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PICROTOXIN AS A POTENT INDUCER OF RAT HEPATIC CYTOCHROME P450, CYP2B1 AND CYP2B2

HYDEYUKI YAMADA, HIROSHI FUJISAKI, HAYATO KANEKO,* YUJI ISHII, TOMOYUKI HAMAGUCHI† and KAZUTA OGURI‡

Faculty of Pharmaceutical Sciences, Kyushu University 62, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan

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Abstract—The induction by the central stimulant picrotoxin of hepatic drug-metabolizing enzymes was studied in rats. The hepatic content of P450 and the activity of benzphetamine N-demethylation increased gradually after administration of picrotoxin dissolved in drinking water (2 mg/mL), to three-times higher levels than the initial values at the third day of treatment. The increase in benzphetamine *N*-demethylase activity by picrotoxin was somewhat higher than the increase produced by phenobarbital. Supporting these results, immunoblot analysis showed that CYP2B1 and 2B2 proteins in the liver microsomes were increased by picrotoxin. Picrotoxinin and picrotin, which are components of the picrotoxin molecule, had the same ability to induce the hepatic activity of benzphetamine N-demethylation. The liver microsomal activities of testosterone 16α- and 16β-hydroxylation were enhanced significantly after treatment with picrotoxinin and picrotin. However, benzo[*a*]pyrene 3-hydroxylation, aniline 4-hydroxylation, and testosterone hydroxylations at the 2α- and 7α-positions were not increased by picrotoxinin and picrotin treatment. In addition to monooxygenase, significant induction of glutathione *S*-transferase activity for 1-chloro-2,4-dinitrobenzene and UDP-glucuronyltransferase activity for 4-hydroxybiphenyl and 4-nitrophenol was also observed by pretreatment of picrotoxin. These results clearly indicate that picrotoxin is an inducer of phenobarbital-inducible liver enzymes.

It is well known that phenobarbital (PB§) markedly induces the CYP2B‖ subfamily of cytochrome P450 (P450) in the rat [2, 3]. While the evidence that PB enhances the transcription of genes coding P450s of the CYP2B subfamily was provided [4–7], the mechanism(s) how this inducer increases the transcription has not yet been determined [8]. A number of diverse synthetic chemicals other than PB have been reported to induce CYP2B P450s [3, 9]. In addition, some natural products are also known to induce the CYP2B subfamily of P450, isosafrole [3, 10], flavonoids [11] and terpenoids [12] are potent inducers for this family of P450. The activity of these natural products was suggested to be a cause of the evolutionary adaptation of the CYP2B subfamily P450 [12]. Connecting with this view, "animal–plant warfare" has been proposed for the evolution of some mammalian P450 enzymes [13, 14]. However, natural products described above are less effective than PB as the inducer of CYP2B P450. We thought that the substances examined so far were comparatively non-toxic and tested whether more potent inducers for CYP2B P450 could be found in highly toxic plant constituents.

Picrotoxin is a natural product contained in plants such as *Cocculus indicus*, *Anamirta cocculus* and *Menispermum cocculus* [15]. This material is a compound consisting of two components, picrotoxinin and picrotin in a ratio of 1:1 (Fig. 1), and the former is more toxic than the latter [16]. Picrotoxin is a stimulant of the central nervous system of mammals due to its inhibitory action toward γ-aminobutyric acid (GABA) receptors [17, 18]. The fact that plants containing such toxic substances are distributed all over the world [15, 19] (Fig. 1) led us to examine the hypothesis mentioned above. The present study examined the inducibility of picrotoxin for CYP2B1 and 2B2. Further, the inducing potencies of picrotoxin components, picrotoxinin and picrotin, were compared.

MATERIALS AND METHODS

Materials. Picrotoxin, picrotoxinin and picrotin were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.) Benzphetamine hydrochloride was a generous gift from the Upjohn Co. (Kalamazoo, MI, U.S.A.). 3-Hydroxybenzo[*a*]pyrene and 2-hydroxytestosterone were kindly donated by Dr N. Kinoshita, (Kyushu University, Fukuoka, Japan) and from Dr Y. Nakamura (Shionogi Pharmaceutical Co., Osaka, Japan), respectively. The following chemicals were purchased from the sources indicated: testosterone, aniline hydrochloride, 4-nitrophenol and 4-hydroxybiphenyl (Wako Pure Chemical Industries, Co. Ltd, Osaka, Japan); 4-aminophenol hydrochloride, 1-chloro-2,4-dinitrobenzene, and benzo[*a*]pyrene (Nacalai Tesque Co. Kyoto, Japan); 6β-, 7α-, 16α-, 16β-hydroxytestosterone and 4-

* Present address: Fujisawa Pharmaceutical Co. Ltd, 2-1-6 Kashima, Yodogawa-ku, Osaka 532, Japan.

† Present address: Shionogi Pharmaceutical Co. Ltd, 3-1 Futaba-cho, Toyonaka 561, Japan.

‡ Corresponding author. Tel. (092) 641 1151, ext. 6136; FAX (092) 641 8154.

§ Abbreviations: P450, cytochrome P450; PB, phenobarbital; GABA, γ-aminobutyric acid; GST, glutathione *S*-transferase; GT, glucuronyltransferase.

‖ A new nomenclature system for designation of cytochrome P450s [1] was used.

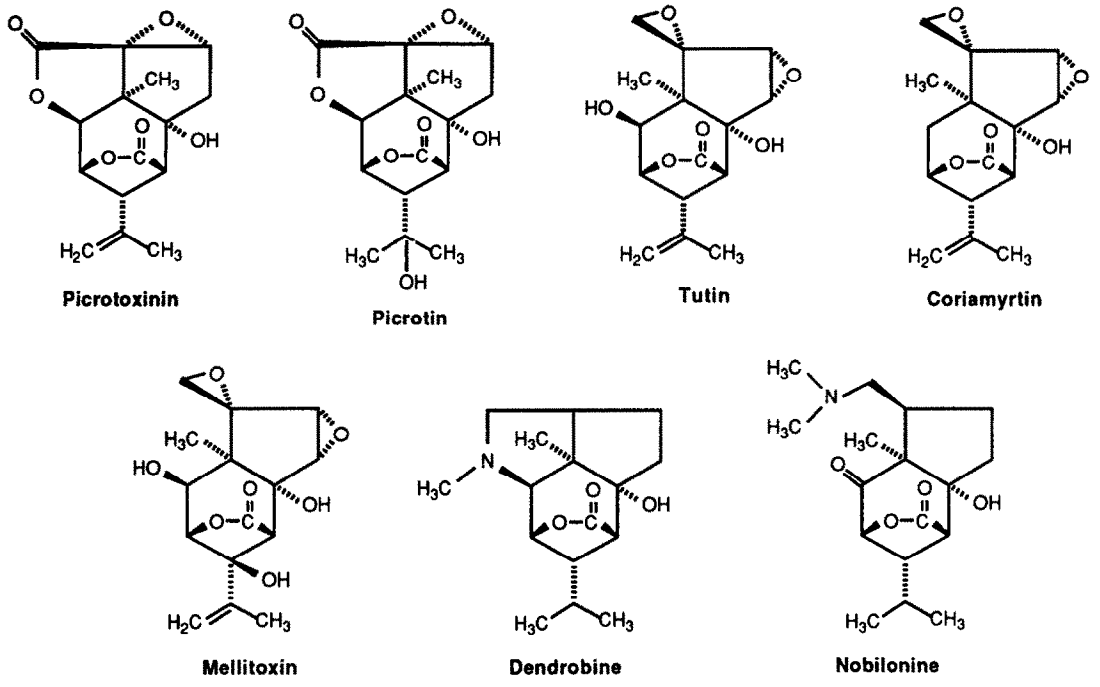

Fig. 1. Structures of picrotoxinin, picrotin and related compounds.

androsten-3, 17-dione (Steraloids Inc. Wilton, NH, U.S.A.). Rabbit anti-CYP2B1 immunoglobulin G was prepared by the method described elsewhere [20]. All other materials were the highest quality commercially available.

Preparation of tissue subfractions. Male Wistar rats weighing 160–180 g were used. Picrotoxin was dissolved in deionized water at a concentration of 2 mg/mL, and this solution was given to rats *ad lib.* together with commercial rat chow. Picrotoxin in (1 mg/mL) or picrotin (1 mg/mL) was similarly administered. The amounts of these toxic compounds ingested by rats were calculated from the volume of drug solution consumed. PB was administered i.p. to rats at a dose of 80 mg/kg/mL saline for 3 consecutive days. After the treatment with the drug for 1–4 days, rats fasted overnight and then the livers were removed. The liver was homogenized in 3 vol. of 1.15% KCl and centrifuged at 9000 *g* for 20 min. The resulting supernatant was further centrifuged at 105,000 *g* for 60 min to prepare microsomes and cytosol fractions. The microsomes were washed once with 1.15% KCl and resuspended in a volume equivalent to the original liver weight of 0.1 M potassium phosphate (pH 7.4) containing 20% glycerol. The subfractions were separated into small quantities and stored at −80° until use.

Analytical methods. The activities of microsomal drug metabolism were assayed by the methods indicated as below: benzphetamine N-demethylation [21], benzo[*a*]pyrene 3-hydroxylation [22], aniline 4-hydroxylation [23], testosterone hydroxylations [24], 4-nitrophenol glucuronidation [25] and 4-hydroxybiphenyl glucuronidation [26]. Cytosolic activity of glutathione *S*-transferase (GST) for 1-chloro-2,4-dinitrobenzene was determined by the method of Habig *et al.* [27]. The content of cytochrome P450 was measured spectrophotometrically by an established method [28]. Protein was determined by the method of Lowry *et al.* [29] using bovine serum albumin as the standard. Western blotting of CYP2B1 and 2B2 was carried out according to reported methods [30, 31].

RESULTS

Picrotoxin was dissolved in drinking water and given to rats for 1–4 days. Daily changes of hepatic P450 content and benzphetamine *N*-demethylase activity were measured (Fig. 2). No rat died during the experimental periods, although the animals given picrotoxin lost body weight within the initial 24 hr of the treatment. From the volume of water consumed, the calculated amounts of picrotoxin ingested at day 1, day 2, day 3 and day 4 were 46.8, 58.1, 64.1, and 84.0 μmol/animal, respectively. The P450 content and the activity of benzphetamine N-demethylation were found to increase progressively up to the third day from the beginning of treatment. As these two parameters at the fourth day were lower than those at the third day, the maximum effect of picrotoxin was indicated at the third day. The increase of CYP2B1 and 2B2 proteins with picrotoxin was confirmed by the western blot analysis as shown in Fig. 3A. The effects of picrotoxin on the induction of benzphetamine *N*-demethylase activity, P450 content (Fig. 2) and of the expression of CYP2B1 and 2B2 proteins (Fig. 3B) demonstrated that picrotoxin is an excellent inducer compared to PB.

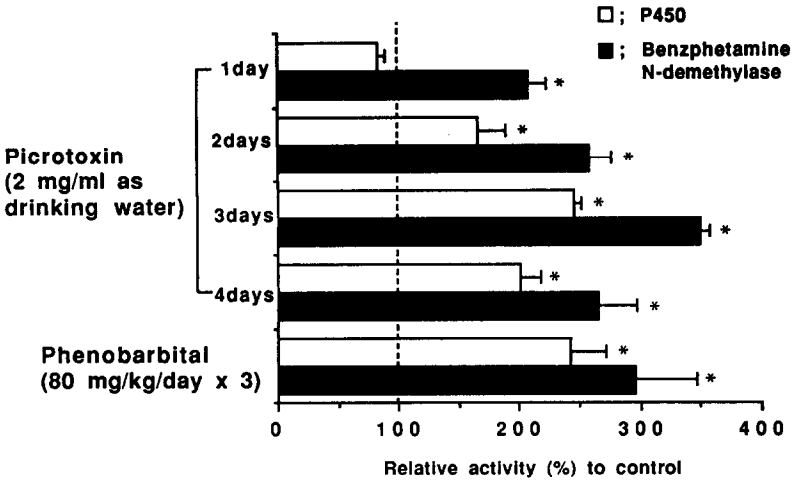

Fig. 2. Change in the P450 content and benzphetamine *N*-demethylase activity of rat liver microsomes by picrotoxin treatment. Picrotoxin was administered to rats in drinking water (2 mg/mL) for the periods indicated. Water without picrotoxin was given to control animals *ad lib*. Phenobarbital, a reference inducer, was administered to rats (N = 4) at a dose of 80 mg/kg/day for 3 consecutive days, and rats in this group were killed on the day after the last injection. Each bar represents the relative value (%) to the control with a standard deviation (N = 4 or 5). The P450 content and benzphetamine *N*-demethylase activity in control animals were 0.69 ± 0.09 nmol/mg protein and 10.6 ± 0.7 nmol/min/mg protein, respectively. *Significantly different from control ($P < 0.01$).

When picrotoxinin and picrotin, which are highly toxic, and the less toxic component of picrotoxin, respectively, were administered to rats, no difference was observed between their abilities to induce benzphetamine *N*-demethylase activity (Table 1). Since the activities of benzo[*a*]pyrene 3-hydroxylation and aniline 4-hydroxylation of rat liver microsomes were not increased by picrotoxinin and picrotin treatments (Table 1), these chemicals were considered not to induce the CYP1A and 2E subfamily. The changes in the microsomal activities of testosterone hydroxylations after picrotoxinin and picrotin administration are also shown in Table 1. As expected, the activities for 16α- and 16β-hydroxylation which are catalysed by CYP2B1 and 2B2 [32, 33] were enhanced more than twice. Testosterone 6β-hydroxylation and 17-oxidation were also increased by picrotoxinin and picrotin treatments, but to a lesser extent than the increase in the activities of 16α- and 16β-hydroxylation.

The ability of picrotoxin to induce GST and glucuronyltransferase (GT) activities is shown in Table 2. The cytosolic activity of GST towards 1-chloro-2,4-dinitrobenzene was increased significantly by picrotoxin pretreatment. The same was true of the activities of microsomal 4-hydroxybiphenyl GT and 4-nitrophenol GT. In addition, the two GT activities were also induced by picrotoxinin and picrotin treatment (Table 2). Noticeable differences were not seen in the induction potency for GT activities by picrotoxin, picrotoxinin and picrotin.

DISCUSSION

The present studies showed that picrotoxin strongly induces rat hepatic CYP2B1 and CYP2B2. Picrotoxin blocks chloride ion channels which are coupled with GABA receptor, and this is considered to be a principle mechanism of picrotoxin toxicity [17, 18]. On the contrary, PB promotes GABA-induced Cl^- current, and picrotoxin antagonizes this effect of PB [34]. Therefore, because of the opposite effect of picrotoxin and PB on the Cl^- ion current, it seems unlikely that the effect of Cl^- ion channel is involved in the mechanism of CYP2B1/2 induction. Supporting this view, the highly toxic picrotoxinin and the less toxic picrotin induced CYP2B1 and 2B2 equally. Since the suppression of the CYP2B subfamily by growth hormone and thyroid hormone has been reported [35–37], it seems possible that picrotoxin induces the CYP2B subfamily through the effect on secretion of these endocrine factors. However, our recent study indicated that picrotoxin competitively inhibits the specific binding of [^{3}H]PB to rat liver,* suggesting picrotoxin directly interacts with the same factor as that involved in PB-mediated induction of the CYP2B subfamily.

The information on the fate of picrotoxin in the animal is limited. However, early studies suggest that this toxin disappeared quickly from the blood by biotransformation [38, 39]. Urinary excretion of the unchanged picrotoxin appears to be very limited [38]. If the absorption of picrotoxin from the gastrointestinal tract of rats was almost complete, the ingested dose would be approximately 50–80 μmol/rat/day in the present study. This is comparable to the dose (60 μmol/rat/day) of PB which was administered intraperitoneally. Segelman

* Hamaguchi T, Oguri K, and Yoshimura H unpublished observation.

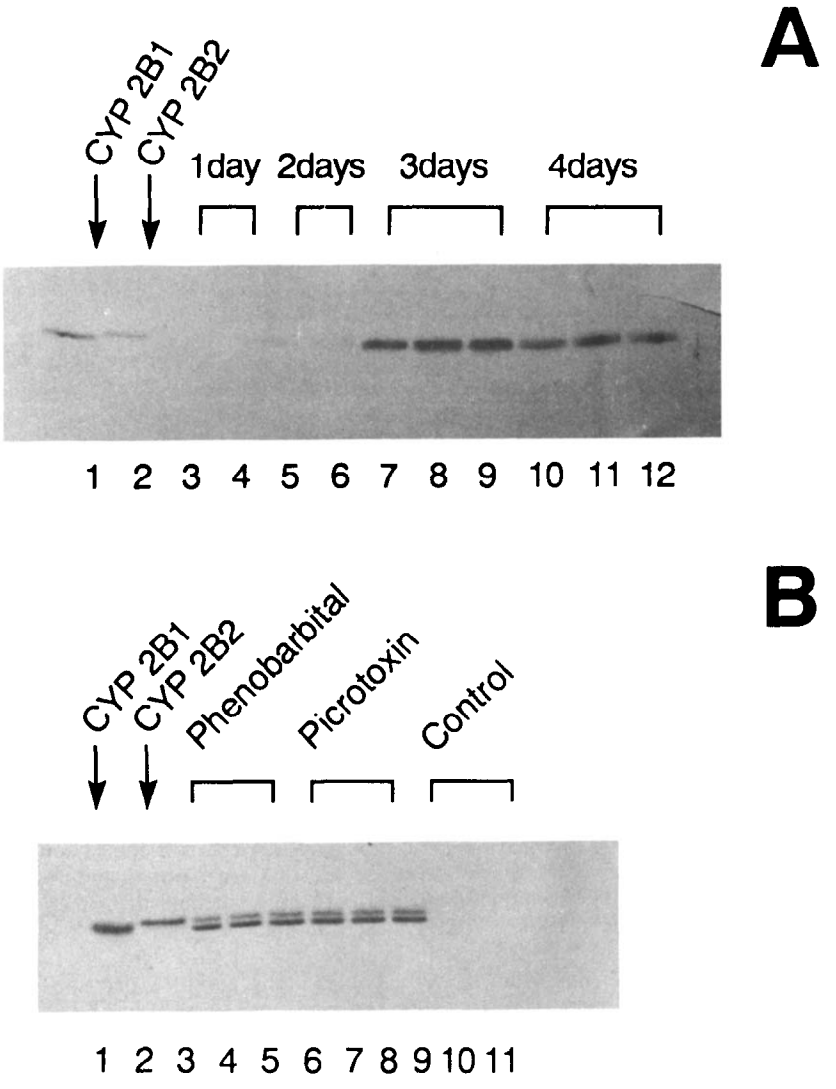

Fig. 3. Immunoblot analysis of liver microsomes from picrotoxin- (A and B) and phenobarbital-pretreated (B) rats with anti-CYP2B1/2 immunoglobin G. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis was performed by using either 9% gel (experiment A) or 7% gel (B). Microsomes were applied to the wells in an amount of 0.5 μg protein. Purified CYP2B1 and 2B2 were electrophoresed in the amount of 0.1 μg of protein. In the experiment shown in A, animals were treated with picrotoxin for the periods indicated. See Materials and Methods and the legend of Fig. 2 for details of animal pretreatments. In the experiment B, picrotoxin-treated microsomes were prepared from rats which were treated with this toxin for 3 days. All microsomal samples were prepared from different animals.

et al. [40] have reported that the administration of PB as 0.1% in drinking water causes maximal induction of rat P450 similar to the injection of 100 mg/kg. In our present study, picrotoxin was given to rats in 0.2% drinking water. This concentration is similar to that of PB in the drinking water described above. Taking the rapid metabolism of picrotoxin in the animal into consideration, the ability of picrotoxin to induce CYP2B1 and 2B2 was estimated to be comparable to PB or higher.

Picrotoxin absorbed in the rat seems to be detoxified by the same enzymes as those responsible for the metabolism of strychnine [41]. Our recent studies indicated that the liver microsomes from PB-pretreated rats efficiently catalysed the metabolism of strychnine [20], and the alkaloid itself was an inducer of CYP2B1 and 2B2 [42]. These observations support the above view. It is known that testosterone 6β-hydroxylation is mainly catalysed by the CYP3A subfamily of P450 [43–45]. As this testosterone hydroxylation was weakly enhanced by picrotoxinin and picrotin, these compounds might induce some forms of P450 belonging to the CYP3A subfamily. The induction of P450 content with picrotin was significantly higher than that with picrotoxinin. This observation may mean that picrotin but not

Table 1. Induction by picrotoxinin and picrotin of hepatic microsomal activities of benzphetamine N-demethylation, benzo[*a*]pyrene 3-hydroxylation, aniline *p*-hydroxylation and testosterone hydroxylations

Reaction	Control (N = 4)	Picrotoxinin (N = 5)	Picrotin (N = 4)
P450 content*	0.71 ± 0.04	1.14 ± 0.11‡§	1.72 ± 0.18‡§
Benzphetamine N-demethylation†	10.9 ± 0.9	19.9 ± 2.7‡	22.1 ± 2.2‡
Benzo[*a*]pyrene 3-hydroxylation†	0.15 ± 0.01	0.19 ± 0.02	0.16 ± 0.01
Aniline 4-hydroxylation†	1.20 ± 0.06	1.25 ± 0.11	1.20 ± 0.07
Testosterone hydroxylation†			
2α-Hydroxylation	0.18 ± 0.01	0.20 ± 0.03	0.19 ± 0.02
6β-Hydroxylation	0.40 ± 0.06	0.62 ± 0.01‡	0.51 ± 0.10
7α-Hydroxylation	0.36 ± 0.01	0.35 ± 0.06	0.30 ± 0.04
16α-Hydroxylation	0.75 ± 0.03	1.81 ± 0.45‡	1.75 ± 0.49‡
16β-Hydroxylation	0.40 ± 0.02	0.82 ± 0.14‡	0.88 ± 0.13‡
17-Oxidation	0.35 ± 0.04	0.54 ± 0.08‡	0.49 ± 0.12

Picrotoxinin and picrotin were administered to rats in drinking water (1 mg/mL) for 3 days. Each value represents the mean ± SD of four to five animals.

* nmol/mg protein.

† nmol/min/mg protein.

‡ Significantly different from the control ($P < 0.01$).

§ Significantly different with each other ($P < 0.01$).

Table 2. Induction of hepatic GST and GT by picrotoxin, picrotoxinin, and picrotin

Reaction	Control (N = 4)	Picrotoxinin (N = 5)	Picrotin (N = 4)	Picrotoxin (N = 4)
Glutathione *S*-transferase	0.78 ± 0.02	ND	ND	1.29 ± 0.08*
Glucuronyltransferase				
4-Nitrophenol	62.3 ± 4.5	118.4 ± 6.7*	101.9 ± 7.8*	97.1 ± 1.3*
4-Hydroxybiphenyl	30.7 ± 1.3	52.8 ± 1.6*	56.1 ± 2.4*	55.6 ± 2.5*

Picrotoxinin and picrotin were administered to rats in drinking water (1 mg/mL) for 3 days. Picrotoxin was administered as 2 mg/mL in drinking water for 3 days. Each value represents the mean ± SD of four to five animals. All activities are shown as nmol/min/mg protein.

ND not determined.

* Significantly different from the control ($P < 0.01$).

picrotoxinin induces P450(s) other than CYP1A, 2B, 2E and 3A subfamily isozymes.

As shown in Fig. 1, picrotoxinin and the related compounds can be classified as terpenoids. Austin *et al.* [12] have studied inducibility by simple terpenoids such as camphor and pinene, and have reported that all the terpenoids are potent inducers of the CYP2B subfamily P450. The potencies were, however, less than that of PB [12]. Their results and our present findings suggest that toxic terpenoids might be stronger inducers of CYP2B P450 than the less toxic terpenoids. Thus, it may be possible to consider that simple prototype terpenoids made by plants evolve to make more toxic compounds such as picrotoxin to avoid being eaten by animals. If this is true, the reverse view that animals made the ancestor form of P450 co-evolve to CYP2B P450 for detoxifying the toxic ingredients of foods might be possible. The fact that picrotin, a less toxic component of picrotoxin, was equally as effective as picrotoxinin, a toxic component, as the inducer for CYP2B1/2 may not agree with the above consideration. However, the LD_{50} value (mouse, i.p.) of picrotin was reported to be 135 mg/kg [16]. This value is far higher than that for picrotoxinin (3 mg/kg) [16], but is much less than the LD_{50} values of the simple terpenoids such as camphor (2200–2400 mg/kg, mouse, s.c.) [46] and menthol (5000–6000 mg/kg, mouse, s.c.) [46]. Therefore, it is possible to consider that picrotin is a toxic ingredient in plants. Besides picrotoxin, strychnine was an inducer for the CYP2B subfamily as mentioned above. Further, another plant constituent, cannabidiol, was also shown to increase the hepatic content of the CYP2B subfamily P450 [47], although this drug initially inhibits hepatic monooxygenase activity [48]. The present results provide evidence for the hypothesis that plant toxins were the driving force for the evolution of the CYP2B subfamily in animals.

REFERENCES

1. Nebert DW, Nelson DR, Coon MJ, Estabrook RW, Feyereisen R, Fujii-Kuriyama Y, Gonzalez FJ,

Guengerich FP, Gusalus IC, Johnson EF, Loper JC, Sato R, Waterman MR and Waxman DJ, The P450 superfamily: update on new sequences, gene mapping, and recommended nomenclature. *DNA Cell Biol* **10**: 1–14, 1991.
2. Thomas PE, Reik LM, Ryan DE and Levin W, Regulation of three forms of cytochrome P450 and epoxide hydrolase in rat liver microsomes. *J Biol Chem* **256**: 1044–1052, 1981.
3. Thomas PE, Leik LM, Ryan DE and Levin W, Induction of two immunochemically related rat liver cytochrome P-450 isozymes cytochrome P-450c and P-450d, by structurally diverse xenobiotics. *J Biol Chem* **258**: 4590–4598, 1983.
4. Adesnik M, Bar-nun S, Maschio F, Zunich M, Lippman A and Bard E, Mechanism of induction of cytochrome P-450 by phenobarbital. *J Biol Chem* **256**: 10340–10345, 1981.
5. Phillips IR, Shephard EA, Mitani F and Rabin BR, Induction by phenobarbital of the mRNA for a specific variant of rat liver microsomal cytochrome P-450. *Biochem J* **196**: 839–851, 1981.
6. Hardwick JP, Gonzalez FJ and Kasper CB, Transcriptional regulation of rat liver epoxide hydrolase, NADPH-cytochrome P-450 reductase, and cytochrome P-450b genes by phenobarbital. *J Biol Chem* **258**: 8081–8085, 1983.
7. Pike SF, Shephard EA, Rabin BR and Phillips IR, Induction of cytochrome P-450 by phenobarbital at the level of transcription. *Biochem Pharmacol* **34**: 2489–2494, 1985.
8. Waxman DJ and Azaroff L, Phenobarbital induction of cytochrome P450 gene expression. *Biochem J* **281**: 577–592, 1992.
9. Lesca P, Fournier A, Lecointe P and Cresteil T, A dual assay for the specific screening of 3-methylcholanthrene- and phenobarbital-like chemical inducers of cytochrome P-450 monooxygenases. *Mutat Res* **129**: 299–310, 1984.
10. Ryan DE, Thomas PE and Levin W, Hepatic microsomal cytochrome P-450 from rats treated with isosafrole. *J Biol Chem* **255**: 7941–7955, 1980.
11. Siess M-H, Guillermic M, Le Bon A-M and Suschetet M, Induction of monooxygenase and transferase activities in rat by dietary administration of flavonoids. *Xenobiotica* **19**: 1379–1386, 1989.
12. Austin CA, Shephard EA, Pike SF, Rabin BR and Phillips IR, The effect of terpenoid compounds on cytochrome P-450 levels in rat liver. *Biochem Pharmacol* **37**: 2223–2229, 1988.
13. Nebert DW, Nelson DR and Feyereisen R, Evolution of the cytochrome P450 genes. *Xenobiotica* **19**: 1149–1160, 1989.
14. Gonzalez FJ and Nebert DW, Evolution of the P450 gene superfamily: animal–plant 'warfare', molecular drive and human genetic differences in drug oxidation. *Trend Genet* **6**: 182–186, 1990.
15. Porter LA, Picrotoxin and related substances. *Chem Rev* **67**: 441–464, 1967.
16. Jarboe CH, Porter LA and Buckler BT, Structural aspects of picrotoxinin action. *J Med Chem* **11**: 729–731, 1968.
17. Fujimoto M and Okabayashi T, Effect of picrotoxin on benzodiazepine receptors and GABA receptors with reference to the effect of Cl^- ion. *Life Sci* **28**: 895–901, 1981.
18. Newland CF and Cull-Candy SG, On the mechanism of action of picrotoxin on GABA receptor channels in dissociated synaptic neurones of the rat. *J Physiol* **447**: 191–213, 1992.
19. Hutchinson J, *Evolution and Physiology of Flowering Plants*. Academic Press, London, 1969.
20. Tanimoto Y, Kaneko H, Ohkuma T, Oguri K and Yoshimura H, Site-selective oxidation of strychnine by phenobarbital inducible cytochrome P-450. *J PharmacobioDyn* **14**: 161–169, 1991.
21. Nash T, The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem J* **55**: 416–421, 1953.
22. Nebert DW and Gelboin HV, Substrate inducible microsomal aryl hydroxylase in mammalian cell culture. *J Biol Chem* **243**: 6242–6249, 1968.
23. Imai Y, Ito A and Sato R, Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. *J Biochem* **60**: 417–428, 1966.
24. Yoshihara S, Nagata K, Wada I, Yoshimura H and Masuda Y, A unique change in steroid metabolism in rat liver microsomes induced with highly toxic polychlorinated biphenyl (PCB) and polychlorinated dibenzofuran (PCDF). *J PharmacobioDyn* **5**: 994–1004, 1982.
25. Isselbacher KJ, Chrabas MF and Quinn RC, The solubilization and partial purification of a glucuronyltransferase from rabbit microsomes. *J Biol Chem* **237**: 3033–3036, 1962.
26. Bock KW, Josting D, Lilienblum W and Pfeil H, Purification of rat liver microsomal UDP-glucuronyltransferase. Separation of two enzyme forms inducible by 3-methylcholanthrene or phenobarbital. *Eur J Biochem* **98**: 19–26, 1979.
27. Habig WH, Pabst MJ and Jakoby WB, Glutathione *S*-transferase: the first enzymatic step in mercapturic acid formation. *J Biol Chem* **249**: 7130–7139, 1974.
28. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. *J Biol Chem* **239**: 2370–2378, 1964.
29. Lowry OH, Rosebrough NJ, Farr AL and Randall R, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
30. Towbin H, Staehelin T and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheet: procedure and some applications. *Proc Natl Acad Sci USA* **76**: 4350–4354, 1979.
31. Guengerich FP, Wang P and Davidson NK, Estimation of isozymes of microsomal cytochrome P-450 in rats, rabbits, and humans using immunochemical staining coupled with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Biochemistry* **21**: 1698–1706, 1982.
32. Wood AW, Ryan DE, Thomas PE and Levin W, Regio- and stereoselective metabolism of two C19 steroids by five highly purified and reconstituted rat hepatic cytochrome P-450 isozymes. *J Biol Chem* **258**: 8839–8847, 1983.
33. Waxman DJ, Ko A and Walsh C, Regioselectivity and stereoselectivity of androgen hydroxylations catalyzed by cytochrome P-450 isozymes purified from phenobarbital-induced rat liver. *J Biol Chem* **258**: 11937–11947, 1983.
34. Rall TW, Hypnotics and sedatives; ethanol. In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics, 8th Edn* (Eds. Gilman AG, Rall TR, Nies AS and Taylor R), pp. 345–382. Pergamon Press, New York, 1990.
35. Yamazoe Y, Shimada M, Murayama N and Kato R, Suppression of levels of phenobarbital-inducible rat liver cytochrome P-450 by pituitary hormone. *J Biol Chem* **262**: 7423–7428, 1987.
36. Schuetz EG, Schuetz JD, May B and Guzelian PS, Regulation of cytochrome P-450b/e and P-450p gene expression by growth hormone in adult rat hepatocytes cultured on a reconstituted basement membrane. *J Biol Chem* **265**: 1188–1192, 1990.
37. Murayama N, Shimada M, Yamazoe Y and Kato R, Difference in the susceptibility of two phenobarbital-inducible forms, P450IIB1 and P450IIB2, to thyroid hormone- and growth hormone-induced suppression in

rat liver: phenobarbital-inducible P450IIB2 suppression by thyroid hormone acting directly, but not through the pituitary system. *Mol Pharmacol* **39**: 811–817, 1991.
38. Dille JM, The inactivation and elimination of picrotoxin. *J Pharmacol Exp Ther* **64**: 319–329, 1938.
39. Duff DM and Dille JM, Distribution and rate of elimination of picrotoxin. *J Pharmacol Exp Ther* **67**: 353–357, 1939.
40. Segelman FH, Kelton E, Terzi RM, Kucharczyk N and Sofia RD, The comparative potency of phenobarbital and five 1,3-propanediol dicarbamates for hepatic cytochrome P450 induction in rats. *Res Commun Chem Pathol Pharmacol* **48**: 467–470, 1985.
41. Kato R, Chiesara E and Vassanelli P, Increased activity of microsomal strychnine-metabolizing enzyme induced by phenobarbital and other drugs. *Biochem Pharmacol* **11**: 913–922, 1962.
42. Fujisaki H, Mise M, Ishii Y, Yamada H and Oguri K, Abstracts of papers, 7th annual meeting of Japanese Society for the Study of Xenobiotics, Kyoto, Japan, October, *Xenobiotic Metab Dispos* **7**(Suppl.): 122 (in Japanese), 1992.
43. Imaoka S, Terano Y and Funae Y, Constitutive testosterone 6β-hydroxylase in rat liver. *J Biochem* **104**: 481–487, 1988.
44. Waxman DJ, Attisano C, Guengerich FP and Lapenson DP, Human liver microsomal steroid metabolism: identification of the major microsomal steroid hormone 6β-hydroxylase cytochrome P-450 enzyme. *Arch Biochem Biophys* **263**: 424–436, 1988.
45. Ciaccio PJ and Halpert J, Characterization of a phenobarbital-inducible dog liver cytochrome P450 structurally related to rat and human enzymes of the P450IIIA (steroid-inducible) gene subfamily. *Arch Biochem Biophys* **271**: 284–299, 1989.
46. Spector WB, *Handbook of Toxicology Vol. I*, WB Sauders Co. Philadelphia and London, 1956.
47. Bernheim LM and Correia MA, Purification and characterization of a mouse liver cytochrome P-450 induced by cannabidiol. *Mol Pharmacol* **36**: 377–383, 1989.
48. Narimatsu S, Watanabe K, Matsunaga T, Yamamoto I, Imaoka S, Funae Y and Yoshimura H, Inhibition of hepatic microsomal cytochrome P450 by cannabidiol in adult male rats. *Chem Pharm Bull* **38**: 1365–1368, 1990.