Halonal is a Phenobarbital Inductor of the Monooxygenase System

A. S. Saratikov, T. P. Novozheeva, and R. R. Akhmedzhanov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 9, pp. 308-310, September, 2002
Original article submitted May 22, 2002

Experiments on rats demonstrated induction of the hepatic monooxygenase system with halonal. The effects of halonal and phenobarbital on the contents of cytochrome P-450 and its isoform P-450b+e and on the rate of substrate metabolism were similar. This suggests that enzyme-inducing activity of halonal is determined by the effect of its major metabolite. It cannot be excluded that halonal molecules possess intrinsic enzyme-inducing activity.

Key Words: *halonal; phenobarbital; monooxygenase system; liver*

Halonal (5-ethyl-5-phenyl-I-*o*-fluoro-benzoyl-barbituric acid) is an original anticonvulsant synthesized in Russia. This preparation prevents convulsions induced by severe electrical shock, Corasol, and camphor. It also produces a depriming effect on the motor cortex, thalamic nucleus, head of the caudate nucleus, and reticular formation of the midbrain [7]. Halonal prevents generalized clonic and tonic seizures and produces an antiarrhythmic effect in patients with extrasystoles and paroxysmal arrhythmias.

Our previous studies showed that halonal acts as a potent inductor of the hepatic monooxygenase system (MOS). The degree and duration of halonal-induced changes are similar to those observed after treatment with phenobarbital [3]. However, the type of MOS induction with halonal was not determined. Type I binding of halonal to cytochrome P-450 (similarly to phenobarbital) was established.

Here were studied the type and mechanism of induction of rat liver MOS by halonal using xenobiochemical approach.

MATERIALS AND METHODS

Experiments were performed on 32 male outbred albino rats weighing 140-160 g. The animals were kept under the natural light/dark regimen, fed standard diet, and had free access to water and food. Inductors were administered 1 time a day for 3 days. The effective doses of inductors were evaluated previously. The animals received 70 mg/kg halonal and 50 mg/kg phenobarbital (suspension in 1% starch gel). Control rats received an equivalent volume of the solvent.

The animals were decapitated under ether anesthesia 48 h after the last injection. The contents of proteins and cytochromes P-450 and b_5, NADPH-dependent cytochrome P-450 reductase activity, rates of aminopyrine N-demethylation, aniline *n*-hydroxylation, and 7-ethoxy- and 7-pentoxyresorufin O-dealkylation, and content of androstenedione metabolites were estimated in the liver microsomal fraction [4]. Molecular isoforms of cytochrome P-450 were isolated from rat liver microsomes induced by phenobarbital (P-450b) and 3-methylcholanthrene (P-450c).

Antibodies against cytochrome P-450 isoforms were obtained after immunization of adult outbred rabbits with cytochrome preparations. Immunoglobulins were isolated from immune sera by ammonium sulfate fractionation and used for immunohistochemical assay of microsomes. The reaction of double immunodiffusion was performed by the method of Ouchterlony. The content of cytochrome P-450 isoforms (P-450b and P-450c) was estimated by immunoelectrophoresis. Microsomal fraction proteins were separated by polyacrylamide electrophoresis with sodium dodecyl sulfate.

Department of Pharmacology, Siberian State Medical University, Tomsk

0007-4888/02/1343-0264$27.00

The results were processed statistically. The arithmetic mean (M) and standard error (m) were calculated. The significance of differences was estimated by Student's t test.

RESULTS

Halonal and phenobarbital increased microsomal protein and cytochrome P-450 contents and NADPH-dependent cytochrome P-450 reductase activity, but had no effect on cytochrome b_5 concentration (Table 1).

Liver microsomes from rats receiving inductors formed the precipitation line with anti-P-450b, but did not interact with anti-P-450c. These results indicate that the isoform of cytochrome P-450 immunologically identical to phenobarbital-induced P-450b is formed in microsomes induced by halonal. However, we found no methylcholanthrene-induced P-450c.

The content of the cytochrome P-450 isoform immunologically identical to phenobarbital-induced P-450b was estimated by rocket electrophoresis using anti-P-450b. It should be emphasized that P-450e induced by phenobarbital inductors is immunologically identical to P-450b [2]. These isoforms have the same molecular weight and are characterized by high homology in the primary structure. They differ only in catalytic activity and substrate specificity. Therefore, we measured the total content of cytochromes P-450b+e. The absolute and relative contents of these hemoprotein isoforms were similar in experiments with halonal and phenobarbital. The content of phenobarbital-induced isoforms was 50% of the total content of cytochrome P-450 estimated by the CO complex (Table 1).

We determined the molecular weight of halonal- and phenobarbital-induced hemoprotein isoforms. The intensity of bands corresponding to a molecular weight of 52 kDa increased, which indicated the induction of cytochrome P-450 isoforms observed under the influence of phenobarbital (P-450b+e) [2].

Halonal and phenobarbital increased activities of aminopyrine N-demethylase and aniline n-hydroxylase (Table 1). However, metabolism of these substrates is not specific for certain isoform of hemoprotein and these changes reflect intensification of metabolism of various substrates, which is typical of phenobarbital inductors [1,2]. To estimate substrate specificity of halonal-induced cytochrome P-450 isoforms we used substrates, whose metabolism is associated with certain isoforms of this hemoprotein. For example, 7-ethoxyresorufin is metabolized only by ethoxyresorufin O-deethylase and serves as the substrate for P-450c induced by 3-methylcholanthrene [8]. In our experiments halonal and phenobarbital did not change catalytic activity of this isoform, which confirms the results of immunochemical assay. The rate of O-dealkylation of 7-pentoxyresorufin that serves as the substrate for P-450b increased, which corresponds to the results of rocket immunoelectrophoresis.

The metabolism of androstenedione reflects activity of four P-450 isoforms (a, b, h, and p). Halonal

TABLE 1. Effects of Halonal and Phenobarbital on Rat Liver MOS ($M\pm m$, n=6-8)

Parameter	Control	Halonal	Phenobarbital
Microsomal protein, mg/liver	56.2±2.5	73.7±407.0*	75.6±7.35*
Cytochrome content, nmol/mg protein:			
P-450	0.53±0.07	1.32±0.07*	1.21±0.08*
P-450b+e	0.020±0.002	0.65±0.06*	0.56±0.07*
b_5	0.440±0.001	0.54±0.04	0.48±0.04
NADPH-dependent cytochrome P-450 reductase, nmol/mg protein/min	153±13	350±38*	321±25*
Monooxygenase activity, nmol product/mg protein/min:			
aminopyrine N-demethylase	1.44±0.15	5.36±0.28*	4.78±0.37*
aniline n-hydroxylase	0.54±0.01	1.12±0.15*	1.12±0.07*
7-ethoxyresorufin O-dealkylase	0.07±0.02	0.06±0.01	0.06±0.01
7-pentoxyresorufin O-dealkylase	0.07±0.01	1.24±0.09*	1.20±0.02*
androstenedione hydroxylase:			
7α-OH	0.17±0.01	0.14±0.05	0.11±0.01
16α-OH	1.96±0.13	1.43±0.42	0.87±0.28*
6β-OH	1.07±0.03	2.22±0.19*	1.36±0.36
16β-OH	0.82±0.05	4.5±0.9*	3.11±0.32*

Note. *p<0.05 compared to the control.

accelerated hydroxylation of androstenedione at 16β-, which is associated with the effects of P-450b [6]. The rate hormone metabolism at positions 7α- and 16α- did not differ from the control. Therefore, halonal acts as a phenobarbital inductor of MOS. The degree of halonal-induced changes does not differ from that observed after phenobarbital administration.

Our results show that halonal produces an inducing effect on liver MOS. However, previous studies demonstrated that in humans and animals halonal is present in the form of its major metabolite phenobarbital, which determines its anticonvulsive effect [6,7]. Moreover, in the intestine halonal is metabolized by alkaline hydrolysis into phenobarbital and benzoic acid [6]. These data suggest that enzyme-inducing activity of halonal is associated with the influence of phenobarbital. This hypothesis is confirmed by our results. Halonal and phenobarbital produce similar changes in the contents of cytochrome P-450 and its isoform P-450b+e and rate of model substrate metabolism (Table 1). However, it cannot be excluded that halonal molecules that undergo type I binding to cytochrome P-450 possess inducing activity. It should be emphasized that induction with phenobarbital is accompanied by a considerable increase in halonal affinity for this hemoprotein.

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