

Effects of *in vivo* Pretreatment with Various Barbiturates on Anaerobic Halothane Metabolism in Rat Liver Microsomes

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ABSTRACT

The effects of *in vivo* pretreatment with phenobarbital (PB), thiopental (TP), thiamylal (TA), pentobarbital (PT), and secobarbital (SB) on hepatic microsomal enzymes, and the effects on anaerobic halothane dehalogenation, aminopyrine N-demethylation, and aniline hydroxylation in the microsomes were studied in male Wistar rats. Three hundred twenty $\mu\text{mol/kg}$ (0.1ml) of PB, TP, TA, PT, SB, or 0.1ml of 0.9% saline were administered daily, intramuscularly, for periods of one day up to ten days. Daily administration of PB, TP, TA, or PT induced cytochrome P-450, NADPH-cytochrome P-450 reductase and/or cytochrome b_5 . However, administration of SB did not induce these enzymes. The potency of these enzyme inductions ranged in descending order as follows: PB, TP, TA, and PT. After five days of daily administration of PB, TP, or TA, the production of the anaerobic halothane metabolite, CDFE, increased to 187%, 134%, and 130% of the control, respectively. The production of another halothane metabolite, CTFE, likewise increased to 197%, 168%, and 163%. However, pretreatment with PT or SB had no effect on anaerobic halothane dehalogenation. Aminopyrine N-demethylation also increased after five days of daily administration of PB, TP, and TA. However, aniline hydroxylation decreased after five days of daily administration of TA. Other barbiturates had no effect on aniline hydroxylation. In this study we showed that whereas PT and SB did not enhance anaerobic halothane dehalogenation, PB, TP and TA did. We conclude that not only PB, and also TP and TA, may be enhancing factors in halothane hepatotoxicity. We recommend that, if barbiturates are necessary, SB and PT be used in the preadministration of halothane anesthesia.

Key words: *Anesthetics; halothane, barbiturates.*
Biotransformation; dehalogenation, induction.
Microsomes; rat, liver.

Since Van Dyke's report²⁰⁾ that halothane was metabolized *in vivo*, it has been assumed that 17-20% of halothane might be metabolized¹⁵⁾. An aerobic metabolite, trifluoroacetic acid (TFAA)^{7,10)}, is detected in the urine. Two anaerobic metabolites, 2-chloro-1, 1-difluoroethylene (CDFE) and 2-chloro-1, 1, 1-trifluoroethane (CTFE)^{11,18)} are detected in expired air; these are produced by a radical intermediate³⁾, which might trigger lipid peroxidation and cause the hepatic damage. Enzyme induction by phenobarbital promotes the formation of the radical intermediate^{3,4,9)}.

In this study we investigated the effects of pretreatment with phenobarbital (PB), thiopental (TP), thiamylal (TA), pentobarbital (PT), and secobarbital (SB) upon rat liver microsomes, and also the effects on anaerobic halothane dehalogenation, aniline hydroxylation, and aminopyrine N-demethylation in the microsomes.

The purpose of this study was to determine which

barbiturate might be used for administration prior to halothane anesthesia.

MATERIALS AND METHODS

1. Experimental animals and reagents.

Five week-old male Wistar rats were used. Authentic halothane was provided by Hoechst Co., Japan. PCR Research Chemical provided the 2-chloro-1, 1-difluoroethylene (CDFE) and 2-chloro-1, 1, 1-trifluoroethane (CTFE). The other reagents were commercial products of analytical grade.

2. Animal treatment.

The rats were divided into six groups: PB, TP, TA, PT, SB, and control group. Each animal received, intramuscularly, a total volume of 0.1ml containing 320 $\mu\text{mol/kg}$ of the designated agent, once daily for periods of one to ten days; the animals in the control group received 0.1ml of 0.9% saline daily.

3. Preparation of microsomes.

The animals fasted for 24 hours after the final drug administration and were then killed by cervical dislocation. Resection of the liver was followed by perfusion with iced saline containing 1mM EDTA; this was homogenized in 0.1M potassium phosphate buffer (pH 7.4). The microsomal fraction was obtained by the method of Ernster and Nordenbrand²⁾. This fraction was suspended, in a concentration equivalent to one g liver per ml, with 0.1M Tris-HCl buffer (pH 7.4). Microsomal protein was measured by Lowry's method⁸⁾, using bovine serum albumin as a standard.

4. Enzyme assay.

Cytochrome P-450 (P-450) and cytochrome b₅ (b₅) were measured by Omura and Sato's method¹³⁾. NADPH-cytochrome P-450 reductase (fp₂) and NADH-cytochrome b₅ reductase (fp₁) were assayed by the methods of Takesue¹⁷⁾ and Omura¹⁴⁾.

5. Assays of drug metabolism.

After five days of daily administration of each barbiturate the following microsome activity was assayed:

a) Anaerobic halothane dehalogenation.

A 15 ml vial which contained 0.3 ml of microsomal suspension, 0.1 ml of 5mM NADPH and 0.6 ml of 0.1 M Tris-HCl buffer solution (pH 7.4) with nitrogen gas was sealed by silicone cap. After preincubation for 10 minutes at 37°C, reaction was initiated by the administration of 4.8 μmol and 47.5 μmol halothane for CDFE and CTFE, respectively¹⁾. Ten minutes after incubation at 37°C, 0.5 ml of the gaseous phase of the vial was subjected to a gas chromatograph, a Shimadzu GC-4A-PTF with a flame ionization detector for analyzing halothane, CDFE, and CTFE¹⁾. Authentic samples were used as standards.

b) Aminopyrine N-demethylation.

Microsomal suspension, equivalent to 0.1 g liver, was transferred into a 50 ml Erlenmeyer flask, into which an NADPH-generating system, consisting of 0.5 mM NADP, 5.0 mM glucose-6-phosphate, 5.0 mM MgCl₂, and 1.5 units of glucose-6-phosphate dehydrogenase, was added. Following preincubation for ten min at 37°C with shaking, reaction was initiated by adding 2 mM of aminopyrine. The total volume of reaction mixture was adjusted to 3.0 ml with potassium phosphate buffer (pH 7.4). At ten min after initiation, the reaction was terminated by addition of trichloroacetic acid. The formation of formaldehyde was measured by Nash's method¹²⁾, using hexamethyltetramine as a standard.

c) Aniline hydroxylation.

The reaction mixture and conditions were the same as for the assay of N-demethylation of aminopyrine except that 1 mM of aniline was used in the substrate instead of aminopyrine. The formation of p-aminophenol was measured by the method of Imai et al⁶⁾. Authentic p-aminophenol

was used as a standard.

6. Data analysis.

The values in this study were expressed in terms of mean ± standard deviation. Statistical analysis was performed using the Student t-test, and differences were considered to be significant when $p < 0.05$.

RESULTS

1. Animal condition and microsomal protein

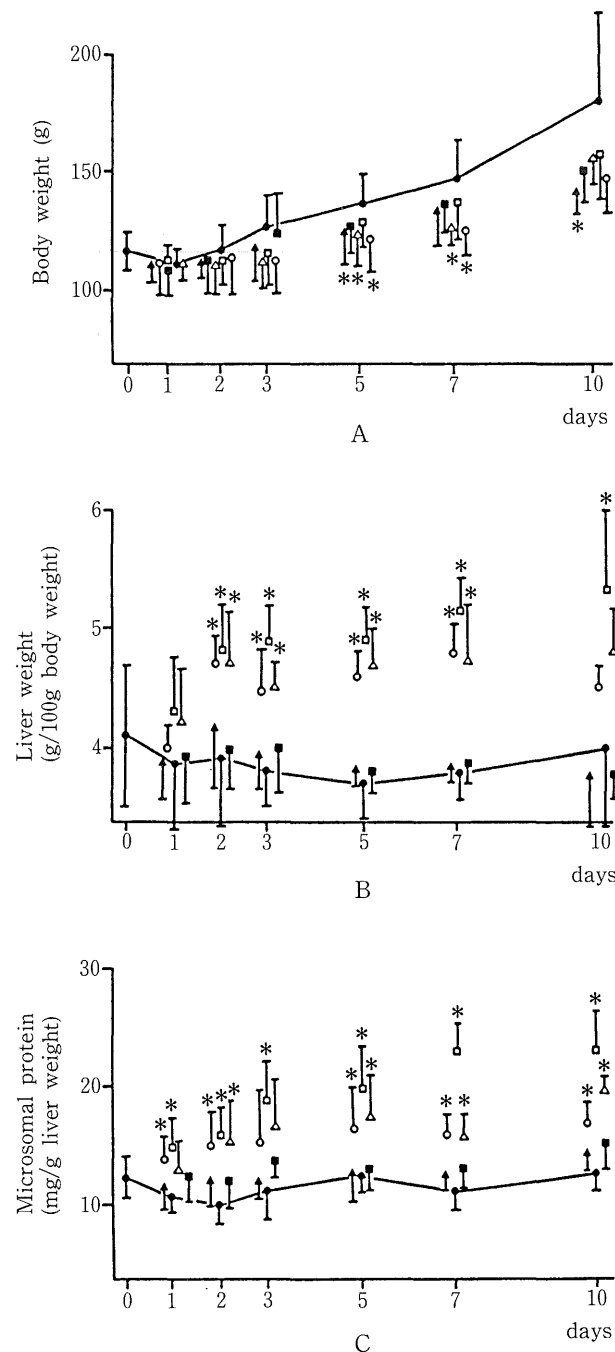


Fig. 1. The effects in rats of pretreatment with various barbiturates on: A) body weight, B) liver weight, and C) microsomal protein.

□ Thiopental ○ Thiamylal
 ■ Pentobarbital ▲ Secobarbital ● Control
 * $p < 0.05$

content.

Body weight in each barbiturate group increased less than that in the control group (Fig. 1 A). The weight of liver (g/100 g body weight), on the other hand, increased in the PB, TP, and TA groups, and reached a plateau on the second day of the treatment. No significant increase of liver weight in the PT, SB, or control groups was observed during the period of administration (Fig. 1 B).

A gradual increase in microsomal protein content (mg/ g liver weight) was observed in the PB, TP, and TA groups. Whereas in the PT, SB, and control groups, no significant change in microsomal protein content was observed (Fig. 1 C).

2. Effects of pretreatment of barbiturates on P-450, b_5 , fp_1 , and fp_2 .

A significant increase of P-450 was observed in the PB, TP, and TA groups until the second or third administration of each agent. On the fifth day of administration, the P-450 content in these three groups was 220%, 174%, and 152% of the control group, respectively. In the PT group, a slight, but significant, increase of P-450 content was observed

on the second, seventh, and tenth days of administration. In the SB group, however, no significant increases were observed (Fig. 2 A).

The activity of fp_2 increased gradually in the PB, TP, and TA groups, whereas no such increase of activity was observed in the PT and SB groups (Fig. 2 B).

In the PB group, b_5 content increased. However, significant increases were observed only on the first and fifth days of administration. In the other groups, there was neither increase nor decrease, except on the first day in the PT group (Fig. 2 C).

No significant change of fp_1 activity was observed in any group.

3. Effects of pretreatment of barbiturates on drug metabolism.

a) Anaerobic halothane dehalogenation (Table 1).

CDFE and CTFE formation per mg of microsomal protein increased significantly to : 187% and 197% in the PB group; 134% and 168% in the TP group; 130% and 163% in the TA group, respectively. In the PT and SB groups no signifi-

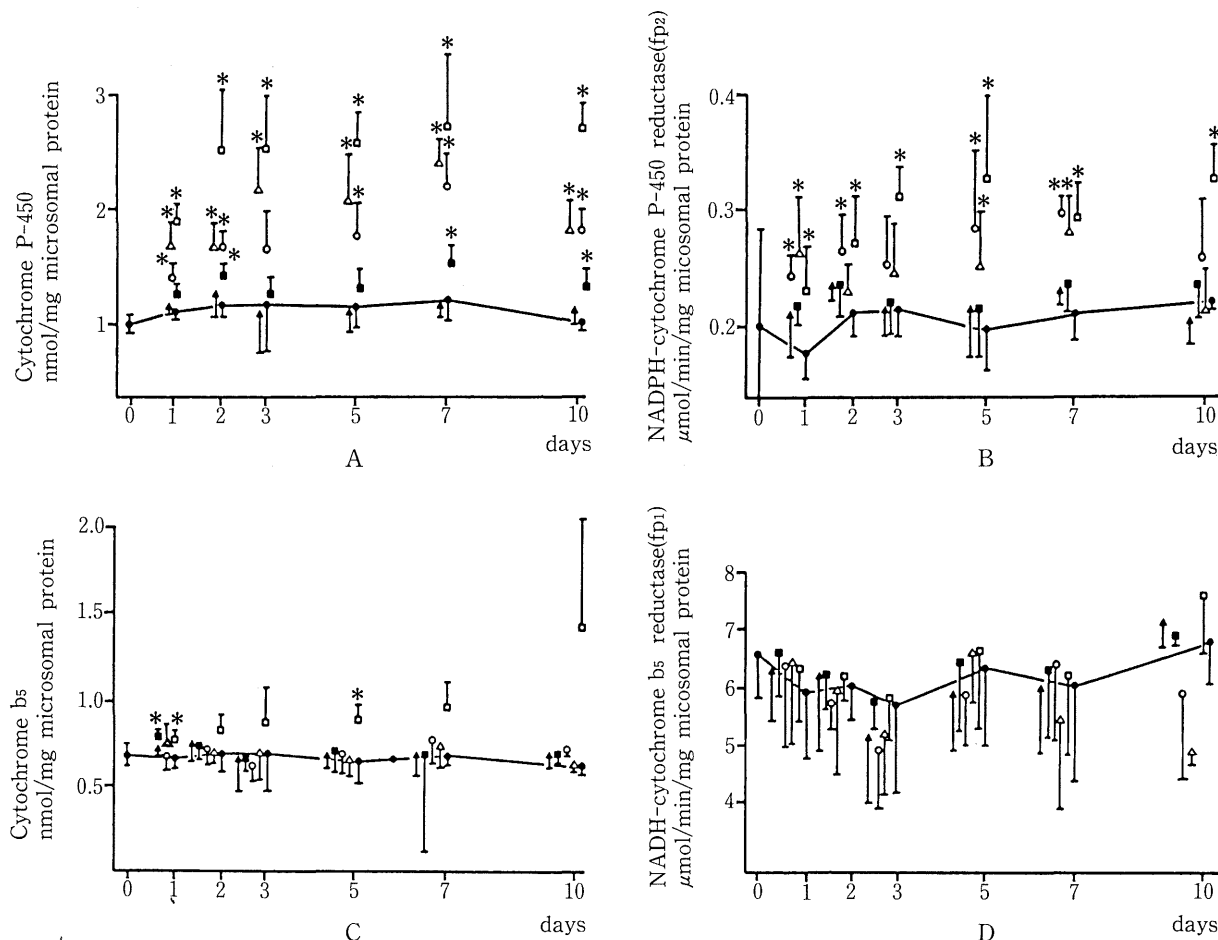


Fig. 2. The effects of pretreatment with various barbiturates on hepatic microsomal enzymes in rats: A) Cytochrome P-450, B) fp_2 , C) Cytochrome b_5 , D) fp_1 .

□ Phenobarbital △ Thiopental ○ Thiamylal
 ■ Pentobarbital ▲ Secobarbital —●— Control
 * $p < 0.05$

Table 1. The effects of five days of daily administration of phenobarbital, thiopental, thiamylal, pentobarbital, and secobarbital on anaerobic dehalogenation, N-demethylation, and hydroxylation.

Barbiturates		Halothane anaerobic dehalogenation				Aniline hydroxylation		Aminopyrine N-demethylation	
		CF ₂ CHCl formation		CF ₃ CH ₂ Cl formation		p-aminophenol formation		HCHO formation	
		a	b	a	b	a	b	a	b
Phenobarbital	mean	0.336*	0.895**	1.56	4.12**	9.8**	22.2	118	226*
	S.D.	0.049	0.119	0.21	0.71	2.3	2.1	28	45
	%	(79)	(187)	(84)	(197)	(46)	(89)	(94)	(180)
Thiopental	mean	0.335*	0.642*	1.75	3.52**	10.3**	19.2	114	226**
	S.D.	0.058	0.147	0.21	0.74	3.6	8.8	23	51
	%	(79)	(134)	(94)	(168)	(49)	(77)	(91)	(153)
Thiamylal	mean	0.343*	0.623*	1.76	3.41**	12.1**	19.8**	119	205**
	S.D.	0.057	0.142	0.35	1.11	2.4	2.4	23	33
	%	(81)	(130)	(95)	(163)	(58)	(80)	(95)	(139)
Pentobarbital	mean	0.412	0.528	1.83	2.39	18.2	22.2	127	158
	S.D.	0.093	0.149	0.1	0.39	3.2	2.5	29	41
	%	(97)	(110)	(98)	(114)	(86)	(89)	(102)	(107)
Secobarbital	mean	0.395	0.482	1.73	2.06	19.6	20.1	131	138
	S.D.	0.088	0.106	0.34	0.42	5.5	5.8	26	23
	%	(93)	(101)	(93)	(99)	(93)	(81)	(104)	(93)
Saline mean		0.424	0.479	1.86	2.09	21.1	24.9	126	148
	S.D.	0.09	0.137	0.28	0.38	3	3	12	26
	%	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)

a: nmol of product/min/nmol of P-450

b: nmol of product/min/mg of microsomal protein

%; % of the control

* p < 0.05

** p < 0.01

cant increase of CDFE or CTFE formation per mg of microsomal protein was observed.

CDFE formation per nmol of P-450 decreased significantly to: 79.2% in the PB group; 79% in the TP group, and 80.9% in the TA group, respectively. There were no significant differences in CDFE formation per nmol of P-450 among the PT, SB, and control groups.

CTFE formation per nmol of P-450 decreased significantly to 83.9% only in the PB group. No significant decrease of CTFE formation per nmol of P-450 was observed in any other group.

b) Aminopyrine N-demethylation (Table 1)

N-demethylation activities per mg of microsomal protein increased significantly to: 180% in the PB group; 153% in the TP group, and 139% in the TA group, respectively. In the PT and SB groups no significant increase of N-demethylation activity per mg of microsomal protein was observed.

N-demethylation activity per nmol of P-450 did not differ from the control in any of the groups.

c) Aniline hydroxylation (Table 1).

Aniline hydroxylation activity per mg of microsomal protein in the TA group decreased significantly to 80% of the control. In the PB, TP, PT, and SB groups neither significant increase nor sig-

nificant decrease in aniline hydroxylation activity per mg of microsomal protein was observed.

Aniline hydroxylation activity per nmol of P-450 decreased significantly to: 46.4% in the PB group; 48.8% in the TP group, and 57.8% in the TA group, respectively. In the PT and SB groups aniline hydroxylation activity did not differ from the control.

DISCUSSION

Hepatic drug metabolizing activity is enhanced by the administration of various kinds of drugs. This action is well accounted for by the mechanism of "drug induced enzymes", that is, enzyme induction of hepatic microsomal P-450 enzyme systems. The P-450 enzyme systems consist of various kinds of molecular species. Methylcholanthrene and PB are well known inducers of P-450s; each agent induced a corresponding and specific P-450⁹.

In our study, P-450s were induced, not only by PB, but also by TP, TA, and PT (Fig. 2 A). However, P-450s were not induced by SB. The P-450s induced by TP, TA, and PT showed a maximum absorption band for the carbon monoxide difference spectrum at 450 nm. This is identical to the P-450 induced by PB. In addition, the activity

of the P-450s induced by PB, TP, and TA, but not by PT in anaerobic halothane dehalogenation and aniline hydroxylation, was lower than that of intact P-450, but the activity of P-450 induced by PT was similar to that of intact P-450. The activity of the P-450s induced by these four barbiturates in aminopyrine N-demethylation was similar to that of intact P-450. It appears then that the P-450s induced by TP and TA displayed similar characteristics to those induced by PB, but those induced by PT did not show these characteristics. The question of whether the P-450s induced by PB, TP, and TA are identical or not, has not been answered in this study.

Valerino et al¹⁹) compared the relative potency of six barbiturates, on a molar basis, in increasing P-450 content in the rat. It was shown that the stimulatory potency for P-450 content ranged in descending order: PB, TP, barbital, PT, SB, and hexobarbital at the same dosage as in our study (320 μ mol). In our study, the potency of induction ranged in descending order: PB, TP, TA, and PT. These findings are partly confirmed in the above-mentioned studies of Valerino et al¹⁹).

Sten et al¹⁶) reported that PB induced fp_1 in addition to P-450. However, they did not investigate other barbiturates. Our study, comparing the effects of various barbiturates, reveals that PB induced fp_2 and b_5 , TP and TA induced fp_2 , PT slightly induced b_5 , but SB did not induce these enzymes (Fig. 2 B, C, D). The reason for such discrepancy among various barbiturates is difficult to explain.

The effects of pretreatment of barbiturates, except for those of PB, on anaerobic halothane dehalogenation have not previously been investigated. Following the administration of PB to the rat, halothane induced hepatic necrosis⁹) occurs. This may be the result of lipid peroxidation induced by the radical³) produced in the process of anaerobic dehalogenation of halothane⁴). In our study, in addition to pretreatment with PB, pretreatment with TP or TA also increased the amount of anaerobic dehalogenation of halothane, although these P-450s showed less anaerobic halothane dehalogenation activity compared to the activity of intact P-450s (Table 1).

These results suggest that not only pretreatment with PB but also pretreatment with TP or TA may be factors in halothane hepatotoxicity. However, PT and SB did not increase anaerobic halothane dehalogenation.

We are able to conclude that, if barbiturates are necessary, we would recommend the administration of secobarbital or pentobarbital prior to halothane anesthesia.

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