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The biotransformation of 8-ethylthio-6-thiotheophylline sodium *in vivo*

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IN THE EVALUATION of the hypnotic properties of 8-ethylthio-6-thiotheophylline sodium (ETTT)[1] it was noticed that the duration of action was rather short.[2] To explain this observation it was postulated that either the compound was redistributed to adipose tissue, excreted unchanged or the compound underwent inactivation by biotransformation. To prove that the compound underwent biotransformation it was decided to see what effect stimulators and inhibitors of drug metabolism would have on the sleeping time of ETTT. Once it had been shown that the compound was inactivated by biotransformation it was decided to attempt to isolate and identify the metabolites.

METHODS

To show the effects of stimulators and inhibitors of drug metabolism on the sleeping time of ETTT, groups of 10 male albino rats were used for each compound tested. A group of 10 rats served as the control for each set of experiments. Also, each compound used was tested alone on groups of 6 rats each, at the dose to be used in the test, to determine what effect if any it had on the animal. Doses used, pretreatment time, and results are given in Table 1.

TABLE 1. EFFECT OF STIMULATORS AND INHIBITORS OF DRUG METABOLISM ON THE SLEEPING TIME OF 8-ETHYLTHIO-6-THIOTHEOPHYLLINE SODIUM

Name	Dose (mg/kg)	Pretreatment time (hr)	Average duration of hypnosis in rats (min ± S.D.)*
Control†	50		29·1 ± 1·5
SKF 525-A	50	1	7·5 ± 2
Lilly 18947	25	1	74·2 ± 2·5
Carbon tetrachloride	‡	24	168·0 ± 5
Phenobarbital sodium	50	b.i.d. for 3 days	26·6 ± 1·5‖
Saline	§	b.i.d. for 3 days	27·0 ± 0·5
Olive oil	§	24	27·7 ± 1·5
3-Methylcholanthrene in olive oil	25	24	3·9 ± 1
Actinomycin D + olive oil	1	25 24	27·5 ± 1
Actinomycin + 3-Methylcholanthrene in olive oil	1 25	25 24	26·1 ± 1

* S.D. for groups of 10 rats.

† 8-ethylthio-6-thiotheophylline sodium.

‡ 0·2 cc given to each rat.

§ Volume of solvent given.

‖ Experiment repeated and confirmed. Under the same conditions, phenobarbital sodium greatly shortened the sleeping time produced by 100 mg/kg hexobarbital sodium.

For the studies *in vivo* of the biotransformation of ETTT, two groups of 3 rats each were used. The urine from 3 rats injected with saline was collected and pooled over a 24-hr period. Similarly, 3 rats weighing about 150 g were injected i.p. with a dose of 100 mg/kg and their urine was collected over a 24-hr period and pooled. The pooled urines were treated similarly. The urine was flash-evaporated to dryness at 40°. The residue was extracted with 30 ml of 95% ethanol. From this volume, 20 μl was pippetted onto a TLC plate coated with Silica gel G according to Stahl.[3] Besides the spotting of the control urine and the test urine, various amounts of other synthetic derivatives were also spotted. Three different solvent systems were used to help identify the spots. The first solvent was a mixture of benzene, acetic acid and ethyl acetate, 5:1:1; the second was similar, but in different proportions, namely 7:2:1; while the third mixture consisted of carbon tetrachloride, toluene, acetic acid and ethyl acetate, 6:1:2:1. Table 2 gives the R_f values of some known compounds along with R_f values for the various spots in the control and test urines in the three solvent systems.

TABLE 2. R_f VALUES FOR 8-ETHYLTHIO-6-THIOTHEOPHYLLINE AND SOME RELATED COMPOUNDS

Compound	Solvent systems		
	Benzene 5 Acetic acid 1 Ethylacetate 1	Benzene 7 Acetic acid 2 Ethylacetate 1	CCl_4 6 Toluene 1 Acetic acid 2 Ethylacetate 1
	(R_f)	(R_f)	(R_f)
8-Ethylthio-6-thiotheophylline	0·87	0·77	0·67
8-Mercapto-6-thiotheophylline	0·80	0·60	0·35
8-Mercaptotheophylline	0·61	0·38	0·19
Test urine	0·87 0·79 0·60 0·50 0·43 0·06	0·77 0·59 0·38 0·31 0·06	0·67 0·35 0·29 0·19 0·06
Control urine	0·43 0·06	0·06	0·06

RESULTS AND DISCUSSION

Redistribution to adipose tissue was disregarded as a major factor in inactivation for three reasons: (1) the compound is poorly soluble in lipid solvents such as ether, carbon tetrachloride, benzene and petroleum ether and is soluble in polar solvents such as alcohols etc.; (2) slight alterations in chemical structure to increase lipid solubility, such as lengthening the alkyl side chain, replacing the other oxygen with sulfur, abolishes activity;[2] (3) the major portion of the administered dose was excreted as metabolic products within 24 hr after administration. No single factor is conclusive proof, but taken together they strongly suggest that redistribution to adipose tissue plays a minor role in inactivation. From Table 1 it can be seen that the enzyme inhibitors, β-diethylaminoethyl-diphenylpropylacetate

(SKF 525-A) and 2,4-dichloro-6-phenylphenoxydiethylamine (Lilly 18947), greatly increase the sleeping time of ETTT. Likewise, CCl_4 which depresses the drug-metabolizing capability of the liver for some drugs also greatly prolongs the action of ETTT. 3-Methylcholanthrene, a stimulator of enzyme activity,[4,5] greatly shortens the duration of action, but pretreatment with phenobarbital has no effect on the sleeping time. The effects of 3-methylcholanthrene was effectively blocked by pretreatment with actinomycin D, which prevents new protein synthesis.[6] Hence, it can be concluded that ETTT is indeed metabolized and that this is the chief route of inactivation. The fact that phenobarbital has no effect on the duration of action suggests that phenobarbital does not stimulate the synthesis of the enzymes required for the metabolism of ETTT. It has been found that pretreatment with thiopental has no effect on the sleeping time of ETTT.[7]

Table 2 gives the R_f values for certain known compounds and also for the spots in the test urine and the control urine for all three solvent systems. It can be seen that some unchanged ETTT is excreted in the urine along with three metabolites. Two of the metabolites have been identified as the 8-mercapto-6-thiotheophylline and 8-mercaptotheophylline. The third metabolite is most likely the *N*-demethylated derivative of 8-mercaptotheophylline, since this is one route of metabolism of related *N*-methylated xanthines.[8-11] By spotting various amounts of the reference compounds, a crude quantitative estimate of the amount of metabolites in the urine could be determined by comparison of spot intensity. A total dose of 45 mg ETTT was administered to the rats. In 24 hr approximately 1 per cent was excreted unchanged, 25 per cent as 8-mercaptotheophylline, and a small amount (about 1 per cent) as the unidentified metabolite. These values are a rough approximation.

A possible pathway of metabolism is through the 8-mercapto-6-thiotheophylline, first by deethylating the 8-thioether and then by dethiating the 6-thio group to the 8-mercaptotheophylline, which is then converted to the unknown metabolite. No 8-ethylthiotheophylline or sulfoxide was found.

The inhibitors of drug metabolism, SKF-525A and Lilly 18947, inhibit the metabolism of ETTT. Carbon tetrachloride also increases its duration of action. 3-Methylcholanthrene, an enzyme stimulator, greatly shortens its action, but phenobarbital has no effect on its sleeping time. These results show that the major path of inactivation is indeed biotransformation. Phenobarbital does not stimulate the enzymes required for the metabolism. Three metabolites were isolated from the urine of rats along with some unchanged compound. Two of the metabolites were identified as 8-mercapto-6-thiotheophylline and 8-mercaptptheophylline. The third was not identified.

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REFERENCES

1. A. J. DIETZ, JR. and R. M. BURGISON, *J. mednl Chem.* **9**, 160 (1966).
2. A. J. DIETZ, JR. and R. M. BURGISON, *J. mednl Chem.* **9**, 500 (1966).
3. E. STAHL, (Ed.) *Thin-layer Chromatography, A Laboratory Handbook*. Academic Press, New York (1965).
4. A. H. CONNEY, C. DAVIDSON, C. GLASGOW and J. J. BURNS, *Pharmacologist* **1**, 64 (1959).
5. A. H. CONNEY, J. R. GILLETTE, J. K. INSCOE, E. R. TRAMS and H. S. POSNER, *Science* **130**, 1478 (1959).
6. I. MERITS, *Biochim. biophys. Acta* **108**, 578 (1965).
7. A. J. DIETZ, JR. and R. M. BURGISON, submitted for publication.
8. H. H. CORNISH and A. A. CRISTMAN, *J. biol. Chem.* **228**, 315 (1957).
9. V. C. MEYERS and R. F. HANZEL, *J. biol. Chem.* **162**, 309 (1946).
10. B. B. BRODIE, J. AXELROD and J. REICHENTHAL, *J. biol. Chem.* **194**, 215 (1952).
11. J. AXELROD and J. REICHENTHAL, *J. Pharmac. exp. Ther.* **107**, 519 (1953).