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Characterization of ketamine induction of hepatic microsomal drug metabolism*

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The pretreatment of rats with ketamine [2-(*o*-chlorophenyl)-2-(methylamino) cyclohexanone] has been shown to decrease the plasma half-life of intravenously administered ketamine and to enhance its rate of *N*-demethylation *in vitro* [1]. Therefore, the following study was undertaken to characterize this apparent induction of hepatic microsomal enzyme activity by ketamine. The influence of ketamine pretreatment on levels of hepatic microsomal cytochrome P-450 and NADPH–cytochrome *c* reductase activity were assessed, as well as the rates of hepatic metabolism *in vitro* of benzphetamine and 3,4-benzo(a)pyrene, two agents used commonly to examine hepatic microsomal metabolism. The binding interaction between ketamine and cytochrome P-450 was determined spectrally. Finally, the *N*-demethylation of ketamine by hepatic microsomal fractions of pretreated rats was evaluated kinetically and for purposes of comparison similar studies were carried out using microsomal fractions prepared from the livers of rats pretreated with phenobarbital.

Microsomal fractions used were freshly prepared from the livers of male Sprague–Dawley rats (110–150 g) pretreated with ketamine (40 mg/kg twice daily for 3 days) or phenobarbital (35 mg/kg twice daily for 3 days) using the procedure described previously [1]. Control animals were injected twice daily for 3 days with equal volumes of isotonic saline. To study the microsomal *N*-demethylation of ketamine *in vitro* at 37°, each 2.5-ml incubation mixture contained: microsomal protein (2.4 mg) glucose 6-phosphate (12.5 m-mole), $MgCl_2$ (25 m-mole), glucose 6-phosphate dehydrogenase (2 I.E.U.), and NADP (1 m-mole) buffered with 160 mM KCl—50 mM Tris–HCl, pH 7.40. The 15-min reaction was initiated by the addition of ketamine to the reaction vessel. Ketamine concentrations were between 3×10^{-5} and 1×10^{-3} M for microsomal fractions from control animals and ranged from 5×10^{-5} to 1.5×10^{-3} M and 1×10^{-4} to 1.5×10^{-3} M in the case of microsomes prepared from the livers of ketamine- and phenobarbital-pretreated animals respectively. Samples were collected and assayed for parent compound and its *N*-demethylated metabolite as previously described [1]. Under these conditions, the rate of demethylation of ketamine (measured as norketamine formed) was linear with time.

The *N*-demethylation of benzphetamine was determined by measuring the rate of formaldehyde formation [2]. Assay conditions for the 10-min incubation, which was initiated by the addition of benzphetamine (12.5 m-mole), were identical to those described above.

Hydroxylation of 3,4-benzo(a)pyrene was assayed using a modification of the method described by Nebert and Gelboin [3]. Glucose 6-phosphate (5 m-mole), MgCl (3 m-mole), NADP (1 m-mole), glucose 6-phosphate (0.7 I.E.U.), and microsomal protein (1.0 mg) were added to the reaction vessel. The final reaction volume of 1.0 ml was buffered to a pH of 6.80 using 0.1 M sodium phosphate buffer, and the 10-min incubation was carried out at 37° in a room darkened except for a yellow light source. The reaction was initiated by the addition of 3,4-benzo(a)pyrene (0.24 μmole in 0.02 ml acetone), and the concentration of the hydroxylated metabolite, 3-hydroxybenzo(a)pyrene, was determined using an SPF 2 Aminco–Bowman spectrophotometer with excitation and emission wavelengths of 386 and 515 nm respectively.

Cytochrome P-450 was determined at room temperature (20–23°) with a Shimadzu model MPS-50L spectrophotometer using the diothionite difference method of Omura and Sato [4]. Final microsomal suspensions had a protein concentration of 1 mg/ml and were buffered to pH 7.70 with 0.1 M potassium phosphate buffer. For determination of the K_s of binding of ketamine to cytochrome P-450, difference spectra were recorded using an Aminco DW-2 dual wavelength spectrophotometer by the method of Schenkman [5].

NADPH–cytochrome *c* reductase activity was determined by the method of Masters *et al.* [6]. The rate of reduction of cytochrome *c*, initiated by the addition of 100 m-moles NADPH to the 1.0-ml spectrophotometric cuvette, was followed at 500 nm and 25° with a Gilford 2000 recording spectrophotometer during the initial linear phase of the reaction.

Student's *t*-test for unpaired data was used to analyze differences between rates of drug metabolism, levels of cytochrome P-450, and NADPH–cytochrome *c* reductase activity. Kinetic data were analyzed from a weighted regression line plotted using a FORTRAN program [7].

As seen from data in Table 1, ketamine pretreatment doubled the maximal rate (V_{max}) of ketamine *N*-demethylation, whereas phenobarbital pretreatment increased the rate of ketamine metabolism by 4- to 5-fold. Pretreatment with ketamine had no influence on the apparent Michaelis constant (K_m) for the *N*-demethylation of ketamine; however, the K_m for the rate of ketamine *N*-demethylation in phenobarbital-pretreated rats was greater ($P < 0.05$) than that of control animals. This may represent an effect of phenobarbital pretreatment in altering the pathways of ketamine metabolism perhaps by an action on enzymes responsible for ring oxidation of norketamine. Alternatively, phenobarbital could stimulate the production of P-450 species with changed affinity for the substrate, ketamine.

Figure 1 shows that the addition of ketamine to washed, nonreduced hepatic microsomal fractions from untreated rats produces a characteristic type I shift in the absorbence spectrum (λ_{max}, 385 nm; λ_{min}, 420 nm). The K_s value for interaction between ketamine and cytochrome P-450 was 1×10^{-5} M.

As can be seen from data presented in Table 2, in addition to increasing levels of hepatic cytochrome P-450 and NADPH–cytochrome *c* reductase activity ($P < 0.05$), both pretreatments also significantly enhanced the hepatic microsomal metabolism of benzphetamine and 3,4-benzo(a)pyrene ($P < 0.01$). However, in all cases, the effects of the ketamine pretreatment on cytochrome P-450 levels and rates of drug metabolism were less than those of phenobarbital. It should be noted that the increases in rates of demethylation and hydroxylation caused by ketamine pretreatment are not stoichiometric with the observed increase in level of cytochrome P-450. A possible explanation for this is that the newly synthesized cytochrome P-450 has

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Table 1. Kinetic analyses of the hepatic microsomal *N*-demethylation of ketamine in phenobarbital- and ketamine-pretreated rats*

Pretreatment	$K_m \pm$ S. E. M. (mM)	$V_{max} \pm$ S. E. M. (nmoles *N*-demethylated/mg of protein/hr)
Control	0.127 ± 0.010	207 ± 16
Ketamine	0.149 ± 0.015	435 ± 33†
Phenobarbital	0.218 ± 0.028‡	894 ± 45†

* The hepatic microsomal metabolism of ketamine was estimated and analyzed as detailed in Materials and Methods. Each pretreatment group consisted of six rats which were randomly assigned to subgroups consisting of two animals. Kinetic constants were calculated from duplicate samples of the microsomes prepared from the pooled livers of each subgroup.

† P value < 0.01.

‡ P value < 0.05.

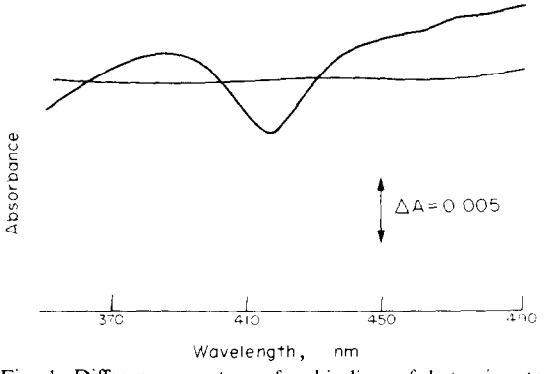

Fig. 1. Difference spectrum for binding of ketamine to hepatic microsomes. Ketamine (0.14 mM) was added to a suspension of nonreduced microsomes (1.1 mg/ml of protein) in 160 mM KCl—50 mM Tris–HCl buffer, pH 7.4. Difference spectra were recorded for the wavelength interval 350–480 nM at 20–23°.

changed properties and exhibits a higher turnover rate for the substrates benzphetamine and 3,4-benzo(a)pyrene than the enzyme present in microsomal fractions from untreated animals. Remmer [8] has listed several general properties that chemical compounds must have if they are to display a potential hepatic microsomal enzyme-inducing capacity. Ketamine fulfills the requirements of a high lipid to water partition ratio [9] as well as a type I cytochrome P-450 binding spectrum (Fig. 1). In addition to increasing levels of hepatic microsomal cytochrome P-450 and NADPH–cytochrome *c* reductase activity, ketamine pretreatment also stimulates the hepatic microsomal metabolism of two substrates used conventionally for the study of hepatic metabolism *in vitro*, 3,4-benzo(a)pyrene and benzphetamine (Table 2). However, the relatively short plasma half-life of ketamine [10] probably limits its potential as an inducing agent.

Inducers of drug metabolism have been classified according to their effects on various components of the electron transport system associated with liver microsomes [11]. Phenobarbital-like inducing agents cause increases in NADPH–cytochrome *c* reductase, cytochrome P-450, and the rate of metabolism of a large number of drugs, including type I substrates such as benzphetamine and ketamine. In contrast, pretreatment of rats with the polycyclic hydrocarbons, such as 3-methylcholanthrene and 3,4-benzo(a)pyrene, does not increase NADPH–cytochrome *c* reductase activity or the metabolism of type I binding compounds. Therefore, ketamine appears to be similar to phenobarbital in its induction of the hepatic microsomal metabolizing enzymes of the rat.

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Table 2. Effect of ketamine and phenobarbital pretreatments on hepatic microsomal cytochrome P-450 levels, NADPH–cytochrome *c* reductase activity, benzphetamine *N*-demethylation, and 3,4-benzo(a)pyrene hydroxylation*

	Control (X ± S. E. M.)	Ketamine (X ± S. E. M.)	Phenobarbital (X ± S. E. M.)
Cytochrome P-450 (nmoles/mg protein)	0.38 ± 0.03	0.57 ± 0.4†	1.13 ± 0.03‡
Cytochrome *c* reductase			
(nmoles cytochrome *c* reduced/mg protein/min)	50.23 ± 4.23	70.27 ± 3.65†	86.19 ± 12.08†
Benzphetamine *N*-demethylation			
(nmoles HCHO formed/mg protein/min)	2.23 ± 0.16	6.03 ± 0.59‡	9.45 ± 0.85‡
(nmoles HCHO formed/nmole P-450/min)	5.86	10.58	8.36
3,4-Benzo(a)pyrene hydroxylation			
(nmoles/mg protein/min)	0.054 ± 0.006	0.119 ± 0.003‡	0.197 ± 0.018‡
(nmoles/nmole P-450/min)	0.142	0.208	0.174

* Cytochrome P-450 levels, NADPH–cytochrome *c* reductase activity, benzphetamine *N*-demethylation, and 3,4-benzo(a)pyrene hydroxylation were determined using hepatic microsomes prepared from pooled livers of phenobarbital- and ketamine-pretreated rats as detailed in the text. Values represent duplicate assays as described in the legends of Table 1.

† P value < 0.05 by Student's paired *t*-test.

‡ P value < 0.01 by Student's paired *t*-test.

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Effects of ethanol and phenobarbital on the metabolism of propranolol by 9000 *g* rat liver supernatant

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Results from previous studies have suggested that propranolol might be of value in the treatment of alcoholism [1,2]. In addition, propranolol is widely used in the treatment of cardiac disorders and it might be expected that many patients consume significant amounts of ethanol while on propranolol. It is possible that ethanol might alter propranolol metabolism. Studies were undertaken to determine the effects of ethanol and phenobarbital administration on propranolol metabolism.

Male Sprague–Dawley rats (175–300 g) were used in all studies except where indicated. All rats were decapitated, the livers quickly removed and a 25% w/w homogenate was prepared. The 9000 *g* supernatant was prepared by centrifugation of the homogenate at 4° for 25 min. This preparation was selected as the source of propranolol-metabolizing enzymes since previous studies have shown that the 9000 *g* supernatant is as active as the microsomal fraction [3]. Aliquots of the 9000 *g* supernatant (1.5 ml) derived from 480 mg wet weight of rat liver were incubated in air at 37° with 500 nmoles propranolol and the following cofactors: NADP+ (0.5 μmole), $MgCl_2$ (150 μmoles), nicotinamide (100 μmoles), glucose 6-phosphate (50 μmoles) and 0.2 M phosphate buffer (pH 7.4) in a total volume of 4 ml as described by Shand and Oates [3]. After 15 min of incubation, the reaction was terminated by the addition of 1 ml of 2N NaOH. The unchanged drug was extracted into 1.5% isoamyl alcohol in heptane (12 ml). Propranolol was extracted from the organic layer (10 ml) with 0.1 N HCl (3 ml), and the fluorescent intensity of the aqueous layer was measured as described by Shand *et al.* [4]. The initial amount of propranolol added was determined from control samples prepared in the same manner except that 1 ml of 2 N NaOH was added before incubation. The amount of propranolol metabolized was the difference between the amount of propranolol present at zero time and that following incubation. Initial experiments established that the concentration of propranolol used was sufficient to saturate the enzyme system.

Aniline hydroxylase activity of the 9000 *g* supernatant fraction was measured as described by Imai and Sato [5] since previous studies have shown this enzyme activity to be induced by phenobarbital [6].

After phenobarbital pretreatment (75 mg/kg, i.p., once daily for 3 days), the rate of propranolol metabolism was increased to 142 per cent of control values (Table 1). Therefore, phenobarbital pretreatment enhances hepatic microsomal propranolol metabolism. In addition, phenobarbital pretreatment increased hepatic microsomal aniline hydroxylase activity by 279 per cent of control values (Table 1).

In order to study the effects of short-term administration of ethanol on the metabolism of propranolol, ethanol (2 g/kg, i.p., twice daily) was given to groups of rats for 3 and 7 days. Weight losses averaging 3–5 g/day were observed during the period of ethanol administration. The rats were sacrificed 24 hr after the last dose of ethanol. No significant decrease in the rate of propranolol metabolism was observed (Table 1). In contrast, a significant reduction in the activity of aniline hydroxylase was noted. This observation differs from that reported by Tobon and Mezey [7]. These workers found a significant increase in microsomal aniline hydroxylase activity after daily administration of ethanol (4 g/kg) either by gastric intubation (3 days) or in the diet (7 days) as an ethanol solution. An explanation for this descrepancy is not readily apparent but could be related to differences in route of administration of ethanol, in dietary intake, and in the source of aniline hydroxylase (9000 *g* supernatant versus washed microsomes).

To study the effect of chronic ethanol administration on propranolol metabolism, female Sprague–Dawley rats (initially weighing 60 *g*) were made chronically alcoholic by employing an adaptation of the Ratcliff method [8]. Drinking water was replaced by ethanol in 10% sucrose in tap water. The initial concentration of ethanol was 2.5%, gradually increasing to 25% during week 7 (the final week of the study). Only 10% sucrose in tap water was given to the control group. There was no significant difference between the weight of control and ethanol-treated rats at the end of the study period. Chronic ethanol administration enhanced the rate of propranolol metabolism to 206 per cent above control values. In contrast, no significant change in the activity of aniline hydroxylase activity was observed, suggesting that propranolol metabolism and aniline metabolism by the hepatic microsomes of the rat proceed via different mechanisms. The absence of change in aniline hydroxylase activity in these chronically treated animal is in agreement with the results of Tobon and Mezey [7]. These workers found an initial rise in aniline hydroxylase activity up to 7 days when ethanol was added