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# STUDIES OF THE INDUCTION OF MICROSOMAL S-, N- AND O-DEMETHYLASES*

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**Abstract**—The effect of treatment of rats with Mesantoin, phenobarbital, 3,4-benzpyrene, or methylthiopurine on the S-demethylase acitvity of liver microsomes was tested. Only 3,4-benzpyrene treatment led to increased activity; the other compounds either had no effect or lowered S-demethylase acitivity. Comparison of the effects of these treatments on S-demethylation with those on two N-demethylases and two O-demethylases led to the conclusion that the S-demethylase was not identical with any of the other enzymes studied.

STUDIES by many workers have shown that treatment of animals with a variety of chemicals leads to increased or decreased activity of various microsomal drug-metabolizing enzymes (reviewed in Refs. 1, 2). The chemicals used in such studies generally do not increase or decrease the activity of all the known microsomal drug-metabolizing enzymes at the same time or to the same extent. Instead, each compound, under a given set of conditions, appears to affect a characteristic spectrum of enzyme activities.[3–5] The characterization of a microsomal system that S-demethylates a variety of compounds[6, 7] prompted this investigation of the effects of certain S-methyl compounds and of other drugs on the activity of the S-demethylating system.

N- and O-Demethylating systems in liver microsomes have each been shown to consist of more than one enzyme.[4, 8, 9] Criteria by which these different enzyme activities were distinguished included individual response to treatments which increased or decreased enzyme activities, and differential response to inhibitors such as SKF-525A.

In order to study the relationship of the S-demethylating system to N- and O-demethylases, the behavior of the S-demethylating system was compared to that of N- and O-demethylases under conditions in which the activities of the latter were altered by pretreatment of animals with various drugs.

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## MATERIALS* AND METHODS

Demethylation was followed by determining the amount of formaldehyde formed during incubation of substrates with microsomes plus soluble fraction from rat liver. Incubation conditions and the procedure for the determination of formaldehyde are the same as described in a previous paper[6] and are summarized in Table 1.

In each experiment, livers from no fewer than four animals were pooled, and the results presented are averages of separate analyses of two to six flasks. The experiments have been repeated two to four times with essentially the same results. Although the absolute amounts of formaldehyde formed from any given substrate varied from day to day with different preparations, the amounts relative to one another remained quite constant, with a variation of not more than 10%. The changes resulting from pretreatment also remained within 10% variation. In a given experiment, variation between formaldehyde analyses on duplicate flasks averaged 3·4%.

## RESULTS

In order to determine if prior exposure to an S-methyl compound or to other drugs would alter the level of S-demethylase activity in rat liver, groups of adult male rats were injected intraperitoneally daily for three days with 6-methylthiopurine, sodium phenobarbital, Mesantoin, or 3,4-benzpyrene. Control rats received injections of the vehicle. The ability of the microsomes plus soluble fraction from treated and control livers to demethylate a S-methyl compound, methylthiopurine, were then compared. In one case a second S-methyl compound, methylthiobenzothiazole, was also used as test substrate. Morphine and methylbarbital were used as substrates for separate N-demethylases; methoxyacetanilide was used as a substrate for an O-demethylase. Codeine is both O- and N-demethylated. Demerol was included as a standard. It should be noted that the two vehicles used, propylene glycol and corn oil, resulted in different basal enzyme levels, which were consistently higher after administration of propylene glycol.

Table 1 presents the results of these experiments. Mesantoin pretreatment increased the N-demethylation of Demerol and methylbarbital but decreased that of morphine. Demethylation of codeine was unchanged under these conditions, whereas that of methoxyacetanilide was increased. S-Demethylation of methylthiopurine was diminished slightly.

Pretreatment with phenobarbital also increased the N-demethylation of Demerol and methylbarbital, but it decreased that of morphine. Phenobarbital differed from Mesantoin by decreasing the demethylation of codeine while not affecting that of methoxyacetanilide; S-demethylation of methylthiopurine was decreased.

Methylthiopurine caused decreased N-demethylation of Demerol and morphine, decreased demethylation of codeine with a marginal decrease in O-demethylation of methoxyacetanilide, and decreased S-demethylation of methylthiopurine itself.

Benzpyrene stimulated the demethylation of morphine, methoxyacetanilide, methylthiopurine, and methylthiobenzothiazole, while causing a slight decrease in that of codeine.

* Codeine phosphate, morphine sulfate, and sodium phenobarbital were obtained from Merck, Sharp and Dohme; SKF-525A (β-diethylaminoethyl diphenylpropylacetate) from Smith, Kline & French, Inc.; Demerol hydrochloride (meperidine hydrochloride) from Winthrop-Stearns, Inc.; methyoxyacetanilide and 3,4-benzpyrene from Eastman Chemical Co.; N-methylbatbital from Abbott Laboratories; and Mesantoin (5-ethyl-3-methyl-5-phenylhydantoin) from Sandoz, Inc. Sources of other chemicals used have been listed elsewhere.[6]

TABLE 1. EFFECT OF PRETREATMENT ON S-, N-, AND O-DEMETHYLATION

| Substrate | Conc. (μmoles/flask) | Formaldehyde formed (mμmoles) | | | |
|---|---|---|---|---|---|
| | | | Pretreatment | | |
| | | Control | Mesantoin | Phenobarbital | Methyl-thiopurine |
| Experiment 1 | | | | | |
| Demerol | 3·53 | 144 | 183 | 197 | 107 |
| Morphine | 2·64 | 134 | 96 | 78 | 53 |
| Methylbarbital | 10·0 | 13 | 136 | 90 | 14 |
| Codeine | 4·72 | 360 | 373 | 247 | 287 |
| Methoxyacetanilide | 12·1 | 133 | 189 | 139 | 115 |
| Methylthiopurine | 23·8 | 144 | 118 | 97 | 108 |
| | | Control | Benzpyrene | | |
| Experiment 2 | | | | | |
| Demerol | 3·53 | 94 | 111 | | |
| Morphine | 2·64 | 33 | 53 | | |
| Methylbarbital | 10·0 | 0·4 | 0·4 | | |
| Codiene | 4·72 | 213 | 179 | | |
| Methoxyacetanilide | 12·1 | 40 | 237 | | |
| Methylthiopurine | 23·8 | 56 | 187 | | |
| Methylthiobenzothiazole | 5·33 | 106 | 155 | | |

Microsomes plus soluble fraction obtained from 1 g of rat liver were incubated for 1 hr at 37° in air with substrate, 0·65 μmole NADP, 1·0 μmole glucose-6-phosphate, 50 μmoles nicotinamide, 45 μmoles semicarbazide hydrochloride, 25 μmoles $MgCl_2$, 3 ml 0·5 M phosphate buffer (pH 7·4) in a total volume of 6·0 ml. In experiment 1 rats had received 100 mg Mesantoin/kg, 100 mg sodium phenobarbital/kg, or 50 mg methylthiopurine/kg in propylene glycol daily for three days prior to sacrifice; control rats received an equal volume of propylene glycol. In experiment 2, rats had received 5 mg benzpyrene/kg in corn oil for three days prior to sacrifice; control rats received an equal volume of corn oil.

TABLE 2. EFFECT OF SKF-525A ON O- AND S-DEMETHYLATION

| | Conc. (μmoles/flask) | Formaldehyde formed (mμmoles) | |
|---|---|---|---|
| | | Control | SKF-525A |
| Codeine | 4·72 | 247 | 63 |
| Methylthiopurine | 23·8 | 97 | 61 |

Experimental conditions are described in Table 1. SKF-525A (β-diethylaminoethyl diphenylpropylacetate) concentration was 1 mM.

It is known that SKF-525A inhibits certain N- and O-demethylases while not affecting the activity of others.[8, 9] The effect of this agent on the S-demethylation of methylthiopurine was therefore tested. Preliminary experiments indicated that this agent at $2 \times 10^{-4}$ M had no effect on this reaction *in vitro*. The results of an experiment at a fivefold higher concentration are shown in Table 2. Codeine was used as a standard whose demethylation is known to be inhibited by this agent. A slight inhibition of the S-demethylation of methylthiopurine was demonstrated.

## DISCUSSION

The results of this study indicate that S-demethylation of at least two substrates can be altered by pretreatment with both related and structurally unrelated drugs.

It must be pointed out, however, that S-demethylation was not so plastic in this respect as the N- and O-demethylases studied.

The variation both in the direction and magnitude of the responses of the S-, N-, and O-demethylation systems studied to pretreatment with four different agents clearly differentiates the S-demethylating enzyme from either of the N-demethylases or the O-demethylases studied. S-Demethylation is also distinguished from certain other demethylases by its relative insensitivity to inhibition by SKF-525A.

The previous papers in this series[6, 7] demonstrated the ability of microsomal drug-metabolizing enzymes to metabolize certain purine antimetabolites. The present results show that the activity of these enzymes may depend on the previous history and current state of the recipient of these drugs. Thus previous or concurrent therapy may alter the rate of metabolism of the drugs and may thereby alter the duration of their pharmacological effects.

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