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Effect of prenatal exposure of deltamethrin on the ontogeny of xenobiotic metabolizing cytochrome P450s in the brain and liver of offsprings

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Abstract

Prenatal exposure to low doses (0.25 or 0.5 or 1.0 mg/kg, p.o.) of deltamethrin, a type II pyrethroid insecticide, to pregnant dams from gestation days 5 to 21 (GD5-21) produced dose-dependent alterations in the ontogeny of xenobiotic metabolizing cytochrome P450 (CYP) isoforms in brain and liver of the offsprings. RT-PCR analysis revealed dose-dependent increase in the mRNA expression of cerebral and hepatic CYP1A1, 1A2, 2B1, 2B2, and 2E1 isoenzymes in the offsprings exposed prenatally to deltamethrin. Similar increase in the activity of the marker enzymes of these CYP isoforms has indicated that placental transfer of the pyrethroid, a mixed type of CYP inducer, even at these low doses may be sufficient to induce the CYPs in brain and liver of the offsprings. Our data have further revealed persistence in the increase in expression of xenobiotics metabolizing CYPs up to adulthood in brain and liver of the exposed offsprings, suggesting the potential of deltamethrin to imprint the expression of CYPs in brain and liver of the offsprings following its in utero exposure. Furthermore, though the levels of CYPs were several fold lower in brain, almost equal magnitude of induction in cerebral and hepatic CYPs has further suggested that brain CYPs are responsive to the induction by environmental chemicals. The present data indicating alterations in the expression of xenobiotic metabolizing CYPs during development following prenatal exposure to deltamethrin may be of significance as these CYP enzymes are not only involved in the neurobehavioral toxicity of deltamethrin but have a role in regulating the levels of ligands that modulate growth, differentiation, and neuroendocrine functions.

Keywords: Deltamethrin; In utero; Ontogeny; Brain; CYPs

Introduction

The developing fetus and neonate during the critical periods in early development may be uniquely sensitive to the effects of chemical substances to which it is exposed in utero (Faustman et al., 2000; Barton, 2005). Fetal damage by chemicals has been shown to modify ontogeny of the enzyme involved in its detoxification (Juchau, 1997; Juchau et al., 1998). Since organ-specific profiles of the biotransformation enzymes continue to evolve and undergo pronounced changes, long after the organs have become morphologically distinct, these enzyme systems are an easy target for chemical insults. Levels of cytochrome P450 (CYP), the major enzyme system involved in biotransformation of the xenobiotics, are much lower in mammalian tissues from early gestational ages in comparison to those observed in adults (Juchau et al., 1998). Environmental agents and known CYP inducers such as phenobarbital (PB), polycyclic aromatic hydrocarbons (PAHs), and ethanol have been found to transplacentally induce the expression of specific CYP isoforms (Cresteil et al., 1986; Miller, 2004). Studies have shown that potentiation of xenobiotic promoted teratogenesis is subject to alterations in the expression of specific fetal CYPs (Krauer and Dayer, 1991).

Synthetic pyrethroids are commonly used insecticides in agriculture and home formulations, and there is widespread exposure to these compounds (Casida and Quistad, 1998). Exposure to pyrethroids has been shown to occur in humans, including exposure to pregnant women, infants, and children (Bradman et al., 2003; Barr et al., 2005). Deltamethrin, a type II synthetic pyrethroid, has been shown to be a neurotoxin acting

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on the axons in the peripheral and central nervous system by interacting with sodium channels (Ray and Cremer, 1979; Vijverberg and van den Bercken, 1990). Deltamethrin produces marked behavioral effects following acute and chronic exposure in laboratory animals (Ray and Cremer, 1979; Gray and Rickard, 1982; Sheets et al., 1994). Deltamethrin has been shown to induce subtle changes in the reproductive behavior and physiology of male offspring in rats at dose levels that do not cause maternal toxicity (Andrade et al., 2002). Neurochemical as well as neurobehavioral impairments have also been reported in the offsprings following gestational exposure of the pyrethroids, suggesting that the pesticide and/or its metabolite reach the conceptus to adversely affect the physiological systems in the offsprings (Eriksson and Fredriksson, 1991; Lazarini and Bernardi, 2001; Aziz et al., 2001a, 2001b). Behavioral deficits associated with alterations in the ontogeny of neurotransmitter systems have been reported in the offsprings after in utero exposure to synthetic pyrethroids (Husain et al., 1992; Aziz et al., 2001a, 2001b).

The neurobehavioral toxicity of deltamethrin was found to correlate with the amount of pyrethroids or its metabolites accumulating in the brain (Anadon et al., 1996; Dayal et al., 2003). Studies from our laboratory have indicated that alterations in the CYPs in rat brain and liver could be correlated with the amounts of the pyrethroids or its metabolites accumulating in the brain and demonstrated the role of CYPs in the neurobehavioral toxicity of deltamethrin (Dayal et al., 2001, 2003). Our data have further shown that differences in the induction of individual CYP isoenzymes in diverse brain regions could play a role in regulating the response of brain to pyrethroid insecticides by modulating their concentration per se or their active metabolites at the target sites (Dayal et al., 2001). Although the role of CYPs in the neurobehavioral toxicity of deltamethrin is relatively well characterized in the adults, limited information exists on the role of CYPs in the developmental neurotoxicity of deltamethrin. As xenobiotic metabolizing CYPs are known to be expressed in the fetus, the present study attempted to investigate the prenatal effects of deltamethrin on the ontogeny of brain and liver xenobiotics metabolizing CYPs, which have been found to be associated with the process of growth and differentiation (Nebert, 1991; Choudhary et al., 2004).

Materials and methods

Chemicals. Deltamethrin formulation [Decis 2.8% EC denotes 2.8% of technical grade deltamethrin (w/w) in emulsifiable concentrate] was procured from Hoescht, India. Trizol reagent was obtained from Life technologies, USA. Oligo$(dT)_{20}$ and RNAse out were procured from Ambion Inc, USA. 1 kb plus ladder, DNase1, Amp. Grade were obtained from Invitrogen, USA. dNTP mix, M-MuLV reverse transcriptase, Taq DNA polymerase were procured from MBI Fermentas, USA. 7-Ethoxyresorufin, 7-pentoxyresorufin, and *N*-nitrosodimethylamine were procured from Sigma, USA. All other chemicals used were of the highest purity commercially available and procured either from BDH (a subsidiary of E. Merck, India) or SISCO Research Laboratories Pvt. Ltd. (India). Phenobarbital sodium salt (PB) was a gift from Biodeal Laboratories (India).

Animals and treatment. Adult male (~12 weeks old) and female (~10 weeks old) Albino Wistar rats of proven fertility were obtained from the Animal House facility of Industrial Toxicology Research Centre, Lucknow. All the animals were maintained on a commercial pellet diet and water ad libitum in a temperature-controlled room with a 12/12-h light/dark cycle and cared for in accordance to the policy laid down by Animal Care Committee of Industrial Toxicology Research Centre. The animal experimentation was approved by the Ethical Committee of the Centre. Female rats were allowed to mate with adult males (3:1). On day 0 of pregnancy (confirmed by a positive vaginal smear), the pregnant rats were randomly divided into four groups. Animals in group 1, 2, and 3 received 0.25 or 0.5 or 1.0 mg/kg body weight of deltamethrin, orally from gestation day 5 (GD5) to GD21. Animals in group 4 served as control and received corn oil in an identical manner. On the day of parturition, the average litter size was adjusted to eight per dam in all the groups with equal number of males and females as far as possible. The male offsprings born to the control and treated dams were sacrificed on postnatal (pnd) 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, and 90. Liver and brain were immediately removed and snap frozen in liquid nitrogen and stored at −80 °C. The tissues were processed for isolation of RNA using Trizol Reagent (Life technologies, USA) using the manufacturer's protocol.

RT-PCR analysis. Prior to reverse transcription (RT), RNA was treated with DNase1, Amp. Grade (Invitrogen, USA), according to the manufacturer's protocol, to avoid contamination with genomic DNA. cDNA was synthesized by first denaturing reaction mixture containing 3 μg of total RNA isolated from brain or liver, 0.5 μg Oligo$(dT)_{20}$ and DEPC treated autoclaved water at 70 °C for 5 min and subsequently incubating at 4 °C. For RT, the reaction mixture in 20 μl contained 1× cDNA synthesis buffer, 0.5 U RNAse Out, 1 mM dNTP mix, 200 U of Revert Aid H Minus M-MuLV Reverse Transcriptase (1 U/μl) of MBI Fermentas and the Oligo $(dT)_{20}$ primed mRNA from the previous step. RT reaction was carried out by incubating the reaction mixture at 42 °C for 60 min. The reaction was terminated by incubating the mixture at 70 °C for 10 min. 1 μl of RNAse H was then added to the cDNA, and the mixture was incubated at 37 °C for 20 min. Reactions without RNA were also carried out which served as the negative RT control. Prior to the amplification of CYPs, normalization was carried out with glyceraldehyde 3-phosphate dehydrogenase (GAPDH), the housekeeping gene. The reaction mixture for PCR of GAPDH in 50.0 μl contained 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.33 μM of each GAPDH primers (Soh et al., 1996), 2.0 μl of cDNA and 1.25 U Taq DNA polymerase. PCR was carried out in PTC-200 (MJ Research, U.S.A) using initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing of primers at 55 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min.

For PCR amplification of the CYPs, cDNA obtained from liver RNA were diluted 5 times for CYP1A1 and 3 times for CYP1A2, CYP2B1, and 2B2 and 20 times for CYP2E1. The cDNA synthesized from RNA extracted from brain tissue was used as such except in the case of CYP2E1 for which brain cDNA was diluted 4 times prior to PCR. The PCR reaction mixture for CYP1A1, 1A2, 2B1, 2B2, and 2E1 in 50 μl contained 1× PCR buffer, 0.2 mM dNTP mix, 0.3 μM of each CYP1A1 or 2B1 or 2B2 or 2E1 primers, or 0.4 μM of each CYP1A2 primers, 2 μl cDNA and 1.5 U Taq DNA polymerase from MBI Fermentas, USA. $MgCl_2$ at the final concentration of 3.0 mM was used for CYP1A1 and 2B2, while 1.5 mM was used for CYP2B1 and 2E1 and 1.0 mM for CYP1A2. The details of the primers for CYPs have been described by Omiecinski et al. (1990a, 1990b), Hodgson et al. (1993), Soh et al. (1996), and Schilter and Omiecinski (1993). PCR was carried out in PTC-200 (MJ Research) using initial denaturation at 94 °C for 3 min, then 30 cycles of denaturation at 93 °C for 45 s, annealing at 58.2 °C for CYP1A1 or 55 °C for CYP2B1 or 53 °C for CYP1A2 or 60 °C for CYP2B2 for 1 min and extension at 72 °C for 1.5 min. 1 cycle of final extension at 72 °C for 10 min was also used. For CYP2E1, nested PCR was carried out similarly using initial denaturation at 94 °C for 3 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing of primers at 50 °C for 30 s, extension at 72 °C for 5 min, and final extension at 72 °C for 5 min. The product from 1st PCR reactions was reamplified using nested primers for CYP2E1 following the same conditions. PCR products were analyzed by agarose gel electrophoresis using VERSA DOC Imaging System Model 1000 (Bio-Rad, USA). The densitometry was performed using Quantity One Quantitation software of Bio-Rad.

Assay of CYP marker enzymes. The activity of CYP1A1-dependent 7-ethoxyresorufin-*O*-deethylase (EROD) and CYP2B1 marker enzyme, 7-

pentoxyresorufin-*O*-dealkylase (PROD) were determined in microsomes prepared from liver and brain of the prenatally exposed offsprings and offsprings born to control mothers by the method of Pohl and Fouts (1980) and Rutten et al. (1992). The activity of *N*-nitrosodimethylamine demethylase (NDMA-*d*) was measured by the method of Castonguay et al. (1991).

Results

RT-PCR analysis with primers specific for rat liver GAPDH resulted in the formation of PCR products of expected band size of 194 bp in the RNA isolated from the liver or brain of control rats or rats prenatally exposed to deltamethrin. PCR amplification resulted in the formation of products of almost equal intensity in the cDNA formed from reverse transcription of RNA extracted from liver or brain isolated from offsprings born to control or deltamethrin treated dams (data not shown).

mRNA expression of CYP1A isoforms

RT-PCR analysis of the RNA extracted from liver or brain isolated from the offsprings born to control or deltamethrin treated dams revealed that PCR products of correct size (341 bp for CYP1A1, 793 bp for CYP1A2) were formed with CYP1A1 and 1A2 primers respectively. As evident from the Figs. 1 and 2 and densitometric analysis of the PCR products, very faint mRNA expression of CYP1A1 and no detectable expression of CYP1A2 were observed in the brain at birth. As shown in Figs. 1 and 2, an increase in the mRNA expression of CYP1A1 and 1A2 was observed in brain with the postnatal development with distinct mRNA expression occurring by pnd 10 to 15. Densitometric analysis further revealed relatively higher adult levels of CYP1A2 mRNA expression when compared to CYP1A1.

Even though 5 and 3 times diluted cDNA was used for amplification of CYP1A1 and 1A2 respectively in the liver, RT-PCR analysis revealed detectable levels of CYP1A1 mRNA (Fig. 1), while very faint mRNA expression of CYP1A2 at birth (Fig. 2). The mRNA for CYP1A1 and 1A2 in rat liver was found to increase with the age of the animal. Densitometric analysis further revealed age-dependent increase in the transcript levels for CYP1A1 and 1A2 from pnd 0 through pnd 90 (Figs. 1 and 2).

Prenatal exposure to 0.25 or 0.5 or 1.0 mg/kg body weight of deltamethrin was found to produce a dose-dependent increase in the mRNA expression of both CYP1A1 and 1A2 in brain at the higher doses of 0.5 or 1.0 mg/kg, while no significant change was observed at the lower dose of 0.25 mg/kg. Densitometric analysis revealed several fold higher increase in CYP1A2 expression as compared to CYP1A1 expression at birth in the brain of offsprings exposed prenatally to the relatively higher doses of deltamethrin (0.5 or 1.0 mg/kg body weight). As revealed by the densitometric analysis, the increase observed in

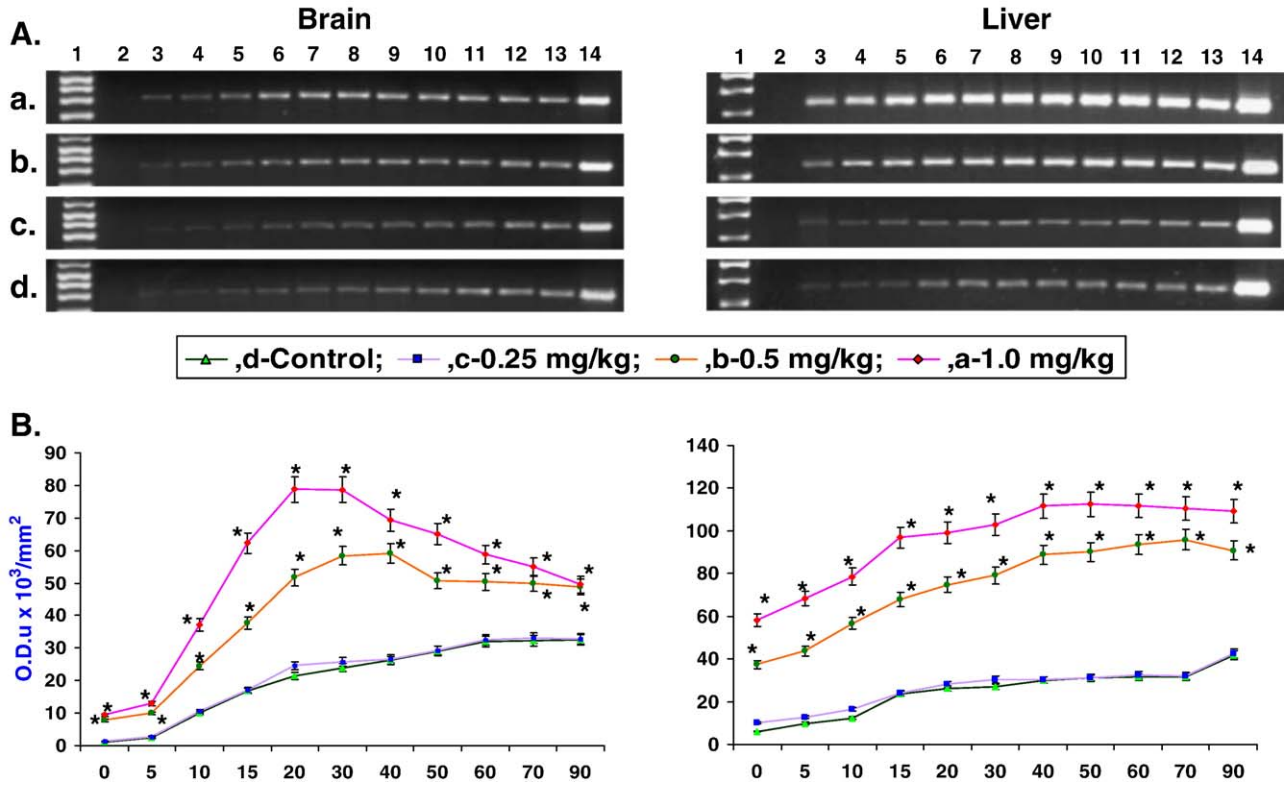

Fig. 1. Effect of prenatal exposure of deltamethrin on brain and liver CYP1A1 mRNA expression in offsprings. (A) Representative ethidium bromide stained agarose gels. Lane 1 contains 1.0 kb plus DNA ladder, lane 2 contains RT-PCR product without RNA. Lanes 3–13 contain 5 μl of RT-PCR product of RNA isolated from brain or liver of 0-, 5-, 10-, 15-, 20-, 30-, 40-, 50-, 60-, 70-, and 90-day-old rat offsprings respectively. Lane 14 contains 5 μl of RT-PCR product of RNA isolated from 3-methylcholanthrene (MC) pretreated animals. (B) Densitometric analysis of the RT-PCR products of RNA isolated from 3 animals. *$P < 0.05$ when compared with controls.

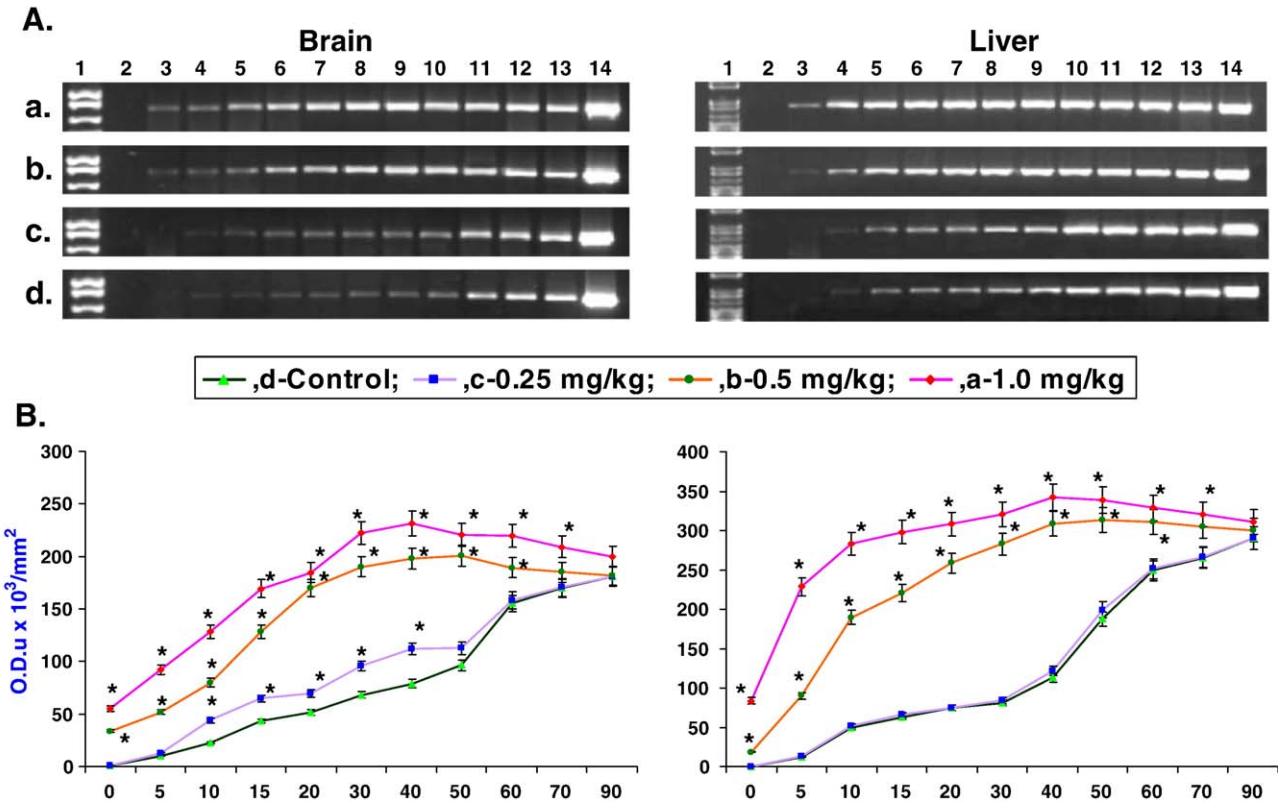

Fig. 2. Effect of prenatal exposure of deltamethrin on brain and liver CYP1A2 mRNA expression in offsprings. (A) Representative ethidium bromide stained agarose gels. Lane 1 contains 1.0 kb plus DNA ladder, lane 2 contains RT-PCR product without RNA. Lanes 3–13 contain 5 μl of RT-PCR product of RNA isolated from brain or liver of 0-, 5-, 10-, 15-, 20-, 30-, 40-, 50-, 60-, 70-, and 90-day-old rat offsprings respectively. Lane 14 contains 5 μl of RT-PCR product of RNA isolated from MC pretreated animals. (B) Densitometric analysis of the RT-PCR products of RNA isolated from 3 animals. *$P < 0.05$ when compared with controls.

CYP1A1 and 1A2 mRNA expression persisted up to pnd 90 though the magnitude of induction observed was several fold less when compared to that observed at pnd 0 (Figs. 1 and 2).

As observed with brain, prenatal exposure to low doses of deltamethrin was found to produce a dose-dependent increase in the mRNA expression of CYP1A1 and 1A2 in the liver of the exposed offsprings at pnd 0. Densitometric analysis revealed almost similar magnitude of induction in rat brain and liver during the postnatal development. As seen with the brain, the increase in the expression of both, CYP1A1 and 1A2 persisted up to pnd 90, the last time point studied (Figs. 1 and 2). Similar to that observed in brain, CYP1A2 was found to be induced to a much greater extent than 1A1 in both brain and liver.

mRNA expression of CYP2B isoforms

RT-PCR analysis of the RNA extracted from liver or brain isolated from the offsprings born to control or deltamethrin treated dams revealed that PCR products of correct size (380 bp for CYP2B1 and 163 bp for CYP2B2) were formed with CYP2B1 and 2B2 primers. RT-PCR analysis revealed that CYP2B1 mRNA expression was barely detectable up to pnd 20 in the brain of the control rat pups (Fig. 3), while detectable though faint mRNA expression of CYP2B2 was visible in the brain at birth (Fig. 4). Very low levels of CYP2B1 mRNA expression were observed at pnd 30. An increase in mRNA expression of CYP2B1 and 2B2 in brain of rat pups was found with the postnatal development with almost similar expression of 2B1 and 2B2 being observed at latter time points (Figs. 3 and 4). The levels of CYP2B1 transcript in brain were found to exhibit a small decrease after pnd 70.

In contrast to that seen with brain, CYP2B1 mRNA expression was detected at birth in the liver of the control rat pups. However, similar to brain, CYP2B1 mRNA expression in the liver increased up to pnd 60, and then a decrease in the expression of CYP2B1 was observed up to pnd 90 (Fig. 3). Expression of CYP2B2 in liver followed a similar pattern as in brain. Low but clearly distinct CYP2B2 mRNA expression was detected by RT-PCR analysis in the liver of the control offsprings at birth which continued to increase with postnatal development (Fig. 4).

As seen with CYP1A1 and 1A2, prenatal exposure to the low doses of deltamethrin was found to produce a dose-dependent increase in the CYP2B1 as well as CYP2B2 levels in the brain of the offsprings. Significantly high mRNA expression of CYP2B1 and 2B2 was detected at birth which continued up to pnd 30 in the offsprings exposed to the relatively higher doses of 0.5 or 1.0 mg/kg body weight deltamethrin. The levels of CYP2B1 and 2B2 then showed a decline though the increase persisted up to pnd 90 in the brain of the offsprings. As compared to CYP2B1, the increase in the CYP2B2 mRNA persisted up to pnd 90 (Figs. 3 and 4).

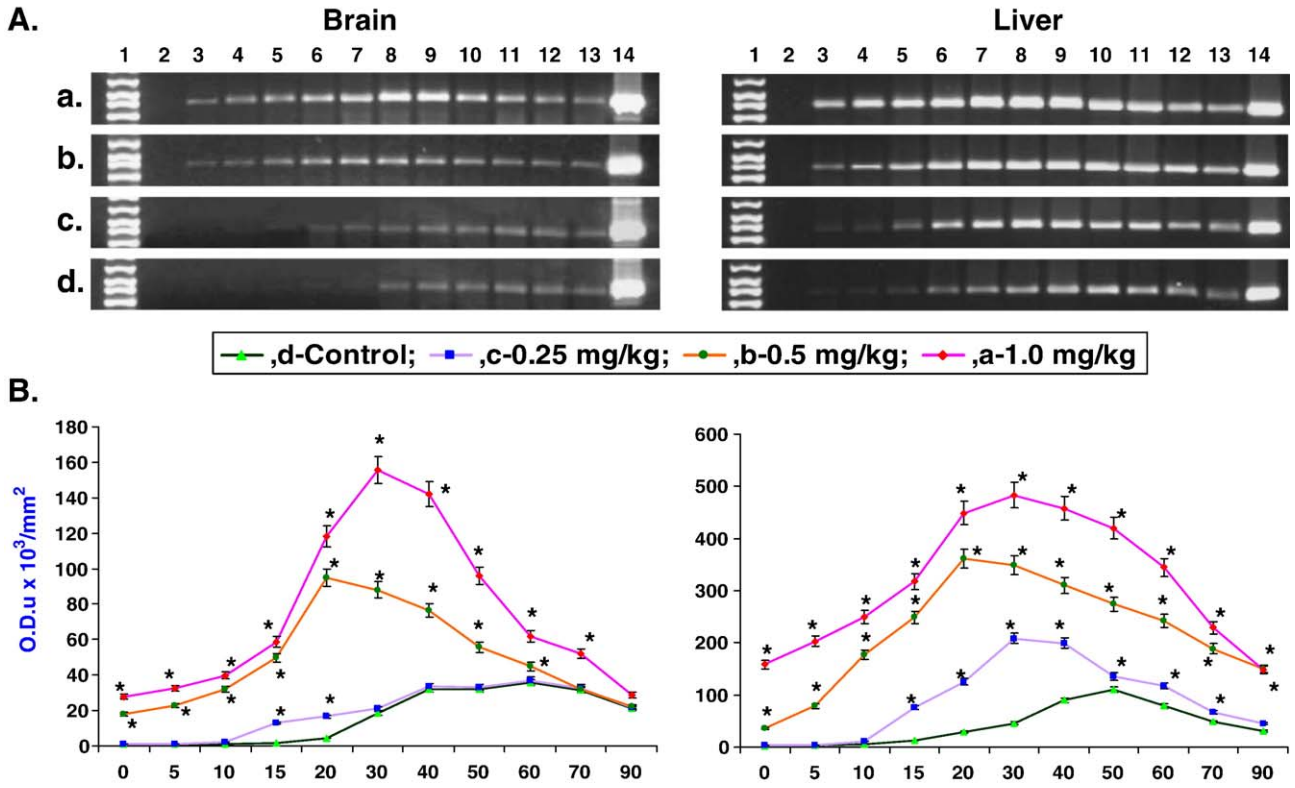

Fig. 3. Effect of prenatal exposure of deltamethrin on brain and liver CYP2B1 mRNA expression in offsprings. (A) Representative ethidium bromide stained agarose gels. Lane 1 contains 1.0 kb plus DNA ladder, lane 2 contains RT-PCR product without RNA. Lanes 3–13 contain 5 μl of RT-PCR product of RNA isolated from brain or liver of 0-, 5-, 10-, 15-, 20-, 30-, 40-, 50-, 60-, 70-, and 90-day-old rat offsprings respectively. Lane 14 contains 5 μl of RT-PCR product of RNA isolated from phenobarbital (PB) pretreated animals. (B) Densitometric analysis of the RT-PCR products of RNA isolated from 3 animals. $*P < 0.05$ when compared with controls.

A dose-dependent increase in CYP2B1 and 2B2 mRNA expression was observed in liver of the offsprings prenatally exposed to different doses of deltamethrin (Figs. 3 and 4). As revealed by densitometric analysis, the increase in the mRNA expression of CYP2B1 and 2B2 continued up to pnd 30, thereafter, the increase in the expression showed a decline, though the increase observed at pnd 90 was found to be significant in the case of CYP2B1 at pnd 90 at the relatively higher doses of 0.5 or 1.0 mg/kg (Figs. 3 and 4).

mRNA expression of CYP2E1 isoform

RT-PCR analysis of the RNA extracted from liver or brain isolated from the offsprings born to control or deltamethrin treated dams revealed that as expected PCR products of correct size (750 bp for CYP2E1) were formed with rat liver CYP2E1 primers (Fig. 5). RT-PCR analysis revealed distinct mRNA expression of CYP2E1 in brain of the control offsprings at birth. Densitometric analysis further indicated an age-dependent increase in the CYP2E1 mRNA expression in brain up to pnd 90. Prenatal exposure to different doses of deltamethrin was found to produce an increase in the CYP2E1 mRNA expression in the brain of the exposed offsprings at the relatively higher doses of 0.5 or 1.0 mg/kg with maximum increase being observed at birth (Fig. 5). The significant increase in the mRNA expression of CYP2E1 in brain of the offsprings was found to persist up to pnd 60, while no significant changes were observed at pnd 70 or above.

As observed with brain, RT-PCR analysis revealed distinct expression of CYP2E1 mRNA in liver of the control offsprings at birth (Fig. 5). Densitometric analysis revealed an increase in the CYP2E1 mRNA expression up to pnd 90. In contrast to the brain, the increase in the CYP2E1 mRNA expression in liver was found to persist up to pnd 90 at the relatively higher doses of 0.5 or 1.0 mg/kg deltamethrin (Fig. 5).

Enzymatic analysis: CYP1A-dependent EROD activity

EROD activity in brain and liver microsomes isolated from the offsprings born to control mothers was found to increase with increasing postnatal age of the offsprings (Fig. 6). Oral administration of different doses (0.25 or 0.5 or 1.0 mg/kg body weight) of deltamethrin to the pregnant rats produced a dose-dependent increase in the CYP1A-dependent EROD activity in the brain microsomes isolated from the offsprings. The increase was found to persist up to pnd 60, with the effect being significant up to pnd 40 at relatively higher doses (0.5 or 1.0 mg/kg body weight) and up to pnd 50 at the highest dose of 1.0 mg/kg body weight (Fig. 6A). Similar increase in the EROD activity was found in the liver microsomes isolated from exposed offsprings, and the magnitude of induction at pnd 90 was less than that observed at early postnatal ages (Fig. 6B).

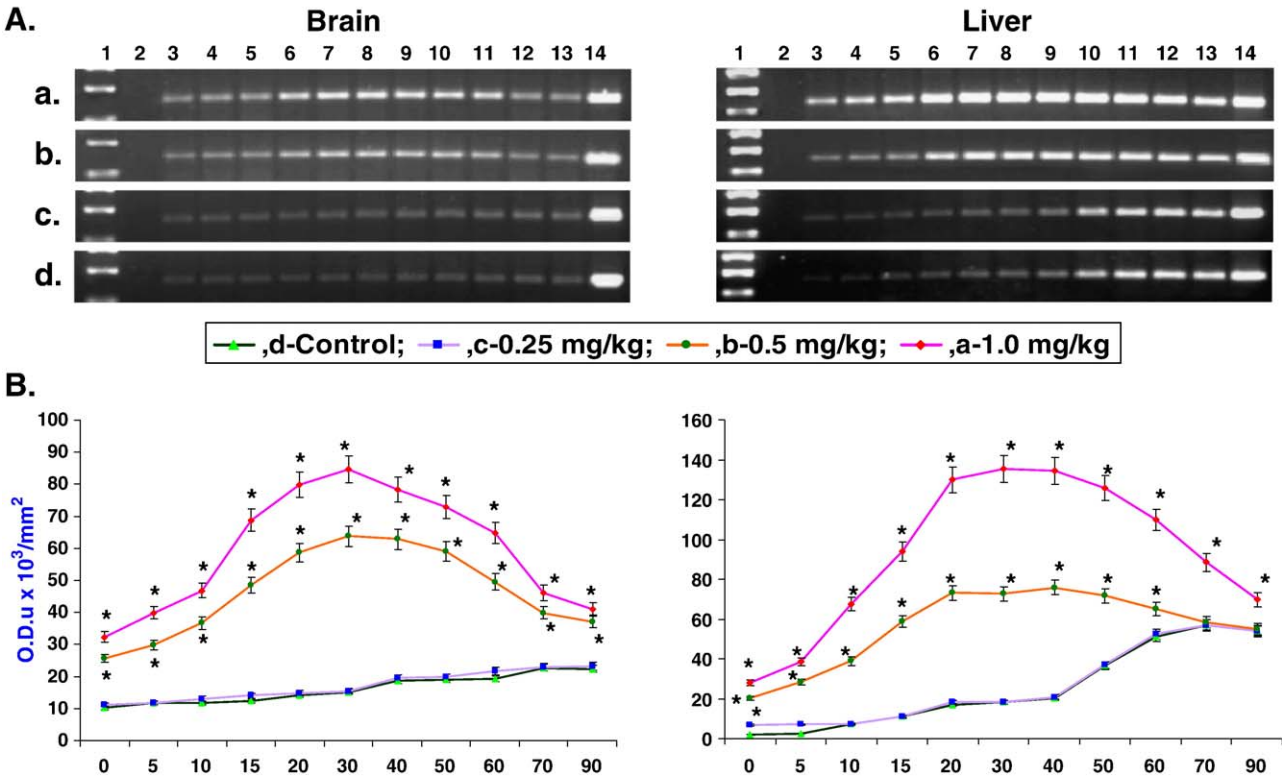

Fig. 4. Effect of prenatal exposure of deltamethrin on brain and liver CYP2B2 mRNA expression in offsprings. (A) Representative ethidium bromide stained agarose gels. Lane 1 contains 1.0 kb plus DNA ladder, lane 2 contains RT-PCR product without RNA. Lanes 3–13 contain 5 μl of RT-PCR product of RNA isolated from brain or liver of 0-, 5-, 10-, 15-, 20-, 30-, 40-, 50-, 60-, 70-, and 90-day-old rat offsprings respectively. Lane 14 contains 5 μl of RT-PCR product of RNA isolated from PB pretreated animals. (B) Densitometric analysis of the RT-PCR products of RNA isolated from 3 animals. *$P < 0.05$ when compared with controls.

Further the increase was significant up to pnd 50 at higher doses (0.5 or 1.0 mg/kg body weight) and up to pnd 90 at the dose of 1.0 mg/kg body weight.

CYP2B-dependent PROD activity

An increase in the CYP2B-dependent PROD activity was observed up to pnd 60 in brain and liver microsomes isolated from the offsprings born to control dams. Thereafter, a decrease was observed in the enzyme activity which continued to decline up to pnd 90 (Fig. 7).

Oral administration of different doses of deltamethrin (0.25 or 0.5 or 1.0 mg/kg body weight) was found to produce a dose-dependent increase in the cerebral PROD activity of the offsprings born to dams exposed to deltamethrin. The increase in PROD activity was found to persist up to pnd 40 in the brain microsomes isolated from offsprings exposed to relatively higher doses of deltamethrin (0.5 or 1.0 mg/kg body weight) however, with the effect being statistically significant only up to pnd 30 at the highest dose of 1.0 mg/kg body weight (Fig. 7A). As seen with brain, PROD activity in liver was found to increase dose dependently. However, in contrast to brain, the effect was found to persist up to pnd 90 in the microsomes prepared from liver of the offsprings prenatally exposed to relatively higher doses of deltamethrin (0.5 or 1.0 mg/kg body weight). The increase in the hepatic PROD activity was found to be statistically significant up to pnd 50 in the offsprings exposed to relatively higher doses of 0.5 or 1.0 mg/kg body weight, while at the highest dose of 1.0 mg/kg body weight, the increase was found to be significant up to pnd 60 (Fig. 7B).

CYP2E-dependent NDMA-d activity

As seen with CYP2E1 mRNA expression (Fig. 5), CYP2E-dependent NDMA-*d* activity was found to increase with increasing postnatal age of the offspring up to pnd 90 in brain and liver microsomes prepared from offsprings born to control mothers (Fig. 8). As observed with EROD and PROD activities, oral administration of different doses (0.25 or 0.5 or 1.0 mg/kg body weight) of deltamethrin to the pregnant rats produced a dose-dependent increase in the CYP2E-dependent NDMA-*d* activity in brain and liver microsomes isolated from exposed offsprings (Fig. 8). The increase in the NDMA-*d* activity was found to be significant up to pnd 30 in the brain microsomes isolated from offsprings exposed to relatively higher doses of deltamethrin (0.5 or 1.0 mg/kg body weight), while at the highest dose of 1.0 mg/kg, the increase persisted and was significant up to pnd 40 (Fig. 8A).

Similar to brain, the dose-dependent increase in NDMA-*d* was found to be significant up to pnd 40 in the liver microsomes isolated from the offsprings born to dams exposed to relatively higher doses of deltamethrin (0.5 or 1.0 mg/kg body weight), while at the highest dose (1.0 mg/kg), the

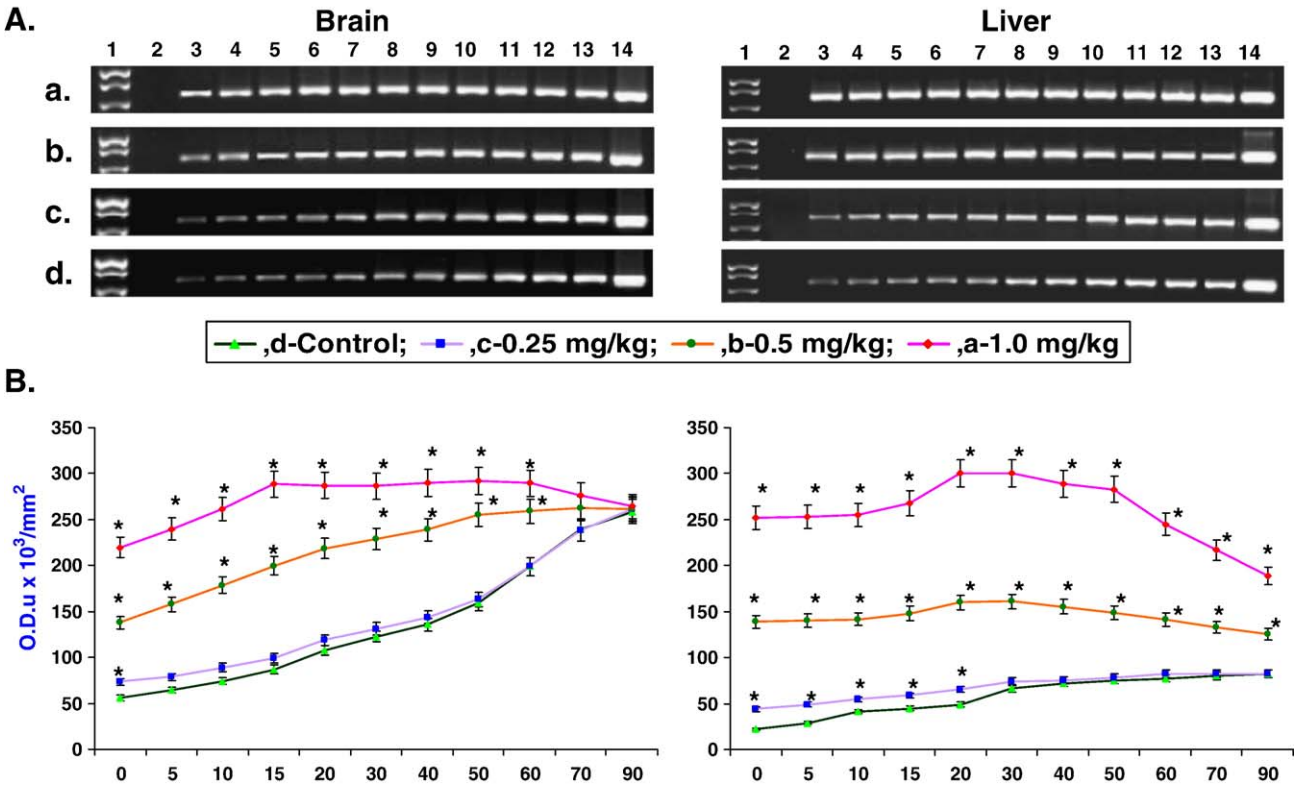

Fig. 5. Effect of prenatal exposure of deltamethrin on brain and liver CYP2E1 mRNA expression in offsprings. (A) Representative ethidium bromide stained agarose gels. Lane 1 contains 1.0 kb plus DNA ladder, lane 2 contains RT-PCR product without RNA. Lanes 3–13 contain 5 μl of RT-PCR product of RNA isolated from brain or liver of 0-, 5-, 10-, 15-, 20-, 30-, 40-, 50-, 60-, 70-, and 90-day-old rat offsprings respectively. Lane 14 contains 5 μl of RT-PCR product of RNA isolated from ethanol pretreated animals. (B) Densitometric analysis of the RT-PCR products of RNA isolated from 3 animals. *$P < 0.05$ when compared with controls.

increase in the hepatic NDMA-*d* activity persisted up to pnd 50 (Fig. 8B).

Discussion

The present study has demonstrated similarities in the postnatal development of xenobiotic metabolizing CYPs in rat brain with the liver, though the levels of mRNA expression of CYP1A, 2B and 2E1 isoenzymes and their catalytic activity were several fold less in brain. As observed with very low mRNA expression in the liver (Hines and McCarver, 2002; Johnsrud et al., 2003; Choudhary et al., 2004), barely detectable or almost negligible mRNA expression of CYP 1A1 and 2B1, the inducible CYP isoforms were observed in the brain at birth or during early neonatal period, which gradually increased with the postnatal development. Similarly, as seen in the liver (Hines and McCarver, 2002; Johnsrud et al., 2003; Choudhary et al., 2004), detectable levels of mRNA of CYP 1A2, 2B2, and 2E1, the constitutive CYPs were observed at birth, and the levels were found to increase with the postnatal development. The early onset of 1A2, 2B2, and 2E1 in brain has suggested that the constitutive expression of these CYPs may be associated with endogenous functions in the brain (Ball and Knuppen, 1978; Sugira et al., 1987; Warner and Gustafsson, 1995; Abu-Abed et al., 2001; Nissbrandt et al., 2001; Shibuya et al., 2003). A dose-dependent increase in the expression of xenobiotic metabolizing CYP isoforms in brain and liver of offsprings following prenatal exposure of low doses of deltamethrin have demonstrated that placental transfer of the pesticide even at these low doses may be sufficient to induce the mRNA expression of CYPs and their catalytic activity in brain and liver of the offsprings. Though the levels of deltamethrin have not been identified, placental transfer of certain pyrethroids to the fetus has been reported earlier (Kaneko et al., 1984; Shiba et al., 1990). However, given the low doses of deltamethrin used in the present study and absence of any visual symptoms of neurotoxicity, associated with accumulation of deltamethrin (Ray and Cremer, 1979; Rickard and Brodie, 1985; Dayal et al., 2003), in the offsprings, suggests that the effects on brain and liver CYPs in the offsprings could be because of the sensitivity of the fetal CYPs during gestation. Interestingly, though the levels of CYPs were several fold lower in brain when compared to the liver, almost equal magnitude of induction in these CYPs in brain have suggested that like in the liver, brain CYPs are responsive to the induction by environmental chemicals, and that the increase is transcriptionally regulated.

The persistence of the increase in mRNA expression of CYPs even up to the adulthood following exposure to low doses of deltamethrin could be correlated with the earlier studies demonstrating imprinting of the CYPs following exposure to low doses of CYP inducers such as PB during early neonatal period (Agrawal and Shapiro, 1996, 2000). It has been shown

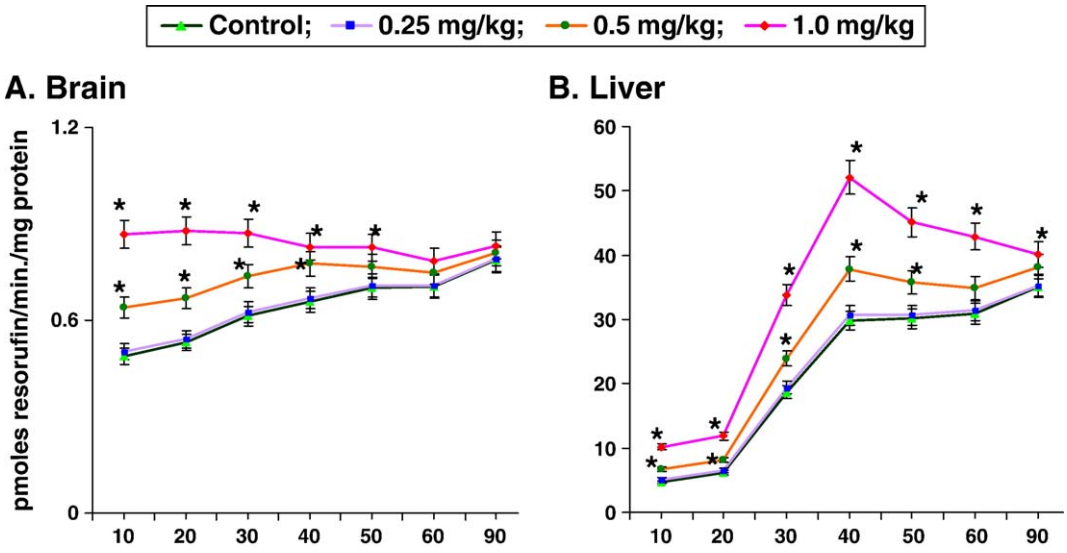

Fig. 6. Effect of prenatal exposure of deltamethrin on EROD activity in brain and liver of offsprings.

that neonatally administered PB results in a silent programming defect leading to a permanent over expression (2-fold) of hepatic CYP2B1 and 2B2 mRNAs (Agrawal and Shapiro, 1996, 2000). This imprinted overinduction of the CYP isoforms has been attributed to the perturbation of the normal endocrine homeostasis (Agrawal et al., 1995). As the pyrethroids have been shown to possess estrogenic activities (Go et al., 1999; Chen et al., 2002; Kim et al., 2004), deltamethrin, as a result of placental transfer, may be disturbing the endocrine environment leading to the persistent increase in the CYPs observed in the brain and liver of the offsprings.

The effects of deltamethrin on the ontogeny of CYPs could also be correlated with its neurochemical and neurodevelopmental effects as these CYPs have been found to be associated with several of the physiological functions of the brain. The alterations in the postnatal development of CYP2E1 could be associated with the specificity of the pyrethroids to alter dopaminergic neurotransmission, known to be functionally linked to CYP2E1 in brain (Nissbrandt et al., 2001; Vaglini et al., 2004). Likewise, the effect of deltamethrin on the postnatal induction of CYP1A1 could be linked to the effect of pyrethroids on the central catecholaminergic system, which has been shown to be involved in the regulation of CYP1A1 induction (Konstandi et al., 2005; Shafer et al., 2005). Similarly, the alterations in the postnatal development of CYP2B1 and 2B2, known to be involved in the metabolism and inactivation of gonadal hormones which determine neuronal differentiation during ontogenesis, suggests the possible influence of deltamethrin on internal regulatory and metabolic pathways in the brain (Rosenbrock et al., 1999; McEwen, 1981). Agundez et al. (1998) have shown that the two indoleamines, serotonin, and tryptamine modulate the activity of CYP1A2 further, suggesting that brain CYPs are susceptible to local regulatory mechanisms.

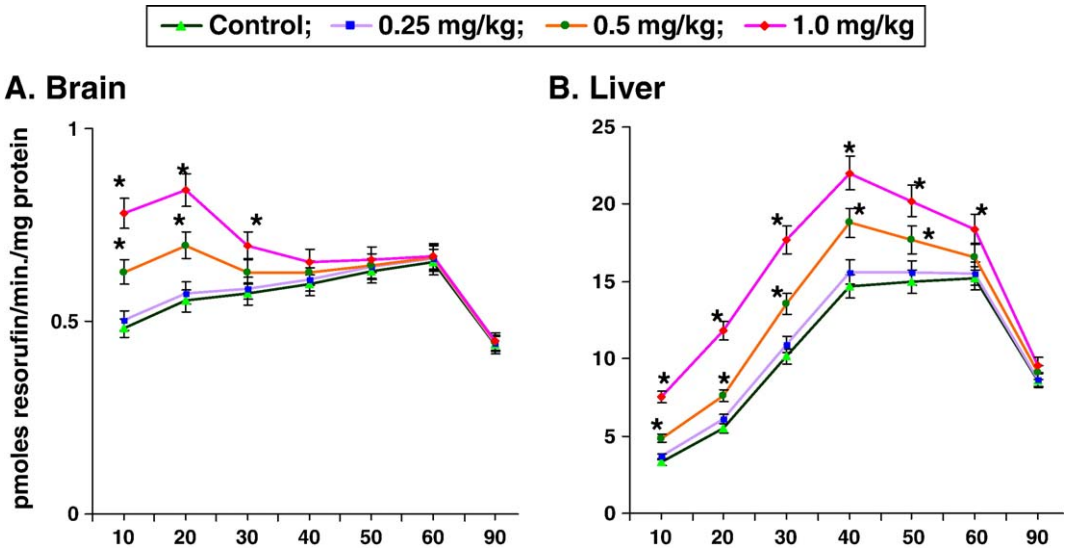

Fig. 7. Effect of prenatal exposure of deltamethrin on PROD activity in brain and liver of offsprings.

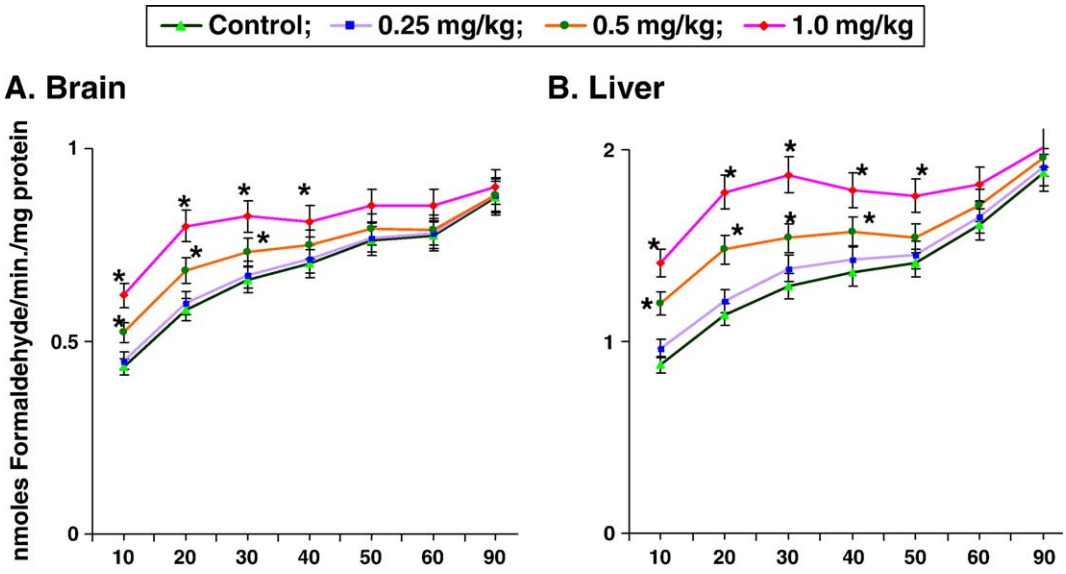

Fig. 8. Effect of prenatal exposure of deltamethrin on NDMA-*d* activity in brain and liver of offsprings.

The alterations in the postnatal development of xenobiotic metabolizing CYPs could also be of significance as these CYPs have a role in regulating growth, morphogenesis, and homeostasis (Nebert, 1991; Choudhary et al., 2004). There are numerous reports indicating that an induced form of CYP metabolizes effectors of growth and differentiation. Dioxin or PAH treatment, associated with increased CYP1A and 1B levels, has been shown to cause decreases in the estrogenic response in cell culture (Spink et al., 1990) and the rats (Astroff and Safe, 1990). Likewise cigarette smoking, known to induce CYP1A isoenzymes, was found to clinically lower the urinary estrogen levels (MacMahon et al., 1982). Choudhary et al. (2004) have further suggested that the presence of xenobiotics metabolizing CYPs in developing conceptus along with the other anabolic CYP isofoms, known to produce molecules with specific physiological functions such as cholesterol, steroids, fatty acids etc., could act as molecule altering enzymes producing and eliminating ligands associated with nuclear receptor activities.

In conclusion, the present study has shown similarities in the postnatal development of cerebral xenobiotic metabolizing CYPs with the liver enzymes. Even though brain exhibit several fold lower mRNA expression and catalytic activity of CYPs when compared with the liver, almost similar magnitude of induction was observed in the brain CYPs, indicating that fetal brain CYPs are responsive to environmental chemicals and are transcriptionally regulated. The dose-dependent effect of deltamethrin on the postnatal development of xenobiotics metabolizing CYPs have further demonstrated that placental transfer of the pesticide even at the low doses used in present study may be sufficient to induce the CYPs in brain and liver of the offsprings. The data indicating persistence in the increase in the mRNA expression of the xenobiotic metabolizing CYPs even up to adulthood are of significance in view of the earlier studies indicating the involvement of these isoenzymes in the neurobehavioral toxicity of deltamethrin (Dayal et al., 2003).

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