carcinogens had an inhibitory effect on the activity of this enzyme. An inhibitory effect on pancreatic deoxyribonuclease activity by actinomycin D and nogalamycin was claimed to be due to their interaction with DNA.[18,19]. Their mode of interaction with DNA, however, differ very much from that of the aflatoxins and they also bind much more strongly to DNA than the aflatoxins do.[12] Aflatoxins B_1, B_2 and M_2 were found to activate pancreatic deoxyribonuclease activity most probably due to its binding to DNA.* Ts'o[20] stated that carcinogens entering into a living cell are more likely to interact with DNA than anything else. The activating effect of versicolorin C, rosenonolactone and cyclopiazonic acid on pancreatic deoxyribonuclease may possibly also be related to interaction with DNA.

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Biochemical Pharmacology, Vol. 20, pp. 246–248. Pergamon Press, 1971. Printed in Great Britain

Tolerance to the hypnotic effect of *l*-methylphenobarbital induced by its nonhypnotic stereoisomer*

(*Received* 29 *April* 1970; *accepted* 19 *June* 1970)

RACEMIC methylphenobarbital N. F. (Mebaral, Winthrop; Prominal, Bayer) has been used for many years as an anticonvulsive and hypnotic. Knabe and Philipson[1] succeeded in resolving the compound in 1966. The effects of the two isomers in rats were then studied by Büch *et al.*[2] Only the levorotatory

* This publication is based upon research activities supported through a grant from the Health Research Council, U-1501 (City of New York Department of Health), and from the New York State Narcotics Addiction Control Commission. Conclusions stated herein are not necessarily those of the Commission.

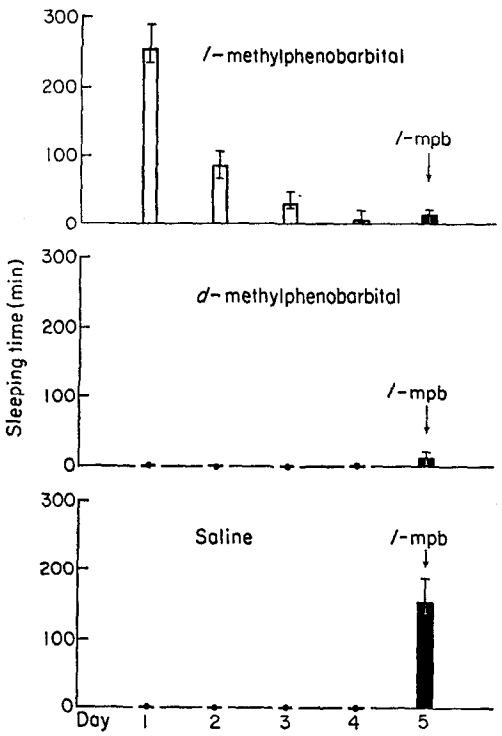

FIG. 1. Tolerance to hypnotic asymmetric barbiturate induced by its nonhypnotic enantiomer. Rats treated for 4 days with either methylphenobarbital enantiomer were equally tolerant on the fifth day to the hypnotic levo isomer. The graph shows means and ranges of sleeping times.

form was found to be hypnotic. The dextrorotatory isomer was not, but in large enough doses caused excitement.

We wished to determine if tolerance to the hypnotic isomer could be induced by the inactive isomer. If the induction were not stereospecific, then the nonhypnotic isomer might provide safe treatment for certain cases of infantile jaundice and congenital hyperbilirubinemia now treated with phenobarbital.[3–5]

Methylphenobarbital enantiomers were prepared by Knabe and Philipson's method[1] of fractional crystallization of their methyl-quinine salts. White Sprague–Dawley male rats weighing about 145 g were divided into three groups. Rats in the first group (six rats) were given four daily intraperitoneal injections of *l*-methylphenobarbital, 14 mg. Those in the second group (six rats) received equal doses of *d*-methylphenobarbital, while those in the third group (five rats) received saline. Barbiturates were prepared daily for injection by dissolving them in saline with 1·1 molar equivalents of NaOH. Sleeping time was defined as the interval between the loss of the righting reflex and its return. Only rats which received *l*-methylphenobarbital slept. During the 4 days, their mean sleeping times decreased from more than 4 hr to less than 10 min. On the fifth day, rats in all groups were injected with 21 mg *l*-methylphenobarbital (Fig. 1). Rats that had received saline during the first 4 days slept 2·5 hr, whereas rats which had received either isomer slept 11 min.

d-Methylphenobarbital, given in the above dose (100 mg/kg, i.p.), produced no visible effects. Rats receiving it behaved no differently from saline controls. When the experiment was performed using higher daily doses of barbiturates (over 150 mg/kg, i.p.), the first injection of *d*-methylphenobarbital produced excitement lasting about 10 min. Subsequent injections were without effect.

Rapidly acquired tolerance to the hypnotic effect of barbiturates results from the induction of microsomal drug-metabolizing enzymes. The ability of both isomers to induce the mitochondrial enzyme, avian hepatic aminolevulinic acid (ALA) synthetase, was also tested. The barbiturates were compared with allylisopropylacetamide, a known potent inducer. For injection, 3 mg of the test compound was dissolved in 0·2 ml propylene glycol. Chick embryos, 16 days old, were divided into

four groups. Embryos in one group received propylene glycol alone. Those in the other groups received allylisopropylacetamide, *d*-methylphenobarbital or *l*-methylphenobarbital. Embryos were incubated 6·5 hr at 37°. Liver homogenates were prepared and their content of ALA synthetase was determined by the procedure of Marver *et al.*[6] The results, shown in Table 1, indicated that both isomers are potent inducers of ALA synthetase.

TABLE 1. INDUCTION OF ALA SYNTHETASE IN CHICK EMBRYO LIVER BY METHYLPHENOBARBITAL ENANTIOMERS

Substance injected	No. of eggs	ALA (mμmoles/g-hr)
Propylene glycol (control)	5	15 ± 3
Allylisopropylacetamide (3 mg/egg)	5	647 ± 60
d-Methylphenobarbital (nonhypnotic) (3 mg/egg)	6	404 ± 47
l-Methylphenobarbital (hypnotic) (3 mg/egg)	4	461 ± 56

In summary, induction of barbiturate tolerance by methylphenobarbital is not stereospecific. It is possible that methylphenobarbital is not the inducer, but that induction is due to its principal metabolite, phenobarbital. This is unlikely for two reasons. First, drug-metabolizing enzymes are induced by many barbiturates, including the nonhypnotic experimental compound, *N*-phenylbarbital.[7] Second, Rifkind* found that racemic methylphenobarbital was 60 per cent as potent as allylisopropylacetamide in inducing avian ALA-synthetase *in vivo*, whereas phenobarbital, in a second set of incubations, was only 25 per cent as potent as allylisopropylacetamide.

Clinically, a safe nonsedative inducer of hepatic microsomal enzymes would be a welcome replacement for phenobarbital in the treatment of appropriate cases of neonatal jaundice. Although it is reasonable to expect that, in proper doses, *d*-methylphenobarbital would be as safe as the racemate, this is not yet proven.

Acknowledgements—The author thanks Dr. Arleen Rifkind of the Rockefeller University for determinations of ALA-synthetase. Mrs. Giedre Stankunas gave skilled technical assistance. Generous gifts of racemic methylphenobarbital were received from Dr. Sidney Archer (Sterling-Winthrop Research Institute, Rensselaer, N.Y.) and Farbenfabriken Bayer AG, Leverkusen, Germany.

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