−0.766
PTH, oral × 8, unfasted	Male	10	1.5	−0.861[c]	12	2.1	−0.852
	Female	14	1.4	−0.667[b]	14	3.3	−0.666[b]
PTHSO, IV	Male	8	1.2	−0.688[d]	12	1.1	−0.774[c]
	Female	10	0.84	−0.851[c]	15	1.0	−0.924
PTHSO, oral	Male	6	0.40	−0.841[d]	10	1.3	−0.898
	Female	9	0.73	−0.798[b]	12	1.7	−0.763[c]

[a] All studies were acute and all rats were fasting unless otherwise noted.
[b] $p < 0.01$; all others were <0.001 unless otherwise noted.
[c] $p < 0.005$.
[d] $p < 0.05$.

in either sex, no substantial differences were noted in the AUCs for PTH, PTHSO, or the total of PTH and PTHSO (third and fourth entries of Table 5). Again, a substantial sex difference was noted with the male rats forming relatively more PTHSO than the female rats, and the latter exhibiting higher totals, especially after only one dose of PTH. Also important was the observation that the AUCs for PTH, PTHSO, and the total of both were substantially less in these unfasted rats compared to the rats receiving a single oral dose after a 24 hr fast. Thus, this study not only demonstrated that PTH did not induce its own metabolism in the rat, it also demonstrated that the absorption of the compound was less in nonfasting animals.

Finally, Table 6 summarizes our limited studies of the disposition of intravenous PTH and PTHSO in armadillos. We found that this species also oxidized PTH to PTHSO, and that the rate of plasma decline of PTH was significantly ($p < 0.01$) more rapid than that of PTHSO. However, from the calculations of the AUCs it is apparent

TABLE 5. *Areas under the plasma curves (AUC) in the rat studies of PTH or PTHSO.*

Drug given and study conditions[a]	Sex	AUC (μg-hr/ml) for 0–8 hr		
		PTH	PTHSO[b]	Total[b]
PTH, IV	Male	11.6	14.0	25.6
	Female	32.0	17.3	49.8
PTH, oral	Male	11.9	18.1	30.0
	Female	17.4	14.1	31.5
PTH, oral, unfasted	Male	2.5	11.6	14.1
	Female	10.2	15.1	25.3
PTH, oral × 8, unfasted	Male	2.0	11.3	13.3
	Female	7.8	9.3	17.1
PTHSO, IV	Male	1.2	9.8	11.0
	Female	6.0	12.6	18.6
PTHSO, oral	Male	1.6	8.0	9.6
	Female	4.2	7.4	11.6

[a] All studies were acute and all rats were fasting unless otherwise noted.
[b] Expressed as PTH equivalents.

TABLE 6. *Plasma levels and kinetic parameters in armdillos receiving 16 mg PTH/kg or 18 mg PTHSO/kg intravenously.*

Drug administered	Time after treatment (hr)	Mean (±AD)[a] level (μg/ml)	
		PTH	PTHSO
PTH	1	3.20 ± 0.20	1.43 ± 0.01
	1.4	2.98 ± 0.34	1.41 ± 0.21
	2	1.50 ± 1.04	1.10 ± 0.06
	3	0.14 ± 0.06	0.64 ± 0.12
	4	0.15	0.31 ± 0.11
	AUC (μg-hr/ml)	5.1	3.1[b]
	t½ (hr)	0.52	1.3
	r	−0.894	−0.932
	p	<0.005	<0.001
PTHSO	1	0.21	3.23
	1.4	0.28 ± 0.12	1.88 ± 0.08
	2	0.34 ± 0.11	1.56 ± 0.12
	3	0.05	0.46 ± 0.12
	AUC (μg-hr/ml)	0.58	4.3[b]
	t½ (hr)	1.0	0.73
	r	−0.610	−0.955
	p	>0.1	<0.001

[a] Mean of two observations ± average deviation from the mean.
[b] Expressed as PTH equivalents.

that the total levels of PTH exceeded those of PTHSO. When an equimolar dose of PTHSO was given, our finding of PTH levels indicates that the armadillo also reduces PTHSO to PTH as does the rat, even though in both species the oxidation pathway apparently predominates. The t½ value of 0.73 hr for the administered PTHSO was significantly shorter ($p < 0.05$) than the value of 1.3 hr for PTHSO formed following PTH administration. These t½ values are of the same magnitude as we found in rats, but the AUC values for armadillos are substantially less than for rats (Table 5) even though the doses were equivalent on a mg/m^2 basis. It should be noted, however, that no 8 hr plasma levels were measured in the armadillo.

DISCUSSION

Our findings that PTH and PTHSO were interconvertible in rats and armadillos are in agreement with earlier studies of ETH in animals and man ([1, 9, 14]). In addition, Rossi and Rubsamen ([27]) recently reported plasma levels of PTHSO in patients that were approximately equivalent to levels of PTH observed during 6 hr following treatment with PTH. Our estimates from their plasma decay curves for PTH and PTHSO suggest t½ values of approximately 3 hr and 4 hr, respectively. It would appear that PTHSO is retained longer than PTH in man as we found in the rats and armadillos. These combined results coupled with the knowledge that the antibacterial activities of these thioamides reside solely in the parent drugs and their sulfoxides ([2, 16, 17]) emphasizes the need to consider the sum of these active compounds rather than the parent drugs alone when considering therapeutic efficacies. Therefore, peak plasma concentrations of active drugs in man following thioamide administration of 2 μg/ml to 3 μg/ml ([13, 30]) should be approximately doubled to 4 μg/ml to 6 μg/ml when estimating ratios of peak concentrations of these compounds to their MIC of 0.05 μg/ml. Under these circumstances, the peak ratios would be 80 to 120 instead of the range of 40 to 60 estimated previously ([31]). However, t½ values in man for the thioamides and their S-oxides of 2 hr to 4 hr ([13, 27]) would still limit the estimated time that plasma levels would be expected to exceed the MICs to about 24 hr. Thus, daily treatment with the thioamides would still appear advisable.

The mechanism of the antibacterial action of the thioamides is unknown, but numerous reports indicate that these compounds exert their antibacterial effects differently from other drugs. Thus, *M. tu-*

berculosis resistant to isoniazid, streptomycin, *p*-aminosalicylic acid, viomycin, cycloserine, or thiacetazone are sensitive to ETH ([26]); *M. leprae* resistant to DDS and RFM are sensitive to ETH ([12]); and combinations of ETH with clofazimine, DDS, or RFM act additively in mice infected with *M. leprae* ([29]). It is apparent that the thioamides are excellent candidates for combination drug regimens in the chemotherapy of either leprosy or tuberculosis.

The *in vivo* interconversion of PTH and PTHSO also parallels the more extensively studied hepatotoxic thioamides, thioacetamide ([25]) and thiobenzamide ([3]). These latter thioamides are activated to toxic S-oxide metabolites by mammalian microsomal oxidases. It is not known whether ETH or PTH are acted on by these oxidases or by those in mammalian microsomes known to oxidize thioether moieties of numerous pesticides to sulfoxides ([10]). In the current studies, no obvious toxicities of PTH or PTHSO were noted in either the rats or the armadillos, although the experiments were not designed to assess the relative toxicities of the compounds. Because ETH, PTH, and their S-oxides are equally active against cultivable bacteria and because PTH and PTHSO were equally active against *M. leprae* in mice, it seems unlikely that ETH and PTH and their S-oxides exhibit markedly different toxicities as do the thioacetamide and thiobenzamide S-oxides from their parent compounds.

Our finding that male rats oxidize PTH to PTHSO more readily than do female rats is apparently another ramification of the well-known sexual dimorphism in rats for the oxidation of many xenobiotics ([11]). Also, our inability to detect any differences in plasma patterns of PTH and PTHSO in rats receiving multiple doses from those found after a single dose indicates that, using the current doses, PTH did not induce its own metabolism.

SUMMARY

A new method for the sensitive and selective measurement of prothioamide (PTH) and its S-oxide metabolite (PTHSO) in biological fluids was described. The limit of sensitivity was approximately 0.01 μg of drug/ml of plasma. Endogenous materials, 2-propylisonicotinamide, ethionamide, dapsone, or monoacetyl dapsone did not interfere or contribute. Rats receiving PTH, intravenously or orally, showed a sexual dimorphism in the ability to oxidize PTH to PTHSO, with males exhibiting greater capacities for this conversion. Both sexes cleared the administered PTH more rapidly from the plasma than the metabolite, PTHSO. Following oral or intravenous administration of equimolar doses of PTHSO, both sexes exhibited an ability to reduce the administered PTHSO to PTH, with the female showing greater capacities for this conversion. Clearances after oral PTHSO administration were again more rapid for PTH than for PTHSO in both sexes. However, the total of PTH and PTHSO in the plasma during 8 hr following PTHSO administration was consistently less than following PTH dosing. Therefore, although PTHSO is retained longer than PTH after either PTH or PTHSO administration, giving PTHSO yielded less total active drug in the circulation. Comparison of plasma patterns of PTH and PTHSO in unfasted rats receiving one oral or eight daily oral doses of PTH did not indicate that PTH induces its own metabolism. Limited studies in armadillos receiving PTH and PTHSO intravenously led to the same general conclusions as those we derived from the rat studies regarding the disposition of PTH and PTHSO.

RESUMEN

Se describe un nuevo método, sensible y selectivo, para la medición de protionamida (PTH) y de su metabolito sulfóxido (PTHSO) en líquidos biológicos. La sensibilidad del método es de aproximadamente 0.01 μg de droga por ml de plasma. Otros materiales endógenos (2-propil-isonicotinamida, etionamida, dapsona y monoacetil-dapsona) no interfieren en la determinación. Las ratas que recibieron PTH por la via oral o intravenosa mostraron un dimorfismo sexual en cuanto a su capacidad para oxidar la PTH a PTHSO; los machos mostraron la mayor capacidad oxidativa. Ambos sexos eliminaron más rápido de su plasma a la PTH que a la PTHSO. Ambos sexos tuvieron la capacidad de reducir al sulfóxido (PTHSO) a PTH, pero las hembras mostraron la mayor capacidad reductora. En ambos sexos, la capacidad de depuración después de la administración oral de PTHSO fue otra vez más rápida para la PTH que para la PTHSO. Sin embargo, el total de PTH y PTHSO fue consistentemente menor que cuando se administró PTH. Por lo

tanto, aunque la PTHSO se retiene más tiempo que la PTH después de la administración de PTH o de PTHSO, la administración de PTHSO produce menos droga activa total en circulación. La comparación de los niveles plasmáticos de PTH y PTHSO en ratas que recibieron 1 u 8 dosis orales de PTH no indicaron que la PTH induzca su propio metabolismo. Algunos estudios en armadillos que recibieron PTH y PTHSO intravenosamente, condujeron a las mismas conclusiones generales que las derivadas de los estudios con ratas en cuanto al manejo de PTH y PTHSO.

RÉSUMÉ

On décrit ici une nouvelle méthode mise au point pour mesurer de manière, à la fois sensible et sélective, le prothioamide (PTH) et son métabolite S-oxydé (PTHSO) dans des liquides biologiques. La limite de la sensibilité de la méthode se situait approximativement à 0.01 μg par ml de médicament dans le plasma. Des produits endogènes, de même que le 2-propylisonicotinamide, l'éthionamide, la dapsone, ou la monoacetyl dapsone, n'interféraient pas avec ces mesures. Des rats recevant de la PTH, soit par voie intraveineuse, soit par voie orale, ont témoigné d'un dimorphisme lié au sexe quant à leur capacité à oxyder le PTH en PTHSO, la capacité de conversion étant plus prononcée chez les males. Les deux sexes éliminaient plus rapidement du plasma, la PTH administrée, que cela n'était le cas pour le métabolite, le PTHSO. A la suite de l'administration de doses équimolaires de PTHSO, par voies orale ou intraveineuse, l'un et l'autre sexe ont témoigné d'une capacité à réduire le PTHSO administré en PTH, cette capacité à convertir le produit en son métabolite étant plus prononcée chez la femelle. L'élimination était également plus rapide pour la PTHSO, après administration orale de PTHSO, et ceci dans les deux sexes. Néanmoins, le contenu total de PTH et de PTHSO dans le plasma au cours des 8 heures suivant l'administration de PTHSO était régulièrement plus faible que la quantité mesurée après l'administration de PTH. Dès lors, on constate que l'administration de PTHSO libère une quantité totale de médicament actif plus faible dans la circulation, malgré le fait que le PTHSO soit retenu plus longtemps que le PTH après l'administration de l'un ou l'autre des produits. La comparaison des profils plasmatiques de PTH et de PTHSO chez des rats non soumis au jeûne et recevant une ou huit doses orales journalières de PTH, ne permet pas de conclure que le PTH entraîne son propre métabolisme. Des études limitées chez les tatous recevant du PTH et de PTHSO par voie intraveineuse a conduit aux mêmes conclusions générales que celles tirées à partir des études menées sur les rats, en ce qui concerne le PTH et le PTHSO.

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