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Studies on the Pharmacology of Methaqualone: Induction of Mammalian Hepatic δ-Aminolevulinic Acid Synthetase (38106)

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Methaqualone (2-methyl-3-*o*-tolyl-4(3H)-quinazolinone) (Fig. 1), a nonbarbiturate sedative hypnotic available in this country as a prescription medication since 1965, has become one of the most highly abused drugs of recent times (1–4). The availability of methaqualone has been limited only most recently by restriction under the Controlled Substances Act. As a quinazoline derivative, methaqualone is among the few drugs in this class of compounds which is available for human consumption. The potential of methaqualone to predispose to psychological and physiological dependence is well documented (1–4), but other pharmacological actions of this drug are not well known. While methaqualone is rapidly absorbed after oral consumption (5), it is relatively slowly excreted (6). The liver microsomal enzymes play a key role in its metabolism (7, 8). Administration of methaqualone to rats induces microsomal protein synthesis and increases the activity of drug-metabolizing enzymes (9). Because of the key role played by heme and hemoproteins in the drug-metabolizing enzyme system, it was of particular interest to examine the possible stimulatory effects of methaqualone on the activity of δ-aminolevulinic acid (ALA) synthetase, the first and rate-limiting enzyme in the heme biosynthetic pathway in adult animals (10). The induction of ALA synthetase by a variety of drugs and chemicals has been demonstrated in laboratory animals (11–13), and increased levels of this enzyme are thought to underlie the etiology of acute intermittent porphyria in man (14).

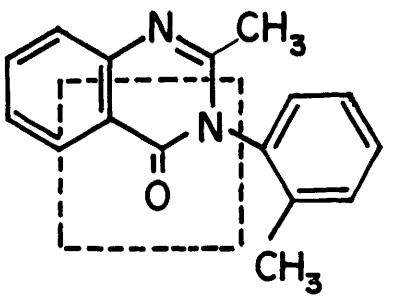

FIG. 1. Structure of methaqualone. Outlined portion resembles chemical configuration thought to induce ALA synthetase.

In the current studies, methaqualone was shown to significantly stimulate the activity of hepatic ALA synthetase in adult rats. The biochemical nature of this stimulation was investigated, and the pharmacologic implications of alterations of hepatic heme synthesis by methaqualone are discussed.

Materials and Methods. Materials. Methaqualone was purchased as 300-mg tablets as either Quaalude (William H. Rorer, Inc.) or as Sopor (Arnar-Stone Laboratories, Inc.). Identical results were obtained using either preparation. Succinyl-CoA synthetase (Succinic thiokinase) (EC 6.2.1.4), pyridoxal-5'-phosphate, adenosine-5'-phosphate (ATP), guanosine-5'-phosphate (GTP), coenzyme-A (CoA), and cycloheximide were obtained from Sigma Chemical Co. Glycine and hemin were purchased from Calbiochem. Other chemicals were of reagent grade and were purchased from standard commercial sources.

Treatment of animals. Sprague–Dawley rats (150–200 g), obtained from the Charles River Breeding Co., were housed in individual cages and were fasted for 24 hr prior to drug treatment. Methaqualone was administered by oral gavage as a suspension in 0.9% sodium chlo-

ride (saline). Test doses were chosen to correspond to a range of dose levels from therapeutic (5 mg/kg) to moderate overdose (50 mg/kg) in man, respectively (3, 4). The results reported here were obtained on studies with male rats, but similar results were obtained on studies when females were used. Crystalline hemin was dissolved in a small volume of 0.01 *M* NaOH and adjusted to pH 7.5 with 0.01 *M* HCl in 0.9% NaCl to a final concentration of 10 mg/ml. It was then administered by intraperitoneal injection at a dose of 20 mg/kg. Cycloheximide was dissolved in 50% ethanol and administered intraperitoneally in a dose of 50 mg/kg. All drugs were given within 2 hr after preparation.

Preparation of tissues. Animals were killed by decapitation. Livers were rapidly excised, washed, weighed, and homogenized in 9 vol of 0.25 *M* sucrose containing 0.02 *M* Tris–HCl buffer (pH 7.6), 0.1 m*M* EDTA, and 0.1 m*M* pyridoxal phosphate in a Potter–Elvehjem homogenizer fitted with a Teflon pestle. The homogenate was centrifuged for 10 min at 600 *g*, and the mitochondria were sedimented from the resulting supernatant solution by centrifugation at 9000 *g* for 15 min. Mitochondria were resuspended and washed twice with the same solution and finally resuspended in 0.02 *M* Tris–HCl buffer (pH 7.6) containing 0.1 m*M* pyridoxal phosphate so that each milliliter of suspension contained 10–15 mg/ml mitochondrial protein.

Enzyme and protein assays. ALA synthetase activity in hepatic mitochondrial fractions was assayed by a modification of the method of Scholnick *et al.* (15). Reaction mixtures contained 0.05 *M* Tris–HCl buffer (pH 7.5), 0.01 *M* $MgCl_2$, 0.1 *M* glycine, 0.01 *M* sodium succinate, 2×10^{-4} *M* pyridoxal phosphate, 0.001 *M* β-mercaptoethanol, 0.25 *M* NaCl, 10^{-4} *M* GTP, 0.005 M EDTA, 6×10^{-5} *M* coenzyme A, 0.003 *M* ATP, sufficient succinyl-CoA synthetase to generate 1 μmole of succinyl-coenzyme A in 30 min and 0.5 ml mitochondrial preparation in a final volume of 2.5 ml. Mixtures were shaken in a metabolic incubator (American Optical Co.) for 60 min at 37°. Reactions were terminated by addition of 0.5 ml cold 10% trichloracetic acid solution. The ALA produced was converted to 2-methyl-3-acetyl-4-propionic acid pyrrole by reaction with sodium acetate and acetylacetone and was then determined colorimetrically by reaction with modified Ehrlich's reagent (16). In some experiments ALA was purified by column chromatography prior to conversion to the corresponding pyrrole. Identical results were obtained using either method. Aminoacetone has been previously shown (17) to represent less than 10% of the total aminoketone present. Hence, aminoketone levels were taken to represent the levels of ALA. All assays were carried out in duplicate using zero time samples as blanks. The molar extinction coefficient for the ALA pyrrole was 5.3×10^4. Proteins were determined by the method of Lowry *et al.* (18).

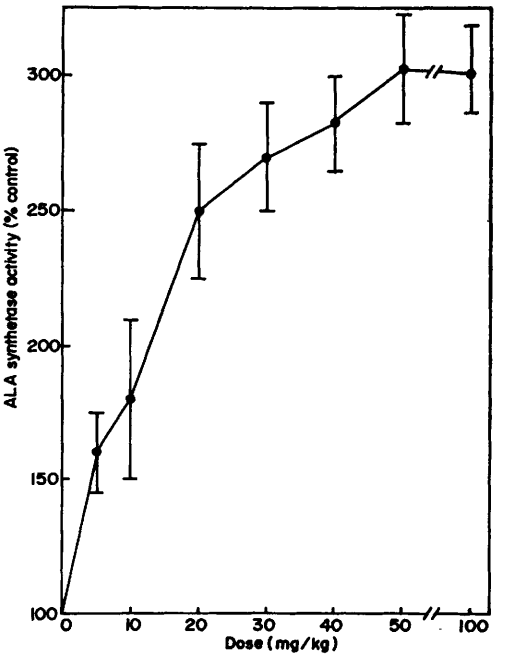

FIG. 2. Dose-response relationship between methaqualone and ALA synthetase activity. Methaqualone was administered orally to fasted male rats 12 hr before sacrifice. ALA synthetase activity was assayed in mitochondria prepared from pooled livers of six animals for each point. Values represent mean percentage of control value ± standard error of five separate determinations. Actual ALA synthetase activity in control adult male rat liver mitochondria was 0.44 ± 0.08 nmoles ALA/mg protein/hr.

Results. Methaqualone produced a dose-related increase in ALA synthetase activity in rat liver mitochondria when measured 12 hr after administration of the drug (Fig. 2). At

20 mg/kg, enzyme activity increased to 250% of the control (saline-treated) values. Administration of 50 mg/kg produced the highest average increase in ALA synthetase activity (approximately 310% of control levels). The maximum increase in ALA synthetase activity observed in a single experiment was to 5.2 times the control value. This increase was observed 8 hr after treatment with 30 mg/kg. Treatment with dose levels as high as 100 mg/kg produced an increase in ALA synthetase activity similar to that seen after 50 mg/kg was given but was often fatal. Test doses were therefore limited to 50 mg/kg for the remainder of the study.

In comparison, phenobarbital (160 mg/kg), which is considered a moderately active inducer of hepatic ALA synthetase in cultured chick embryo cells (19), produced a 285% increase in ALA synthetase activity 24 hr after intraperitoneal injection of male rats with a saline solution of the drug.

The time course of induction of ALA synthetase after methaqualone treatment at 5, 25, or 50 mg/kg dose levels is shown in Fig. 3. A significant increase in ALA synthetase activity was observed shortly after administration of methaqualone at all three dose levels. The major increase in enzyme activity occurred shortly after methaqualone was given, and in each case a peak value was reached within 12 hr after drug treatment. These values corresponded to 195, 270, and 288% of control levels for 5, 25, and 50 mg/kg doses, respectively. A relatively slow decline in enzyme activity occurred at each dose level. It is particularly interesting that enzyme levels were significantly greater than control values for as long as 48 hr after drug treatment, even at the 5 mg/kg level.

Daily administration of methaqualone in doses of 5, 25, or 50 mg/kg for 7 consecutive days produced a maximal increase in ALA synthetase activity similar to that observed 12 hr after a single dose of the drug. However, this increased enzyme activity was sustained for as long as the drug was given. When the drug was withdrawn, at least 4 days was required before enzyme levels approached those seen in control rats.

In *in vitro* studies, methaqualone (5×10^{-4} *M*) did not significantly alter ALA synthetase activity when it was incubated with isolated rat liver mitochondria and the appropriate reaction mixture. In addition, the increase in ALA synthetase activity normally seen 2 hr

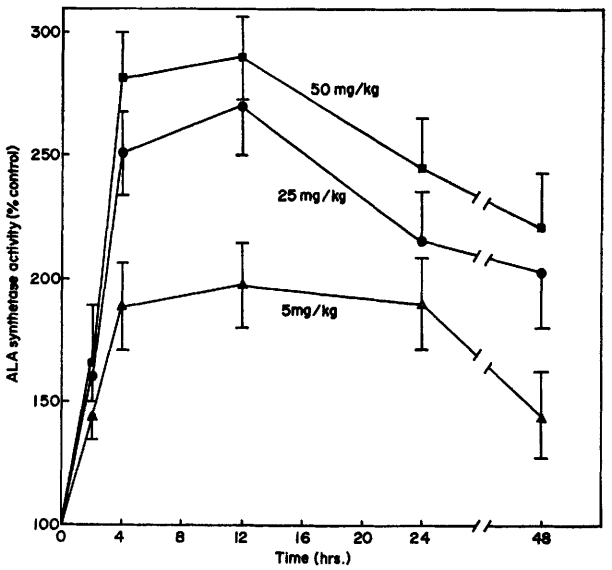

FIG. 3. Time course of induction of ALA synthetase at three dose levels of methaqualone. Fasted male rats were treated orally with a single dose of 5, 25, or 50 mg/kg methaqualone and were killed at the indicated intervals. ALA synthetase activity was assayed in mitochondria prepared from pooled livers of six rats for each time point. Values represent mean percentage of control value ± standard error of five separate determinations.

TABLE I. Effects of Methaqualone and Hemin on ALA Synthetase Activity.[a]

Treatment	ALA synthetase activity (nmoles ALA/mg protein/hr ± SE)		
	Hours after drug treatment		
	0	2	4
Methaqualone (30 mg/kg)	0.51 ± 0.09	0.87 ± 0.05	1.83 ± 0.20
Methaqualone and Hemin (20 mg/kg)	0.48 ± 0.11	0.52 ± 0.08	0.50 ± 0.01

[a] Mitochondrial fractions were prepared as described under Materials and Methods. Rats were divided into six treatment groups and were treated simultaneously with the indicated drugs. Animals were sacrificed after the appropriate time interval. Values represent the mean ± standard error of at least four experiments. Livers of three rats were pooled for each experimental value.

after treatment of rats with methaqualone (30 mg/kg) was completely prevented by pretreating rats with cycloheximide (50 mg/kg) 1 hr before methaqualone was given. Similar results were seen when hemin (20 mg/kg) was administered simultaneously with methaqualone. Previous studies from this laboratory (17) and others (20, 21) have shown that hemin prevents the drug-induced increase in ALA synthetase activity in adult rats, presumably by acting as a feedback repressor of the enzyme (22). Table I demonstrates that when hemin was administered together with methaqualone (30 mg/kg), the increase in ALA synthetase produced by methaqualone alone is prevented.

Discussion. The ability of many drugs to stimulate ALA synthetase activity in both chick embryo cells (19) and in mammalian liver (11, 13) has been previously demonstrated. This study represents the first demonstration of induction of ALA synthetase by a quinazoline derivative. The specific chemical properties which render methaqualone, a substituted quinazolinone, an inducer of ALA synthetase activity are not known. However, Granick (20) has suggested several types of chemical configurations common to many compounds which effectively increase ALA synthetase activity in cultured chick embryo cells. The portion of the methaqualone molecule outlined in Fig. 1 resembles one of the most potent of these configurations, a fact which may account for its ALA synthetase-inducing ability. The integrity of this structure does not seem to be altered by enzymatic metabolism of the drug which, in man, involves relatively nonspecific hydroxylation of the tolyl substituent (6). Thus, the inductive substituents of methaqualone may be sustained in the liver until the majority of the metabolites are excreted, a possibility which might account for the relatively slow decline in ALA synthetase activity after withdrawal of methaqualone treatment.

These studies suggest that methaqualone probably acts by increasing the rate of synthesis of ALA synthetase in liver cells, rather than by activation or stabilization of the enzyme. The observations that methaqualone did not alter ALA synthetase activity *in vitro,* and that cycloheximide and hemin prevented the increase in enzyme activity normally observed in animals treated with methaqualone alone, support the concept that methaqualone stimulated an increased synthesis of the enzyme.

The present studies may bear on the problems of acute intermittent porphyria and drug abuse in man. Although a direct correlation cannot be drawn between results obtained in rats and the effects of drugs on humans, these studies suggest that it is doubtful that the increase in ALA synthetase activity which follows ingestion of a single dose of methaqualone in the prescribed therapeutic dose range (5–10 mg/kg/day) is of sufficient magnitude to warrant contraindication of the drug in careful usage. However, caution is recommended in the use of this drug for prolonged management of patients with acute intermittent porphyria, since exacerbation of the disease may result.

On the other hand, the persistent elevation of ALA synthetase which characterizes continuous use of methaqualone for periods as brief as even 1 week may pose a special health hazard in cases of continuous use or abusive

overdose of the drug. A relationship between a drug-induced increase in ALA synthetase and alterations in microsomal mixed-function oxidase activity has been suggested by several investigators (23, 24). In light of these studies, it seems likely that the sustained induction of ALA synthetase by methaqualone might interfere with normal hepatic drug-metabolizing function and possibly affect the rate or degree to which certain drugs are activated or detoxified. The overproduction of heme precursors and the coincidental occurrence of neurological complications associated with both methaqualone overdose (25) and with acute intermittent porphyria (26, 27) might also be considered a potentially dangerous consequence of sustained elevation of ALA synthetase activity which results from methaqualone abuse.

In conclusion, these studies provide information regarding a specific pharmacologic property of methaqualone which has not been previously described. The capacity of this, and possibly other quinazoline derivatives, to induce ALA synthetase in mammalian liver suggests that this class of compounds may exert widespread effects on hepatic heme metabolism. These effects may be especially deleterious when such drugs are abused through continuous overdose or when used in the management of acute intermittent porphyria. On the other hand, identification of the specific porphyrigenic properties of the quinazoline derivatives may render these chemicals useful pharmacologic tools for further investigating the regulation of mammalian hepatic heme synthesis.

Summary. Methaqualone, a highly abused quinazoline derivative with demonstrated therapeutic efficacy, significantly stimulates the activity of hepatic δ-aminolevulinic acid (ALA) synthetase, the first and rate-limiting enzyme in heme biosynthesis in adult mammals. In adult rats, methaqualone produces a rapid and sustained increase in hepatic ALA synthetase activity in doses which correspond in man to both therapeutic (5–10 mg/kg) or overdose (25–50 mg/kg) levels. The major increase in enzyme activity occurs within 12 hr after drug treatment. Elevated enzyme levels are sustained for at least 48 hr after administration of a single dose and for several days after daily ingestion of the drug for 1 wk. The increase in ALA synthetase activity produced by methaqualone can be prevented by simultaneous administration of hemin or by pretreatment with cycloheximide and cannot be demonstrated *in vitro*. This study represents the first demonstration of stimulation of mammalian ALA synthetase activity by a quinazoline derivative. The mechanism of action of methaqualone appears to be by induction of enzyme synthesis. Although single therapeutic dose levels (5–10 mg/kg/day) probably do not induce the enzyme to a significant extent as to warrant contraindication of this drug in prescribed usage, ingestion of abusive doses may significantly alter hepatic heme metabolism. These studies suggest, however, that quinazoline derivatives may serve as useful pharmacologic tools for the further investigation of the regulation of mammalian heme synthesis.

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