Phenobarbital-Mediated Induction of the CYP2B Subfamily Is Not Antagonized by Picrotoxin, A Potent Antagonist of Barbiturates in the Central Nervous System

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Our previous study indicated that picrotoxin competes with phenobarbital (PB) for the binding to liver microsomes, although the target of binding and the physiological role were not determined (unpublished observation). To seek information on the target site of PB, reflecting the induction of hepatic enzymes, we examined here the effect of picrotoxin on PB-mediated induction of hepatic cytochrome P450 and UDP-glucuronosyltransferase in vivo in rats. The induction of the CYP2B1/2 was estimated by immunoblot analysis and by measuring the activity of testosterone 16β-hydroxylations. Intraperitoneal injection of picrotoxin alone slightly increased CYP2B1/2 protein. An experiment on co-treatment of picrotoxin and PB showed that picrotoxin enhanced rather than antagonized the inducing effect of PB. The results suggest two possibilities: that 1) picrotoxin increases the CYP2B subfamily by binding to the same site as PB; or 2) the site in microsomes for the competition between PB and picrotoxin does not reflect the induction of P450.

Key words: picrotoxin; phenobarbital; induction; CYP2B1/2; glucuronosyltransferase; 4-hydroxybiphenyl

Hepatic cytochrome P450 (P450) belonging to the CYP2B subfamily is one of the isoenzymes which catalyze a wide variety of xenobiotics.1–3 As is widely known, this subfamily is induced by various xenobiotics.4 Because of the wide spectrum of substrate specificity and its inducible nature, the CYP2B subfamily has commanded the interest of researchers for many years, however, the mechanism by which it is induced is largely unknown.

Phenobarbital (PB) is not only a hypnotic and anticonvulsant drug, but also a typical inducer for the CYP2B subfamily. The former is thought to be due to the action on the chloride ion channel and/or on the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor of central nervous system.5 Picrotoxin antagonizes the central effect of PB by competing with the binding at the specific site of chloride ion channel.5 On the other hand, our previous study indicated that 1) there is a specific binding site(s) for 3H-PB in the microsomal fraction but not in other subcellular fractions of rat liver; and 2) picrotoxin antagonizes the binding of 3H-PB to microsomes (unpublished observation). The site for this competition has not been identified, and the physiological meaning of the competition is also unknown. However, it is conceivable that picrotoxin binds to the same site as that for PB, which reflects on the induction of the CYP2B subfamily. To address this possibility, we examined here the effect of picrotoxin on PB-mediated increase in the CYP2B subfamily in vivo in rats.

MATERIALS AND METHODS

Materials The following chemicals were purchased from the sources indicated: picrotoxin (Sigma Chemical Co., St Louis, MO, U.S.A), sodium PB (Tokyo Kasei Industries Co., Tokyo, Japan), hydroxytestosterone except for 2α-hydroxy isomer (Steraloids Inc., Wilton, NH), and 4-hydroxybiphenyl (Wako Pure Chemical Industries, Co., Ltd., Osaka, Japan). 2α-Hydroxytestosterone was donated by Dr. M. Nakano, Shionogi Pharmaceutical Co., Osaka). 4-Hydroxybiphenyl glucuronide was prepared from the urine of rabbits treated with 4-hydroxybiphenyl by the method of Dodgson et al.7 Rabbit anti-CYP2B1/2 IgG was prepared by the method described.8

Animal Treatments and Preparation of Hepatic Microsomes Male Wistar rats of 6 weeks old were used and were acclimatized one week prior to the start of treatment. Picrotoxin was injected i.p. to the rats at doses of 3, 5 and 10 mg/kg/ml corn oil for 4 consecutive days (once per day). PB was injected i.p. at a dose of 80 mg/kg/ml saline for 4d. The animals were fasted overnight, and the liver was removed. Hepatic microsomes were prepared as described.9

Analytical Methods The activity of testosterone metabolism was assayed by the method reported.10 The activity for glucuronidation of 4-hydroxybiphenyl was determined according to the method of Bock et al.11 Hepatic content of total P450 was determined by an established method.12 SDS–PAGE13 and immunoblotting14 were performed. In the experiment shown in Fig. 1, the immunoblot bands were read by a scanner (EPSON GT-8000) and quantitated using NIH Image (version 1.45) installed in a personal computer.

RESULTS

Immunoblot analysis of liver microsomes with anti-CYP2B1/2 antibody is shown in Fig. 1. Intraperitoneal injection of picrotoxin at doses of 3 and 5 mg/kg for 4 d slightly increased the hepatic content of CYP2B1/2. The increase in CYP2B1 content was greater than that in 2B2. The magnitude of the increase in the CYP2B1 content was somewhat greater at 5 mg/kg than at 3 mg/kg. Probably due to the small increase of some P450 isozymes, significant change in hepatic content of total P450 was not observed following picrotoxin treatment, although an increasing tendency (less than 20% of control value) was seen (data not shown). Higher doses including 10 mg/kg could not be examined because of the lethal effect (not shown). A band with higher mobility than the CYP2B1/2 was not identified. The result indicated

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Fig. 1. Immunoblot Analysis of CYP2B1/2 in Microsomes of Rats Treated with Picrotoxin
The bands immunohistochemically detected (A) and their intensity (B) are shown. Microsomes were applied to the wells at an amount of 8 μg protein.

the inducing potency of picrotoxin, although it is not great. This was supported by the observation that hepatic microsomal activity of testosterone 16β-hydroxylase, a marker of the CYP2B subfamily, was significantly increased by picrotoxin treatment (5 mg/kg×4) (Fig. 2). The magnitude (2.7-fold over the control) of the increase was consistent with that of the immunoblot band shown in Fig. 1B. As for the activity at positions other than 16β, hydroxylation at the 7α-position was also increased by the high dose of picrotoxin (Fig. 2).

When PB and picrotoxin were co-administered to rats for 4 d, an additive effect was observed for the hepatic content of total P450 (Table 1). In this experiment, 3 and 10 mg/kg of picrotoxin were administered. As described, when picrotoxin alone was administered to rats, the latter dose could not be used due to the toxicity; however, this amount could be administered when given together with PB. Immunoblot analysis indicated that the hepatic level of the CYP2B1/2 was somewhat increased by co-treatment with PB and picrotoxin in comparison with treatment with PB alone (Fig. 3). Com-
Fig. 5. Change in 4-Hydroxybiphenyl UDP-Glucuronosyltransferase Activity of Hepatic Microsomes of Rats Treated with Picrotoxin and Phenobarbital

The activity (nmol/min/mg protein ± S.E., n = 3) in control rats was 18.3 ± 5.6. *, Significantly different from the control (p<0.01); †, Significantly different from the PB-treated group (p<0.01).

crotinin (10 mg/kg) and PB than treatment with PB alone, although the difference was not significant (Fig. 4). The increase in enzyme activity by treatment with the two drugs was also seen for 4-hydroxibiphenyl glucuronidation which is one of the substrates for PB-inducible UDP-glucuronosyltransferase (UGT). The activity of rats co-treated with picrotoxin and PB was significantly higher than that of rats treated with PB alone (Fig. 5). However, treatment with picrotoxin alone showed almost no effect on the activity. This was different than the effect on P450.

DISCUSSION

This study clearly indicated that picrotoxin does not antagonize PB in the induction of hepatic CYP2B subfamily. Co-treatment with the two drugs increased the level rather than decreasing it. We observed previously that picrotoxin competes with the binding of 4-H-PB to hepatic microsomes from rats (unpublished data). In that experiment, the specific binding of 3H-PB which had disappeared with the adding of non-labeled PB was detected in abundance in microsomes. Observations made in this study together with this previous data suggests that 1) picrotoxin is a pure agonist for the induction of the CYP2B subfamily: that is, picrotoxin increases the subfamily by binding to the same site as PB; or 2) the site in microsomes for the competition between PB and picrotoxin does not reflect on the induction of P450.

Our previous study indicated that administration of picrotoxin in drinking water increased hepatic CYP2B1/2 as markedly as did i.p. injection of PB.9 This was inconsistent with the present work in which i.p. injection of picrotoxin caused only a minor increase in CYP2B1/2. This seems to be due to the difference in the ingested amount of picrotoxin. The amount of picrotoxin absorbed by rats could be around 150 mg/kg, consuming the absorption was complete in the earlier study. Although it is unknown whether this assumption is reasonable due to the lack of metabolism study on picrotoxin, the dose (3 and 5 mg/kg) used in this study (i.p. injection) was far smaller.

A similar difference was seen in the induction of 4-hydroxybiphenyl UGT. This activity was not increased by i.p. injection of picrotoxin alone in this study, whereas we previously found that oral administration of picrotoxin in drinking water significantly increased the activity.9 This difference in the inducibility is also thought to be due to the amount of picrotoxin used in the two studies. As shown in Fig. 5, co-treatment of picrotoxin and PB significantly raised the hepatic UGT activity for 4-hydroxybiphenyl over that produced by treatment with PB alone. These results suggest that picrotoxin is a more potent enhancer of 4-hydroxybiphenyl UGT induction than of the induction of CYP2B1/2. The proteins and cDNAs of PB-inducible UGT isoforms capable of catalyzing 4-hydroxybiphenyl glucuronidation have been isolated/clone.11-15 However, the mechanism by which PB induces UGTs, including the role of the gene upstream, remains largely unknown, although the role of the promotor region of the UGT2B1 gene has recently been reported.9 The reason that picrotoxin more markedly enhances PB-mediated increase in the UGT than in the CYP will not be resolved until the molecular mechanisms of the induction of these proteins are understood.

REFERENCES