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Benactyzine inhibition of microsomal meprobamate metabolism

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INTERACTION of therapeutic effects when drug combinations are administered is well known. Recently, the metabolic aspects of simultaneous or concurrent administration of various compounds have received wide attention. The unique synergistic pharmacological properties[1] shown by the combination of benactyzine and meprobamate, coupled with the extensive use of this combination as a therapeutic agent (Deprol), has led us to study the effects of benactyzine on microsomal meprobamate metabolism. Our findings are reported in this manuscript.

MATERIALS AND METHODS

Microsome preparations. Male Sprague–Dawley rats weighing 150–180 g, fed *ad libitum*, were decapitated and exsanguinated. The livers were immediately removed, rinsed with cold 1·15% (w/v) potassium chloride, and homogenized at 0° with 2 vol. of KCl/g liver. The homogenate was centrifuged at 14,000 *g* in the cold and the supernatant, containing microsomes and the soluble fraction, was used as the source of the drug-metabolizing enzymes. A protein determination of each supernatant, by the method of Folin-Ciocalteau with bovine serum albumin as a standard, was used to standardize preparations from different animals. All results are reported on the basis of μmole hydroxymeprobamate formed per mg protein in the enzyme source.

Assay procedure. The incubation mixture contained 6·0 ml supernatant, 33 μM magnesium sulphate, 0·6 μM nicotinamide adenine dinucleotide phosphate, 50 μM glucose-6-phosphate, 200 μM nicotinamide, 2 μM adenosine-5-triphosphate, 90 μM meprobamate-^{14}C-carbamate (Isotope Specialties, Inc.), benactyzine hydrochloride in the concentrations indicated, and sufficient 0·2 M, pH 7·4 phosphate buffer to give a final volume of 10·0 ml. Flasks were shaken under oxygen at 37° and 0·5-ml aliquots removed at specified times. An equal volume of acetone was added to the sample to stop the reaction and to precipitate the protein. The precipitated protein was found to bind negligible amounts of radioactivity. Fifty-μl aliquots of the acetone–water supernatant were chromatographed on paper in *n*-butanol–acetic acid–water (4:1:5). This solvent system separates hydroxymeprobamate (R_f 0·78) and meprobamate (R_f 0·94). The radioactivity present in the area of the paper chromatogram corresponding to each of these two compounds was quantitated with a Vanguard model 880 Autoscanner equipped with an integrating recorder. No other radioactive areas were detected.

Feeding. Male Sprague–Dawley rats were fed *ad libitum* a normal laboratory ratio–diet augmented with either 0·06% (w/w) phenobarbital or 0·16% (w/w) benactyzine hydrochloride for 2–3 weeks. Microsomes from the livers of these treated animals were prepared as described above.

RESULTS

The inhibitory effect of benactyzine hydrochloride on the metabolic oxidation of meprobamate to hydroxymeprobamate in pooled rat liver microsomes is shown in Fig. 1. This effect is not immediately evident as there is a period of about 15 min before the onset of inhibition. At a concentration of 1·25 mM, the maximum solubility of benactyzine hydrochloride under these conditions, the oxidation is inhibited to about 44 per cent of the control after 1 hr. Lower concentrations of benactyzine hydrochloride have correspondingly less activity, the relationship between activity and concentration being linear.

The induction period can be overcome by incubating the microsomes with benactyzine hydrochloride for 15 min prior to the addition of the radioactive substrate. However, the overall extent of inhibition is not altered to an appreciable degree since the oxidation is also decreased about 40 per cent in this experiment.

The time lag in benactyzine inhibition of meprobamate oxidation could be due to the saponification of the inhibitor *in vitro*. We therefore investigated the effects of diethylaminoethanol and benzilic acid (diphenylglycolic acid) on this microsomal system. Fig. 2 shows the rate of oxidation of

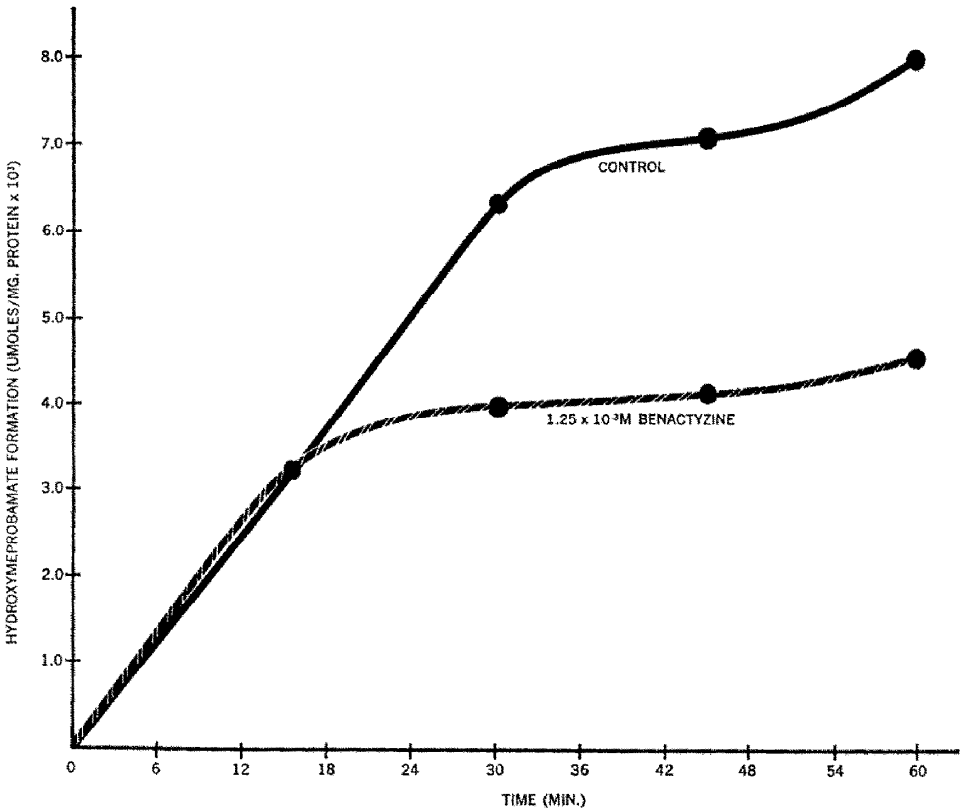

FIG. 1. Effect of benactyzine on meprobamate oxidation in rat liver micromosomes.

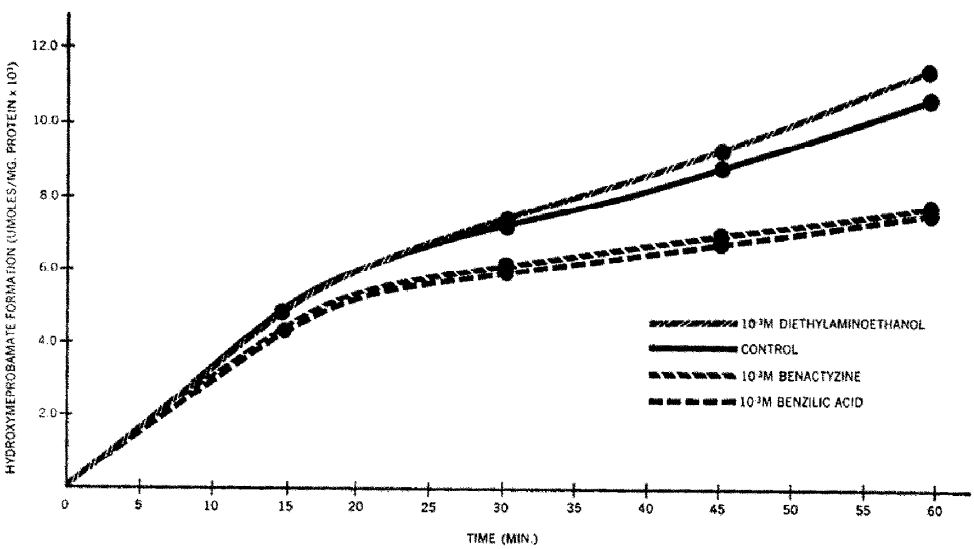

FIG. 2. Effect of the hydrolyis products of benactyzine on meprobamate oxidation in rat liver microsomes.

meprobamate in the presence of these compounds. Diethylaminoethanol does not affect the reaction, whereas benzilic acid is as effective as benactyzine hydrochloride, on a molar basis, in inhibiting hydroxymeprobamate formation. In the presence of benzilic acid, the rate of meprobamate oxidation is not impaired for the first 15 min (lag period), after which the inhibition becomes evident.

The effect of benactyzine hydrochloride on meprobamate oxidation by stimulated microsomes was investigated. In this study the increased rate of hydroxymeprobamate formation by the rat hepatic microsomal enzyme system from the phenobarbital pretreatment group (4 animals) fell to 83 per cent of the control level ($P < 0.01$) when incubated for 60 min in the presence of 10^{-3} M benactyzine hydrochloride.

Effects of benactyzine *in vivo* were studied in rats which were fed a diet containing 0·16 per cent of the compound. After drug therapy, the livers were excised and the rate of microsomal meprobamate oxidation was measured. In our experiments, after 2–3 weeks of feeding, hydroxymeprobamate formation is reduced by about 25 per cent ($P < 0.001$) after 60 min of incubation. The effect of feeding benactyzine is not immediate since injection of this inhibitor into rats, 50 mg/kg i.p., gives no inhibition of meprobamate metabolism when the animals are sacrificed at ½, 1, or 3 hr after injection.

DISCUSSION

The effect of benactyzine on metabolism of meprobamate was studied in rat liver microsomes. The results indicate that there is a small but significant inhibition by benactyzine both *in vivo* and *in vitro*.

The data showed that *in vitro* there is a lag period of about 15 min during which the inhibitor is not effective. Incubation of the microsomes with benactyzine for the same period prior to addition of substrate overcomes the induction period. Although the nature of the lag has not been determined, it may be due either to the production of a metabolite of benactyzine or to the slow interaction of benactyzine itself with the active oxidative site of the hepatic microsome. It is apparently not due to hydrolysis of the drug since the equally effective cleavage product, benzilic acid, also requires an induction period of 15 min.

The stimulation of drug metabolism by a number of compounds, including phenobarbital, is well documented. Other investigators have indicated that the increased detoxification of drugs after treatment with phenobarbital is due to an increase in the microsomal drug-metabolizing enzymes,[2] which may be due to an increase in smooth endoplasmic reticulum.[3] It is therefore likely that the decreased inhibitory action of benactyzine in the case of animals pretreated with phenobarbital (17 per cent) when compared to nonstimulated animals (44 per cent) is simply due to a difference in the quantity of enzyme available for the conversion of meprobamate to hydroxymeprobamate.

Although meprobamate is partially conjugated with glucuronic acid to form an *N*-glucuronide in the intact animal,[4] this reaction does not appear to play a significant role in the microsomal metabolism of this drug *in vitro* under our experimental conditions.

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REFERENCES

1. F. M. BERGER, M. KLETZKIN and S. MARGOLIN, *Medna pharmac. Exp.* **13**, 103 (1965).
2. H. REMMER and H. J. MERKER, *Science* **142**, 1658 (1963).
3. J. R. FOUTS and L. A. ROGERS, *J. Pharmac. exp. Ther.* **147**, 112 (1965).
4. H. TSUKAMOTO, H. YOSHIMURA and K. TATSUMI, *Chem. pharm. Bull., Tokyo* **11**, 421 (1963).