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The effect of chronic phenobarbital administration on some hepatic drug-metabolizing enzyme activities in the rat*

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PROLONGED administration of phenobarbital produces an increase in hepatic microsomal drug-metabolizing enzyme activity in some animals. Burns and Conney[1] gave phenobarbital to dogs for 2 months and found enhanced metabolism of phenylbutazone for as long as 6 weeks after cessation of therapy. In a similar study, Remmer *et al.*[2, 3] noted that canine microsomal enzyme activity, with hexobarbital and aminopyrine as substrates, remained elevated for 2 to 4 months after stopping a 2–3-month course of phenobarbital treatment. A single overdose of hypnotics in man can enhance 4-aminoantipyrine production from Novalgin for as long as 15 days.[2]

The activity of microsomal drug-metabolizing enzymes in rat liver during and after chronic phenobarbital administration has not been studied. Using rats, Orrenius and Ernster[4] have shown that formation of formaldehyde from aminopyrine falls to control levels within 6 days after short-term administration of phenobarbital (every day for 5 days). In view of this, four microsomal drug-metabolizing pathways were measured in livers from rats given phenobarbital for varying periods of time up to 2 months. A plateau in rate of hexobarbital metabolism was reached during the treatment with phenobarbital, and a rapid decline in enzyme activity was observed after stopping this treatment.

METHODS

Male Long-Evans rats (200–300 g) were injected i.p. with phenobarbital sodium, 75 mg/kg/day, for 8 days and then every other day (with this same dose) for an additional 52 days. This dose of phenobarbital (75 mg/kg/day) was shown to provide maximal stimulation of the metabolic pathways studied. Thus, the level of enzyme activity reached after 4 days of treatment with this dose was greater than that seen with 40 mg/kg/day, but the same as that after 100 mg/kg/day. Another group of rats received an injection of water, buffered to the pH of the phenobarbital solution on the same injection schedule. Control and experimental groups were of comparable age, and three animals of each group were used for each time period studied.

Livers were excised 24 hr after the last injection of phenobarbital and homogenized in the cold, with 2 ml 1·15% KCl/g liver. Incubation media and the 9000 *g* supernatant fraction of liver homogenate were prepared according to the method of Dixon *et al.*[5] The metabolism of hexobarbital by side-chain oxidation was measured by determining the amount of substrate disappearance.[6] Benzpyrene hydroxylation was measured according to the method of Juchau *et al.*, adapted from Conney, *et al.*[8, 9] *p*-Aminobenzoic acid (PABA) formed from *p*-nitrobenzoic acid (PNBA) was estimated according to the method of Fouts and Brodie.[10] Morphine produced by the O-dealkylation of codeine was extracted from the incubation mixture and determined with the phenol reagent of Snell and Snell.[11] All results were expressed as micromoles drug biotransformed per gram liver per 30-min incubation. Statistical methods are described in Snedecor;[12] the level of significance chosen was $P < 0.05$.

RESULTS

Figure 1 shows the amount of hexobarbital metabolized by livers from control v. animals treated with phenobarbital for varying periods of time from 1–60 days. Maximal enzyme activity was reached after 4 days of treatment, and this level was maintained throughout the treatment period. No gradual increase in activity occurred with repeated doses of phenobarbital, nor was any decreased response to phenobarbital stimulation observed. Withdrawal of phenobarbital resulted in a decline to control levels within 7 days.

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Untreated rats, and those which had been injected with the buffered water solution for 57 days, were given phenobarbital, 75 mg/kg/day, for 4 days and found to have similar levels of hexobarbital-metabolizing activity: 7·24 and 8·07 μmoles/g liver per 30 min respectively. Intraperitoneal injections and attendant handling for 60 days, therefore, did not interfere with the ability of rats to respond to phenobarbital stimulation of hexobarbital metabolism.

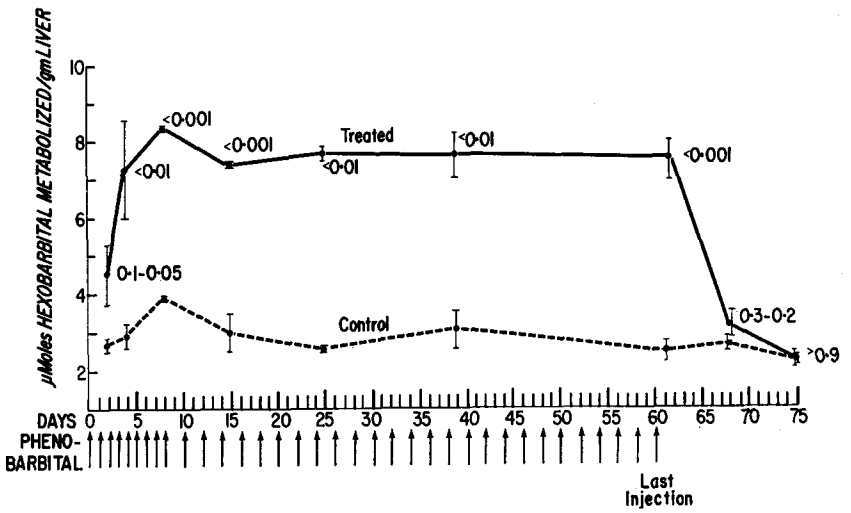

FIG. 1. Effect of chronic phenobarbital administration on hexobarbital metabolism. Phenobarbital injections are indicated by arrows. The standard error of the mean (vertical lines) and level of significance as compared with the control are indicated for each determination. The upper line denotes the phenobarbital-treated group and the lower line the control group.

The effect of prolonged phenobarbital administration on benzpyrene, PNBA, and codeine metabolism is depicted in Fig. 2. After 2 days of treatment, PNBA metabolism was increased; benzpyrene hydroxylation was enhanced within 4 days. The metabolism of both compounds remained at a

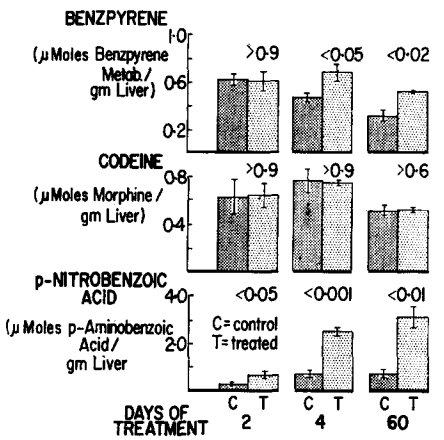

FIG. 2. Effect of chronic phenobarbital administration on the metabolism of benzpyrene, codeine, and *p*-nitrobenzoic acid. Days of phenobarbital treatment are indicated for the control (C) and treated (T) groups. Standard error of the mean and level of significance are shown as in Fig. 1.

constant but elevated level during phenobarbital administration. Codeine biotransformation to morphine was not significantly increased by phenobarbital treatment.

DISCUSSION

The duration of stimulation, as well as the level to which hepatic microsomal drug-metabolizing enzyme activity can be raised by a maximally effective dose of phenobarbital, varies with the species studied. As noted above, the dog may maintain increased levels of hepatic microsomal enzyme activity for 3 months after phenobarbital treatment is stopped but, in the rat, at least one such enzyme activity appears to fall to normal within 7 days after the last dose of phenobarbital. A plateau of hepatic enzyme activity is maintained in the rat as long as phenobarbital is continued, but this activity declines rapidly after stopping therapy. Other experiments have indicated that some stimulation of hexobarbital metabolism by rat liver may still be detected 4 days after the last dose of phenobarbital, but the exact rate of decline of enzyme activity between 61 and 68 days (Fig. 1), has not been determined. Although unfortunate, such a lack of data does not alter the fact that there is a marked difference between rats and dogs in the duration of enhanced activity of certain hepatic microsomal enzymes after the cessation of chronic phenobarbital treatment. An explanation of this difference is not available from the data presented here, but might involve a difference in the persistence of phenobarbital (or other inducer) in rats vs. dogs. Thus, the inducer might have a longer half-life in dogs after the last phenobarbital injection than it had in rats. For day-to-day constancy of hepatic microsomal enzyme activity in phenobarbital-treated rats, however, four or more doses of phenobarbital should be given.

Benzpyrene and PNBA metabolisms were enhanced by four doses of phenobarbital, but codeine biotransformation to morphine was unaffected. This is at first glance in contrast to the findings of Dixon *et al.*,[5] who were able to enhance significantly (but only 1·3-fold) metabolism of codeine to morphine with 2 days of phenobarbital therapy. This may be explained by the fact that, in their study, all determinations were made 12 hr after the last injection, while in the present investigation, determinations of enzyme activity were made 24 hr after the last dose of phenobarbital. Also, fewer animals were used in the present study than were used by Dixon *et al.* Orrenius *et al.*[13] have observed a twofold increase in hepatic codeine metabolism after treating rats for 2 days with phenobarbital (100 mg/kg/day), but they measured formaldehyde production, which may result from both O- and N-demethylation of codeine. Only morphine formed from the O-dealkylation of codeine is reported here, and phenobarbital may stimulate both the O- and N-demethylation of codeine.

The species, total number of doses and time of assay following the last dose of phenobarbital appear to influence the level of hepatic microsomal enzyme activity in animals. Even without phenobarbital stimulation, the amount of enzyme activity depends on the species studied,[14] and with or without phenobarbital pretreatment, certain differences in microsomal drug-metabolizing activity have been observed among various rabbit strains.[15] These differences may also be age-dependent.[16] When comparisons of microsomal drug-metabolizing enzyme activity are made it may be necessary, therefore, to consider not only the dose and duration of treatment with phenobarbital but also the age, species, and strain of the animal.

The interaction of one drug given simultaneously with or shortly before another drug has been previously emphasized.[1] However, the influence of a drug given several weeks before the onset of other therapy may be overlooked. The action of an enzyme-inducing agent after acute administration (1 to 3 doses) may persist for one or more weeks in man[2] or dogs.[1] Duration of enhanced enzyme activity may also depend on the length of drug administration, because Remmer[3] has shown that the increased hexobarbital-metabolizing activity in dogs treated for 3 months with phenobarbital may persist for 2–4 months. The purpose of this paper has been to describe the effect of chronic phenobarbital administration of hexobarbital metabolism in the rat, and to emphasize that the duration of stimulation of enzyme activity by a drug given acutely or chronically may be species-dependent.

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Study of the cellular action of drugs with protozoa—III. Comparison of the effect of SKF 525-A and related compounds on the multiplication of *Ochromonas danica**

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THE diethylaminoethanol ester of diphenylpropylacetic acid (SKF 525-A) as well as its analog SKF 3301-A inhibited the metabolism of unsaturated fatty acids in the phytoflagellate *Ochromonas danica*[1] and the synthesis of cholesterol in mammals.[2] The purpose of this study is to compare the effects of several analogs of SKF 525-A on *O. danica* to determine (1) if the analogs act on the same metabolic site as SKF 525-A and (2) what part of the molecule is necessary for inhibition.

EXPERIMENTAL

The organism used was *O. danica* Pringsheim. The methods for studying inhibition of multiplication and its annulment have been described.[3, 4] Chemicals were purchased from commercial sources. Fatty acids (99% pure by gas–liquid chromatography) were purchased from the Hormel Institute, Austin, Minn. The SKF compounds: 525-A, 3301-A, 16467-A, 7732-A_3, and 799-$7A_3$ were generously supplied by Dr. W. L. Holmes, Smith, Kline and French Laboratories, Philadelphia, Pa. SKF 2314 was dissolved in 95% ethanol and the other SKF compounds in distilled water. Experiments reported are typical of a minimum of three separate trials giving the same results.

RESULTS

The concentrations of analogs of SKF 525-A causing a 50 per cent inhibition of multiplication are shown in Table 1. The most active compounds were SKF 525-A and its acid SKF 2314 which has

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