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Effects of cypermethrin on monoamine transporters, xenobiotic metabolizing enzymes and lipid peroxidation in the rat nigrostriatal system

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Abstract

Long-term exposure to cypermethrin induces the nigrostriatal dopaminergic neurodegeneration in adult rats and its pre-exposure in the critical periods of brain development enhances the susceptibility during adulthood. Monoamine transporters, xenobiotic metabolizing enzymes and oxidative stress play critical roles in the nigrostriatal dopaminergic neurodegeneration. The study was undertaken to investigate the effects of cypermethrin on DAT, VMAT 2, CYP2E1, GST Ya, GST Yc and GSTA4-4 expressions, CYP2E1 and GST activities and lipid peroxidation in the nigrostriatal system of adult rats with/without post-natal exposure to cypermethrin. Cypermethrin reduced VMAT 2 and increased CYP2E1 expressions without causing significant change in DAT. Although GSTA4-4 mRNA expression and lipid peroxidation were increased, no significant changes were observed in GST Ya and GST Yc expressions and total GST activity. The results obtained demonstrate that long-term exposure to cypermethrin modulates VMAT 2, CYP2E1, GSTA4-4 expressions and lipid peroxidation, which could contribute to the nigrostriatal dopaminergic neurodegeneration.

Keywords: *Cypermethrin, dopamine transporter, vesicular monoamine transporter 2, oxidative stress, lipid peroxidation, Parkinson's disease*

Introduction

Dopaminergic neurodegeneration of the nigrostriatal pathway is one of the critical anatomical features of sporadic and chemically-induced Parkinson's disease (PD) [1–4]. Environmental exposures to pesticides and heavy metals are implicated in the aetiology of PD both in humans and experimental animals in addition to age and genetic factors [3,5–10]. Cypermethrin, a class II pyrethroid pesticide, enters the brain from blood through the blood–brain barrier, possibly owing to its hydrophobic nature and small molecular size. Cypermethrin crosses the biological membrane, reaches the nucleus and leads to DNA damage [11]. Although class II pyrethroids are metabolized by cytochrome P-450s (CYPs) in the brain, their metabolites produce more pronounced neurotoxicity than that of the parent compounds [12]. On the other hand, clearance of class II pyrethroids and their metabolites from the brain is limited due to interference of the blood–brain barrier [13,14]. Cypermethrin induces neurotoxicity in multiple ways, including the inhibition of calcium uptake and sustained opening of the Na^+-channels [15–19]. Cypermethrin induces free radical generation and oxidative stress, which may lead to loss of coordination, muscle tremor, convulsion, salivation and neuronal degeneration [20–26].

Dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT 2) regulate dopamine level in the nigrostriatal dopaminergic neurons [27–31]. DAT mediates the removal of dopamine from the synapse to the intracellular space for recycling and metabolism.

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VMAT 2, on the other hand, transports cytoplasmic dopamine into vesicles for storage and release and protects it from oxidation [27–31]. DAT or VMAT 2 dysfunctions or their abnormal expressions lead to dopamine oxidation, thereby free radical generation, one of the major causes of the nigrostriatal neurodegeneration [32,33].

CYP2E1 and glutathione-S-transferases (GSTs), including GSTA4-4, are implicated in sporadic and chemically-induced nigrostriatal dopaminergic neurodegeneration [7,34]. CYP2E1 participates in the generation of free radicals, which bind to macromolecules and further enhance lipid peroxidation [34–39]. Alteration in GSTA4-4 in various chemically-induced PD models could be an adaptive mechanism to encounter lipid peroxides and affect their subsequent detoxification [7,34,40].

Long-term exposure to cypermethrin induces dopaminergic neurodegeneration in adult rats and its post-natal pre-exposure enhances the susceptibility during adulthood [41]. Cypermethrin-mediated modulation of dopamine regulating genes and their contribution to short-term acute toxicity are known [42,43]; however, its effect on long-term exposure with or without post-natal pre-exposure leading to dopaminergic neurodegeneration have not yet been investigated. Similarly, the roles of CYP2E1, GSTA4-4, GSTYa and GSTYc in cypermethrin-induced nigrostriatal dopaminergic neurodegeneration have not yet been investigated. The present study was therefore undertaken to investigate the effects of cypermethrin on DAT, VMAT 2, CYP2E1, GSTA4-4, GSTYa, GSTYc and lipid peroxidation in the rat nigrostriatal system.

Materials and methods

Chemicals

Acrylamide, ammonium persulphate (APS), 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) alkaline phosphatase substrate solution chromogen, bromophenol blue, chloroform, cypermethrin, diethyl pyrocarbonate (DEPC), DL-dithiothreitol (DTT), ethylene-diamine-tetra-acetic acid (EDTA), formamide, glycine, 3-hydroxytyramine hydrochloride (dopamine), isopropanol, N,N'-methylene bisacrylamide, 3-(N-morpholino) propanesulphonic acid (MOPS), 4-nitrocatechol, p-nitrophenol, polyvinylidene difluoride (PVDF) membrane, sodium acetate, sodium dodecyl sulphate (SDS), sucrose, N,N,N',N'-tetramethyl-ethylene-diamine (TEMED), thio-barbituric acid (TBA), TRI reagent, tris-base and antibody for β-actin were purchased from Sigma-Aldrich (St. Louis, MO). The antibodies for DAT and VMAT 2 were procured from Santa Cruz Biotechnology (Santa Cruz, CA). The antibody for GSTA4-4 was procured from Abnova Corporation (Taipei city, Taiwan) and antibody for CYP2E1 was purchased from Millipore (Temecula, CA). Deoxy-NTPs, Taq polymerase and reverse transcription-polymerase chain reaction (RT-PCR) kits were purchased from Fermentas Inc. (Glen Burnie, MD). Forward and reverse primers for β-actin, DAT, VMAT 2, GSTA4-4, GSTYa, GSTYc and CYP2E1 genes were procured from Hysel India Pvt. Ltd. (New Delhi, India). Perchloric acid and sodium hydroxide (NaOH) were purchased from Ranbaxy Pvt. Ltd. (New Delhi, India), Other chemicals, such as agarose, ethanol, methanol, dimethyl sulphoxide (DMSO), heptane sulphonic acid, nitric acid, 1-chloro-2, 4-dinitrobenzene (CDNB), hydrogen peroxide, nicotinamide adenine dinucleotide phosphate (NADPH), di-sodium hydrogen phosphate (Na_2HPO4), sodium di-hydrogen phosphate (NaH_2PO4), potassium chloride, acetic acid, sodium pyrophosphate, bovine serum albumin (BSA), glycerol, etc. were procured locally either from Bangalore Genei (Bangalore, India), Qualigens (Mumbai, India) or Sisco Research Laboratories (SRL) (Mumbai, India).

Animal treatment

Wistar female pregnant rats (170–180 g) were obtained from the animal colony of Indian Institute of Toxicology Research (IITR), Lucknow. The animals were maintained in the animal house at IITR. The Institutional ethics committee for use of laboratory animals approved the study. The animals were maintained in standard conditions of temperature and humidity (temperature: 22°C ± 2°C; humidity: 45–55%; light intensity: 300–400 lx) and given proper pellet diet and water *ad libitum*. Females were reared up to the delivery of pups. Four post-natal treatments were given from day 5–19 intraperitoneally, twice a week, with or without cypermethrin (1.5 mg/kg), along with respective controls. Male pups were allowed to grow up for a further 2 months without any exposure and under normal conditions. The male animals were re-challenged with cypermethrin (15 mg/kg), twice a week, for 4, 8 and 12 weeks, along with respective controls that were treated intraperitoneally with corn oil (vehicle). The animals were sacrificed via cervical dislocation; brain was taken out and kept in liquid nitrogen for the study.

Measurement of dopamine content in the striatum

The dopamine content was measured in controls and all treated groups, as described previously [41]. The values are expressed in ng/mg of tissue.

RT-PCR analysis

Total RNA was extracted from the nigrostriatal tissue using Trizol reagent following manufacturer's instructions. Revert aid^{TM} minus MuLV reverse transcriptase was used to synthesize cDNA from total RNA. Primers

for DAT, VMAT 2, CYP2E1, GSTA4-4, GST Ya, GST Yc and β-actin were synthesized, as reported elsewhere [44–47]. PCR products were visualized in 1.5% agarose gel using ethidium bromide (10 mg/ml) under UV light. Images were captured and the band density was calculated by a computerized densitometry system (Alpha Imager System, Alpha Innotech Corporation, South Africa). Band densities for DAT, VMAT 2, CYP2E1, GSTA4-4, GST Ya and GST Yc were normalized to β-actin.

Western blot analysis

The nigrostriatal tissue was homogenized, sonicated and the homogenate was centrifuged at 2000 × g for 5 min at 4°C. The pellet was dissolved and re-centrifuged at 30 000 × g for 30 min at 4°C. The supernatant was collected; protein content was measured and protein (100 µg) was subjected to 10% polyacrylamide gel electrophoresis. Samples were electrophoretically transferred on to a PVDF membrane. Non-specific sites were blocked in 5% non-fat dry milk in tris-buffered saline (135 mM NaCl, 2.5 mM KCl, 50 mM Tris and 0.1% Tween 20, pH 7.4). Membranes were incubated either with goat monoclonal antibody for DAT, rabbit-polyclonal antibody for VMAT 2, rabbit-polyclonal antibody for GSTA4-4 or rabbit-polyclonal antibody for CYP2E1 in tris-buffered saline (TBS). Specific DAT/VMAT 2/GSTA4-4 bindings were detected using anti-rabbit/anti-goat alkaline phosphatase conjugated secondary antibody and BCIP/NBT as substrate. The western blotting for CYP2E1 was done with microsomal protein and developed by enhanced chemiluminescence. Images were captured and the band density was calculated by computerized densitometry system. Band densities of DAT, VMAT 2, GSTA4-4 and CYP2E1 were normalized to β-actin.

CYP 2E1 activity

Brain was perfused with normal saline, microsomes were prepared and CYP2E1 enzyme activity was determined according to the method described elsewhere [48,49]. In brief, the reaction mixture containing 4-nitrophenol (0.2 mM) was mixed with tris–HCl (50 mM; pH 7.4) and $MgCl_2$ (25 mM). Microsomal protein (200–250 µg) was added to the mixture and incubated at 37°C for 5 min. The reaction was initiated by the addition of NADPH (50 mM) and was further incubated for 10 min. Perchloric acid (500 µl) was added to stop the reaction. The supernatant was obtained following centrifugation at 825 × g for 20 min and sodium hydroxide (100 µl of 10 N) was added to the reaction mixture. The absorbance was recorded at 510 nm and activity was calculated in terms of nM/min/mg protein.

Total GST activity

GST activity was determined in the homogenate (10% w/v) of the nigrostriatal tissues according to literature reported protocol [40]. The reaction mixture containing phosphate buffer (0.2 M, pH 6.5), glutathione (9 mM), CDNB (150 mM) and protein (100 µg) was used for the assay. The enzymatic activity was determined by measuring an increase in absorbance at 340 nm for 3 min and calculated in nM/min/mg protein.

Lipid peroxidation

Lipid peroxidation was assayed in the nigrostriatal tissues, as described previously [50]. In brief, homogenate (10% w/v) was mixed with SDS (10% w/v) and incubated for 5 min followed by the addition of glacial acetic acid (20% v/v). The mixture was incubated for 2 min; TBA (0.8% w/v) was added and kept on a boiling water bath for 1 h. The content was centrifuged at 10 000 × g for 5 min at 4°C. The absorbance of the supernatant was recorded at 532 nm against the control blank. The values are expressed as TBA reactive substances (TBARS).

Statistical analysis

Two-way analysis of variance (ANOVA) with Bonferroni post-test was used for comparisons involving multiple groups. The data are expressed as means ± standard error of means (SEM). The differences were considered statistically significant when p-value was less than 0.05.

Results

Measurement of dopamine content in the striatum

Cypermethrin reduced dopamine content in the adult animals in a time of exposure dependent manner. The adult animals already exposed to cypermethrin during the critical periods of development exhibited more pronounced reduction. The dopamine level in 4 weeks controls was 10.43 ± 0.71, in adulthood alone treated rats was 9.80 ± 0.48 and in post-natal as well as adulthood treated animals was 7.77 ± 0.44 ($^{*}p < 0.05$) ng/mg of tissues ($n = 3$–5). Similarly, the dopamine values for 8 and 12 weeks control animals were 10.60 ± 0.61 and 10.16 ± 0.46, 8 and 12 weeks adulthood alone treated animals were 8.11 ± 0.41 ($^{**}p < 0.01$ as compared with control) and 6.22 ± 0.51 ($^{***}p < 0.001$ as compared with control) and for 8 and 12 weeks post-natal as well as adulthood treated animals were 6.90 ± 0.31 ($^{***}p < 0.001$ as compared with control) and 4.75 ± 0.30 ng/mg of tissues ($^{***}p < 0.001$ as compared with control and $^{\#}p < 0.05$ as compared with adulthood alone), respectively ($n = 3$–5).

DAT expression

Cypermethrin treatment did not alter the expression of DAT both at mRNA and protein levels (data not shown). Similarly, no significant change was observed in DAT mRNA and protein expressions in animals treated during post-natal periods as well as adulthood as compared with adulthood alone (data not shown).

VMAT 2 expression

Cypermethrin treatment significantly reduced the expressions of VMAT 2 mRNA and protein (Figures 1A–D). More pronounced reduction was observed in VMAT 2 mRNA and protein expressions in animals exposed to cypermethrin during post-natal periods and adulthood as compared with adulthood alone (Figures 1A–D).

CYP2E1 expression and catalytic activity

Cypermethrin treatment significantly increased the CYP2E1 mRNA and protein expressions as well as catalytic activity (Figures 2A–D). A more pronounced increase in CYP2E1 mRNA and protein expressions and catalytic activity was noted in animals exposed to cypermethrin during post-natal periods and adulthood as compared with adulthood alone (Figures 2A–D).

GSTA4-4, GSTYa and GSTYc mRNA expressions, GSTA4-4 protein expression and GST activity

Cypermethrin treatment significantly increased the GSTA4-4 mRNA and protein expressions without any significant change in total GST activity (Figures 3A–D). No significant change in GSTYa and GSTYc mRNA expressions (Figure 3F–I) was observed in adults as well as post-natal and adult cypermethrin treated animals. More pronounced increase in GSTA4-4 mRNA and protein expressions without any significant alteration in GST catalytic activity was noted in animals exposed to cypermethrin during post-natal days as well as adulthood as compared with adulthood alone (Figures 3A–E).

Lipid peroxidation

Lipid peroxidation was increased significantly in cypermethrin-treated animals as compared with controls (Figure 4). Lipid peroxidation activity was more pronounced in post-natal cypermethrin-treated and adulthood re-challenged animals.

Discussion

The dose, route, schedule and time of treatments to the experimental animals were used for assessing the contribution of DAT, VMAT 2, CYP2E1, GST and lipid peroxidation in the nigrostriatal dopaminergic neurodegeneration, as described previously [41]. Although the degeneration of the nigrostriatal dopaminergic neurons is reported, the dopamine level was measured, which was reduced significantly in the cypermethrin-treated rats, as reported previously [41].

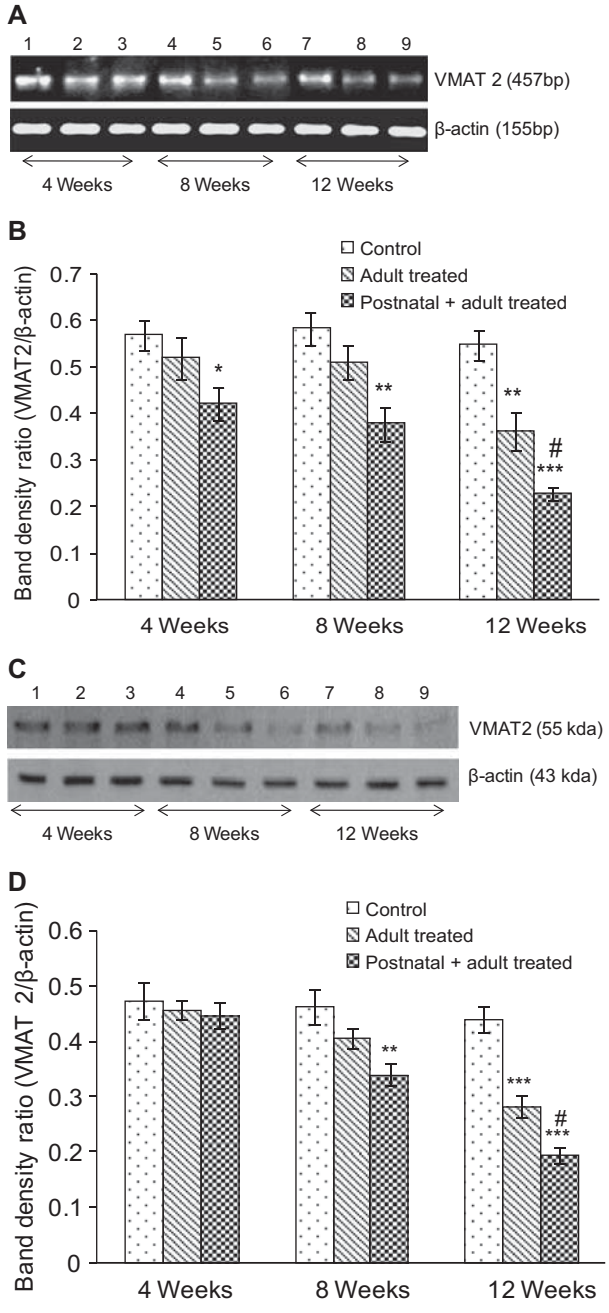

Figure 1. Effect of cypermethrin on VMAT 2 expression in the nigrostriatal tissue of control and treated rats. Agarose gel electrophoretogram of VMAT 2 mRNA is shown in (A) and band density ratio in (B). Similarly, protein expression pattern is shown in (C) and band density ratio in (D). Lanes 1, 4 and 7 represent control; 2, 5 and 8 represent adulthood treated and 3, 6 and 9 represent post-natal + adulthood treated animals. The data are expressed as means ± SEM (n = 3–5). Significant changes are expressed as $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared with control and $^{\#}p < 0.05$ in comparison with adulthood alone treated animals.

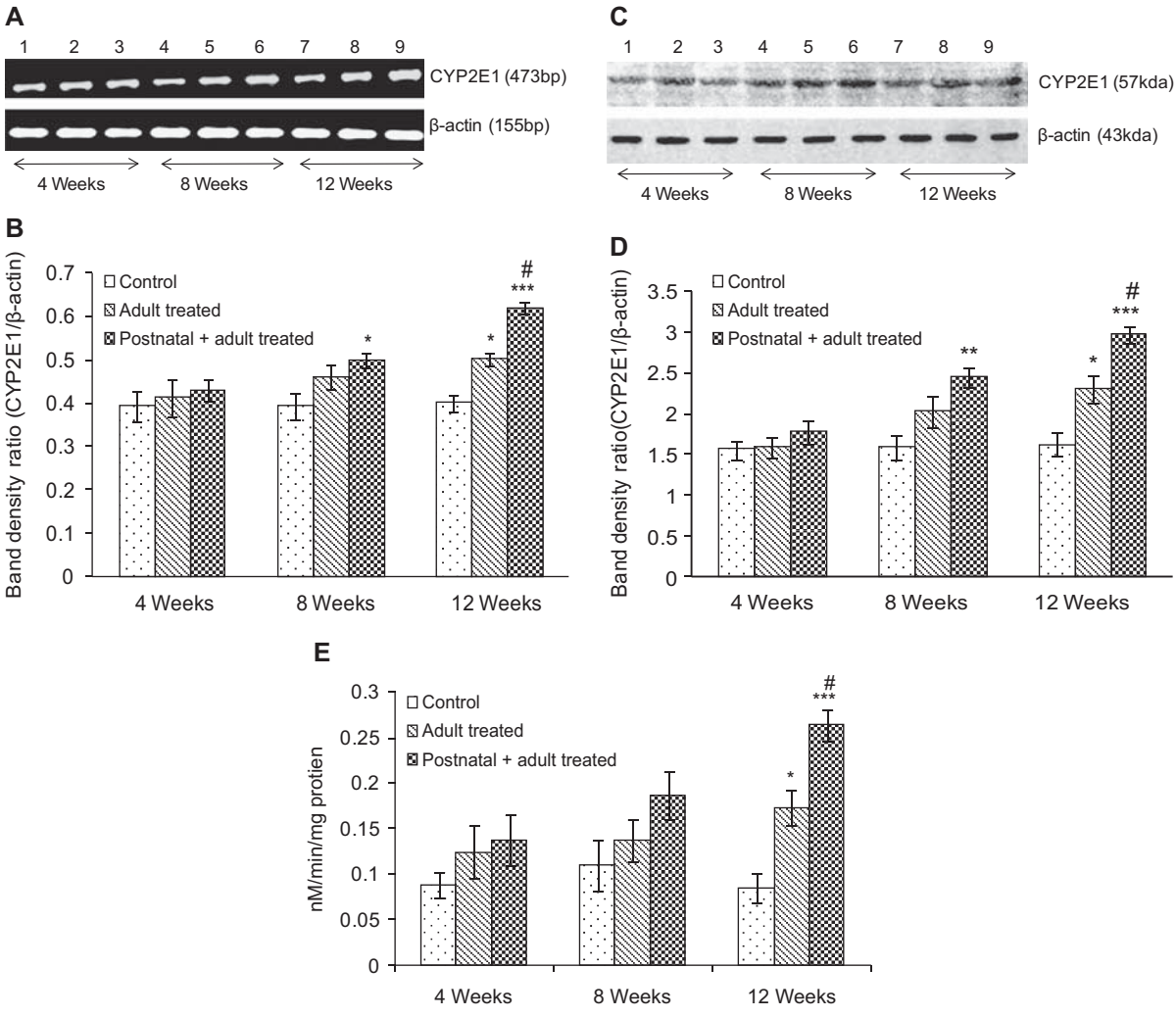

Figure 2. Effect of cypermethrin on CYP2E1 in the nigrostriatal tissue of control and treated rats. CYP2E1 mRNA expression pattern is shown in (A) and band density ratio in (B). Similarly, protein expression pattern is shown in (C), band density ratio in (D) and CYP2E1 catalytic activity in (E). Lanes 1, 4 and 7 represent control; 2, 5 and 8 represent adulthood treated and 3, 6 and 9 represent post-natal + adulthood treated animals. The data are expressed as means ± SEM (n = 3–5). Significant changes are expressed as $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared with control; $^{\#}p < 0.05$ in comparison with adulthood alone treated animals.

Cypermethrin crosses the blood–brain barrier without specific active machinery and is known to exert neurotoxicity [51]. DAT and VMAT 2 are known to regulate the level of dopamine and any change in their level could act as an indicator of neurotoxicity of dopaminergic neurons [27–33]. No significant change in DAT and reduced level of VMAT 2 were observed in the present study that showed a higher DAT/VMAT 2 ratio. The ratio of DAT and VMAT 2 has been used to predict the likelihood of the nigrostriatal dopaminergic neurodegeneration, as a higher ratio of DAT/VMAT 2 is observed in the terminals of dopaminergic neurons in pesticide-induced PD phenotype [51]. DAT expression was slightly increased, but was not statistically significant in cypermethrin-treated rats as compared with controls. This is in accordance with previous observations with pesticides-mediated neurodegeneration showing variable DAT expression in paraquat alone treated rat and maneb and paraquat co-treated mouse nigrostriatal tissues [7,52]. This could be because of differences in their entry in the brain directly or through transporters, metabolic fate and further transport in the nigrostriatal tissues. The decreased VMAT 2 level directly reflects loss of the ability of dopaminergic neurons to protect against cypermethrin-induced neurotoxicity after prolonged exposure, as VMAT 2 is well known to protect dopaminergic neurons through vesicular sequestration of toxic metabolites [53]. The reduced VMAT 2 is not an unusual phenomenon, as it is involved in the transportation of dopamine into vesicles for storage, release and to protect from autooxidation [27–31]. The reduced VMAT 2 could also be explained by the fact that cypermethrin reduced the level of dopamine [41].

The role of oxidative stress in the nigrostriatal dopaminergic neurodegeneration using various model systems is well known [3,4,9]. The increased levels of CYP2E1 and GSTA4-4 observed in the study are in

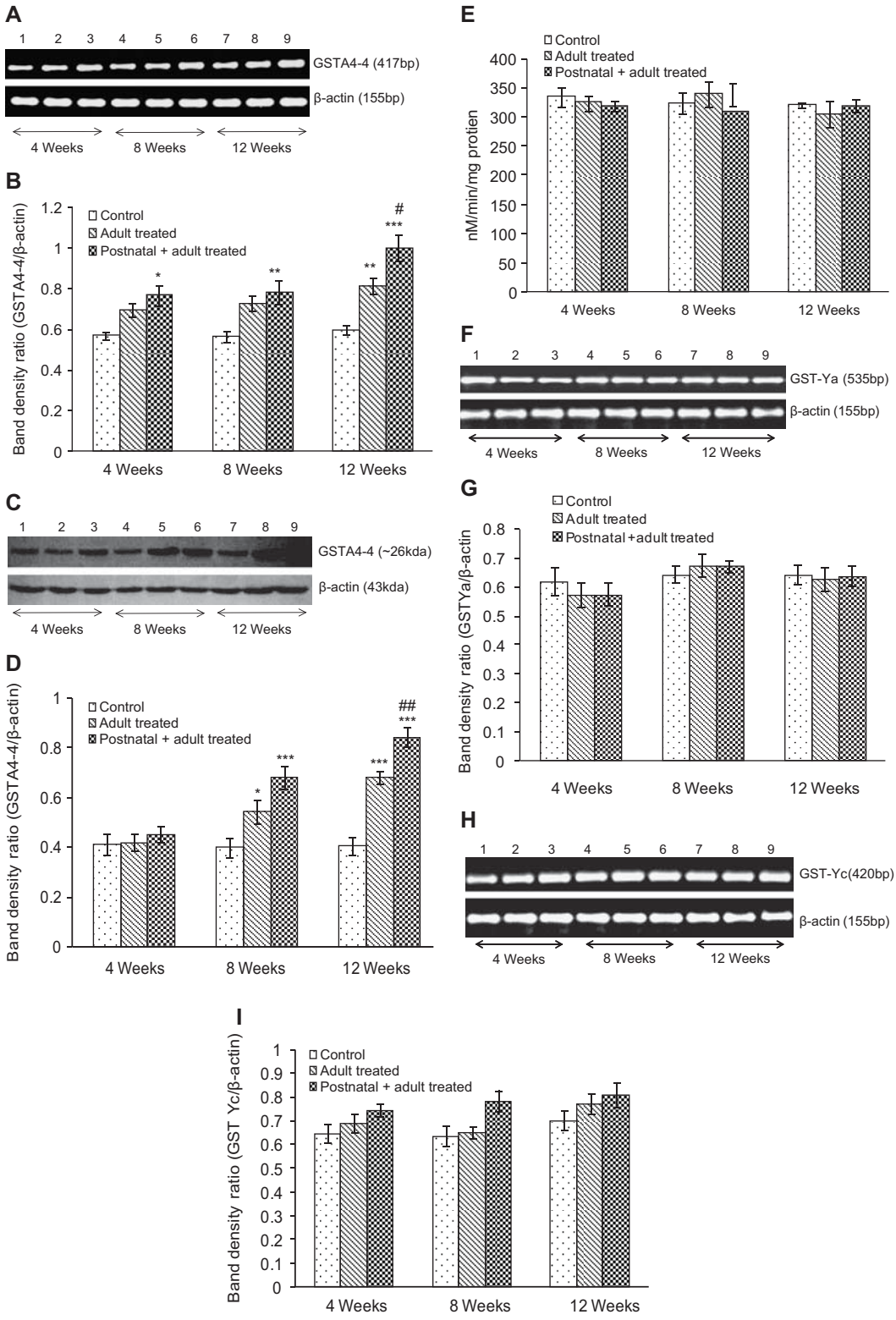

Figure 3. Effects of cypermethrin on GSTA4-4, GST-Ya and GST-Yc expressions and GST catalytic activity in the nigrostriatal tissue of control and treated rats. The GSTA4-4 mRNA expression pattern, band density ratio, GST A4-4 protein expression and band density ratio are shown, respectively, in (A–D). Total GST activity is shown in (E); GST-Ya mRNA expression and band density ratio are shown in (F) and (G) and GST-Yc mRNA expression and band density ratio are shown in (H) and (I). Lanes 1, 4 and 7 represent control; 2, 5 and 8 represent adulthood-treated and 3, 6 and 9 represent post-natal + adulthood-treated animals. The data are expressed as means ± SEM (n = 3–5). Significant changes are expressed as $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared with control and $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ as compared with adulthood alone treated animals.

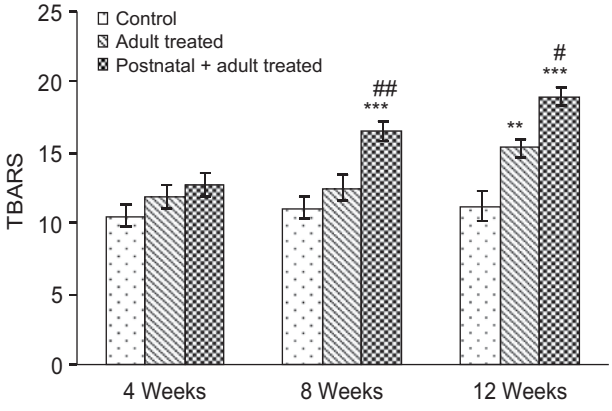

Figure 4. Effect of cypermethrin on TBARS in the nigrostriatal tissue of control and treated rats. The data are expressed as means ± SEM (n = 3–5 separate experiments; 3–5 rats per experimental group). Significant changes are expressed as $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared with control and $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ as compared with adulthood alone treated animals.

accordance with previously reported observations in environmental chemicals-induced nigrostriatal dopaminergic neurodegeneration [7,34]. The increased CYP2E1 could be one of the contributors of the nigrostriatal dopaminergic neurodegeneration, as it is known to generate free radicals owing to its mixed function oxidase activity and to enhance lipid peroxidation [34–39]. The increase level of CYP2E1 could be associated with the specificity of the pyrethroids to alter dopaminergic neurotransmission, which is functionally linked to brain CYP2E1 [54,55]. The observed differences in significance levels between mRNA expressions and protein blots of a few genes at some time points could be due to the fact that RT-PCR, which measured mRNA expression in this study, is a semi-quantitative tool even under the linear range.

GSTA4-4 is a phase II enzyme that is involved in the detoxification of the products of the phase I reactions [40]. An increased level of GSTA4-4 in cypermethrin-treated animals could be a compensatory response to overcome the toxic reactions, as reported in various chemically-induced PD models [7,34,40]. It may be further supported by the fact that GSTA4-4 has high affinity for the lipid peroxidation products and cypermethrin exposure induces lipid peroxidation [56]. Statistically significant change in GST A4-4 expression and lack of significant changes in GST-Ya and GST-Yc expressions along with GST activity in cypermethrin-treated animals showed the importance of GSTA4-4 among the studied isoforms in the cypermethrin-induced nigrostriatal dopaminergic neurodegeneration. The lack of alteration in GST level is supported by the previous observation, which has shown no significant change in GST following maneb or paraquat alone exposures in the mice striatal tissues [34]. More pronounced changes in CYP2E1 and GSTA4-4 levels in animals treated both during post-natal days as well as during adulthood as compared with adulthood only are supported by the previous observations that post-natal cypermethrin exposure enhances the susceptibility during adulthood [41]. The increased level of toxicant responsive proteins in animals treated both in post-natal periods as well as during adulthood could be similar to the studies that have demonstrated the imprinting of CYP in rats [57,58]. Overall changes in CYP2E1 and GSTA4-4 are in agreement with the previous observations, which have shown that cypermethrin-mediated neurotoxicity could be due to an increase in oxidative stress and lipid peroxidation [59–62]. This is supported by experimental evidence that showed a time of exposure dependent increase in lipid peroxidation in the present study. Lipid peroxidation could be one of the responsible factors for cypermethrin-induced dopaminergic neurodegeneration in rats, as it is well known to contribute to the pathogenesis of chemically-induced and sporadic PD [34,36].

Most of the changes observed after cypermethrin exposure were time of exposure dependent. This is in accordance with the previous observation that cypermethrin exposure did not produce the nigrostriatal dopaminergic neurodegeneration for up to 3 weeks [51]; however, its long-term exposure reduced the number of dopaminergic neurons and thereby neurodegeneration in a time of exposure dependent manner [41]. The pronounced changes in animals treated during the post-natal period as well as in adulthood as compared with adulthood alone treated animals showed that, during post-natal exposure; cypermethrin possibly exerts some irreversible changes that appear in the animals during adulthood re-exposure. Cypermethrin-mediated modulation of the dopaminergic system during the post-natal days observed in this study is also in accordance with the previous observations [41,63]. This could possibly be because cypermethrin produces some irreversible and invisible changes in motor and sensory abilities, the synthesis of brain lipids and the turnover of proteins, as these are at their highest levels during post-natal days [41,64,65]. Although we do not have any direct evidence to support the hypothesis, the possibility of more pronounced changes owing to cypermethrin exposure in post-natal as well as adult treated animals could be due to imprinting of a few genes and proteins involved in the nigrostriatal dopaminergic neurodegeneration.

Conclusively, the study demonstrates that long-term exposure to cypermethrin modulates the expression of VMAT 2, CYP2E1, GSTA4-4 and lipid peroxidation in a time of exposure dependent manner and its pre-exposure during post-natal periods exhibits more pronounced changes in these indices. Since modulation in these variables are reported to be associated with the nigrostriatal dopaminergic neurodegeneration in experimental animals [34], therefore, the study shows that cypermethrin induces nigrostriatal dopaminergic neurodegeneration in adult

animals and post-natal pre-exposure enhances the susceptibility during adulthood.

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References

[1] Burke RE. Parkinson's Disease. In: Koliatsos VE, Ratan RR, editors. Cell death and diseases of the nervous system. Totowa, New Jersey: Humana; 1999. p. 459–475.

[2] Jankovic J, Wooten M, Van der Linden C, Jansson B. Low body weight in Parkinson's disease. South Med J 1992;85: 351–354.

[3] Singh MP, Patel S, Dikshit M, Gupta YK. Contribution of genomics and proteomics in understanding the role of modifying factors in Parkinson's disease. Indian J Biochem Biophys 2006;43:69–81.

[4] Srivastava G, Singh K, Tiwari MN, Singh MP. Proteomics in Parkinson's disease: current trends, translational snags and future possibilities. Expert Rev Proteomics 2010;7: 127–139.

[5] Aschner M. Manganese in health and disease. In: Massaro EJ, editor. From transport to neurotoxicity. Handbook of neurotoxicology. Totowa, New Jersey: Humana: 2000. p. 195–209.

[6] Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Richardson RJ. The risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. Neurology 1998;50: 1346–1350.

[7] Kumar A, Ahmed I, Shukla S, Singh BK, Patel DK, Pandey HP, Singh C. Effect of zinc and paraquat co-exposure on neurodegeneration: modulation of oxidative stress and expression of metallothioneins, toxicant responsive and transporter genes in rats. Free Radic Res 2010; DOI: 10.3109/10715762.2010.492832.

[8] Patel S, Pandey A, Bajpayee M, Parmar D, Dhawan A. Cypermethrin-induced DNA damage in organs and tissues of the mouse: evidence from the comet assay. Mutat Res 2006;607: 176–183.

[9] Singh C, Ahmad I, Kumar A. Pesticides and metals induced Parkinson's disease: involvement of free radicals and oxidative stress. Cell Mol Biol 2007;53:19–28.

[10] Thiruchelvam M, Richfield EK, Goodman BM, Baggs RB, Cory-Slechta DA. Developmental exposure to the pesticides paraquat and maneb and the Parkinson's disease phenotype. Neurotoxicology 2002;23:621–633.

[11] Saxena PN, Chauhan LK, Gupta SK. Cytogenetic effects of formulation of cypermethrin in root meristem cells of Allium sativum: spectroscopic basis of chromosome damage. Toxicology 2005;216:244–252.

[12] Dayal M, Parmar D, Dhawan A, Ali M, Dwivedi UN, Seth PK. Effect of pretreatment of cytochrome P450 (P450) modifiers on neurobehavioral toxicity induced by deltamethrin. Food Chem Toxicol 2003;41:431–437.

[13] Parkinson A. Biotransformation of xenobiotics. In: Klaassen CD, editors. Casarett and Doull's toxicology - The basic sciences of poison. New York: McGraw Hill; 2001. p. 133–224.

[14] Walker AP, Bacherald HS. Studies on DNA damage and repair in mammalian brain. J Neurochem 1988;51:1394–1399.

[15] Crawford MJ, Croucher A, Hutson DH. Metabolism of cis- and trans-cypermethrin in rats. Balance and tissue retention study. J Agric Food Chem 1981;29:130–135.

[16] Gowland B, Webster L, Fryer R, Davies I, Moffat C, Stagg R. Uptake and effects of the cypermethrin-containing sea lice treatment Excis in the marine mussel Mytilus edulis. Environ Pollut 2002;120:805–811.

[17] Ramadan AA. Action of pyrethroids on K^+ simulated calcium uptake by, and [3H] nimodipine binding to, rat brain synaptosomes. Pest Biochem Physiol 1988;32:114–122.

[18] Rao GV, Rao KSJ. Inhibition of monoamine oxidase-A of rat brain by pyrethroids-an in vitro kinetic study. Mol Cell Biochem 1993;124:107–114.

[19] Smith TJ, Soderlund DM. Potent actions of the pyrethroid insecticides cismethrim and cypermethrin on rat tetrodotoxin-resistant peripheral nerve (SNS/PN3) sodium channels expressed in Xenopus oocytes. Pest Biochem Physiol 2001; 70:52–61.

[20] Barbeau A, Roy M, Cloutier T, Plasse L, Paris S. Environmental and genetic factors in the etiology of Parkinson's disease. Adv Neurol 1987;45:299–306.

[21] Bocchetta A, Corsini GU. Parkinson's disease and pesticides. Lancet 1986;2:1163.

[22] Desi I, Dobronyi I, Varga L. Immuno-, neuro-, and general toxicologic animal studies of a synthetic pyrethroid, cypermethrin. Ecotoxicol Environ Saf 1986;12:220–232.

[23] Giray B, Gurbay A, Hincal F. Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin E or allopurinol. Toxicol Lett 2001;118:139–146.

[24] Kale M, Rathore N, John S, Bhatnagar D. Lipid peroxidation and antioxidant enzymes in rat tissues in pyrethroid toxicity: possible involvement of reactive oxygen species. J Nutr Environ Med 1999;9:37–46.

[25] Koller W, Vetere-Overfield B, Gray C, Alexander C, Chin T, Dolezal J, Hassanein R, Tanner C. Environmental risk factors in Parkinson's disease. Neurology 1990;40:1218–1221.

[26] Sian J, Dexter DT, Lees AJ, Daniel S, Agid Y, Javoy-Agid F, Jenner P, Marsden CD. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann Neurol 1994;36:348–355.

[27] Fon EA, Pothos EN, Sun BC, Killeen N, Sulzer D, Edwards RH. Vesicular transport regulates monoamine storage and release but is not essential for amphetamine action. Neuron 1997;19: 1271–1283.

[28] Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 1996;379: 606–612.

[29] Sora I, Wichems C, Takahashi N, Li XF, Zeng Z, Revay R, Lesch KP, Murphy DL, Uhl GR. Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. Proc Natl Acad Sci USA 1998;95:7699–7704.

[30] Takahashi N, Miner LL, Sora I, Ujike H, Revay RS, Kostic V, Jackson-Lewis V, Przedborski S, Uhl GR. VMAT2 knockout mice: heterozygotes display reduced amphetamine-conditioned reward, enhanced amphetamine locomotion, and enhanced MPTP toxicity. Proc Natl Acad Sci USA 1997;94:9938–9943.

[31] Wang YM, Gainetdinov RR, Fumagalli F, Xu F, Jones SR, Bock CB, Miller GW, Wightman RM, Caron MG. Knockout of the vesicular monoamine transporter 2 gene results in neonatal death and supersensitivity to cocaine and amphetamine. Neuron 1997;19:1285–1296.

[32] Liu Y, Peter D, Roghani A, Schuldiner S, Prive GG, Eisenberg D, Brecha N, Edwards RH. A cDNA that suppresses MPP+ toxicity encodes a vesicular amine transporter. Cell 1992;70: 539–551.

[33] Xu Z, Cawthon D, McCastlain KA, Slikker W, Jr, Ali SF. Selective alterations of gene expression in mice induced by MPTP. Synapse 2005;55:45–51.
[34] Patel S, Singh V, Kumar A, Gupta YK, Singh MP. Status of antioxidant defense system and expression of toxicant responsive genes in striatum of maneb and paraquat-induced Parkinson's disease phenotype in mouse: mechanism of neurodegeneration. Brain Res 2006;1081:9–18.
[35] Mari M, Cederbaum AI. Induction of catalase, alpha, and microsomal glutathione S-transferase in CYP2E1 overexpressing HepG2 cells and protection against short-term oxidative stress. Hepatology 2001;33:652–661.
[36] Gupta SP, Patel S, Yadav S, Singh AK, Singh S, Singh MP. Involvement of nitric oxide in maneb- and paraquat-induced Parkinson's disease phenotype in mouse: is there any link with lipid peroxidation? Neurochem Res 2010;35:1206–1213.
[37] Kaplowitz N. Biochemical and cellular mechanisms of toxic liver injury. Semin Liver Dis 2002;22:137–144.
[38] Lieber CS. CYP2E1: from ASH to NASH. Hepatol Res 2004; 28:1–11.
[39] Lieber CS. Cytochrome P-4502E1: its physiological and pathological role. Physiol Rev 1997;77:517–544.
[40] Habig WH, Pabst MJ, Jacoby WB. Glutathione-S-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130–7139.
[41] Singh AK, Tiwari MN, Upadhyay G, Patel DK, Singh D, Prakash O, Singh MP. Long-term exposure to cypermethrin induces the nigrostriatal dopaminergic neurodegeneration in adult rats: postnatal exposure enhances the susceptibility during adulthood. Neurobiol Aging 2010;DOI: 10.1016/j.neurobiolaging.2010.02.018.
[42] Elwan MA, Richardson JR, Guillot TS, Caudle WM, Miller GW. Pyrethroid pesticide-induced alterations in dopamine transporter function. Toxicol Appl Pharmacol 2006;211:188–197.
[43] Gillette JS, Bloomquist JR. Differential up-regulation of striatal dopamine transporter and alpha-synuclein by the pyrethroid insecticide permethrin. Toxicol Appl Pharmacol 2003; 192:287–293.
[44] Gonzalez-Hernandez T, Barroso-Chinea P, De La Cruz Muros I, Del Mar Perez-Delgado M, Rodriguez M. Expression of dopamine and vesicular monoamine transporters and differential vulnerability of mesostriatal dopaminergic neurons. J Comp Neurol 2004;479:198–215.
[45] Garcon G, Gosset P, Zerimech F, Grave-Descampiaux B, Shirali P. Effect of Fe(2)O(3) on the capacity of benzo(a) pyrene to induce polycyclic aromatic hydrocarbon-metabolizing enzymes in the respiratory tract of Sprague-Dawley rats. Toxicol Lett 2004;150:179–189.
[46] Thomas T, Jurgen B. Testosterone, cytochrome P450, and cardiac hypertrophy. FASEB J 2002;16:1537–1549.
[47] Kumar A, Patel S, Gupta YK, Singh MP. Involvement of endogenous nitric oxide in myeloperoxidase mediated benzo(a)pyrene induced polymorphonuclear leukocytes injury. Mol Cell Biochem 2006;286:43–51.
[48] Upadhyay G, Kumar A, Singh, MP. Effect of silymarin on pyrogallol- and rifampicin-induced hepatotoxicity in mouse. Eur J Pharmacol 2007;565:190–201.
[49] Koop DR. Hydroxylation of p-nitrophenol by rabbit ethanol-inducible cytochrome P-450 isozyme 3a. Mol Pharmacol 1986;29:399–404.
[50] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–358.
[51] Mun JY, Lee WY, Han SS. Effects of cypermethrin on the dopaminergic neurons in the progressive hemiparkinsonian rats. Toxicol Mech Method 2005;15:399–404.
[52] Patel S, Singh K, Singh S, Singh MP. Gene expression profiles of mouse striatum in control and maneb + paraquat-induced Parkinson's disease phenotype: validation of differentially expressed energy metabolizing transcripts. Mol Biotechnol 2008;40:59–68.
[53] Miller GW, Gainetdinov RR, Levey AI, Caron MG. Dopamine transporters and neuronal injury. Trends Pharmacol Sci 1999;20:424–429.
[54] Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. Neuron 2003;39:889–909.
[55] Nissbrandt H, Bergquist F, Jonason J, Engberg G. Inhibition of cytochrome P4502E1 induces an increase in extracellular dopamine in rat substantia nigra: a new metabolic pathway? Synapse 2001;40:294–301.
[56] Vaglini F, Pardini C, Viaggi C, Bartoli C, Dinucci D, Corsini GU. Involvement of cytochrome P450 2E1 in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced mouse model of Parkinson's disease. J Neurochem 2004;91:285–298.
[57] Hubatsch I, Ridderstrom M, Mannervik B. Human glutathione transferase-A4-4: an alpha class enzyme with high efficiency in the conjugation of 4-hydroxynonenal and other genotoxic products of lipid peroxidation. Biochem J 1998;330: 175–179.
[58] Agrawal AK, Shapiro BH. Imprinted over induction of hepatic CYP2B1 and 2B2 in adult rats neonatally exposed to phenobarbital. J Pharmacol Exp Ther 1996;279:991–999.
[59] Agrawal AK, Shapiro BH. Latent over expression of hepatic CYP2C7 in adult male and female rats neonatally exposed to Phenobarbital: a developmental profile of gender dependent P450s. J Pharmacol Exp Ther 2000;293:1027–1033.
[60] Chinopoulos C, Tretter L, Rozsa A, Adam-Vizi V. Exacerbated responses to oxidative stress by an Na+ load in isolated nerve terminals: the role of ATP depletion and rise of [Ca^{2+}]. J Neurosci 2000;20:2094–2103.
[61] Fonne-Pfister R, Bargetzi MJ, Meyer UA. MPTP, the neurotoxin inducing Parkinson's disease, is a potent competitive inhibitor of human and rat cytochrome P450 isozymes (P450 buf1, P450db1) catalyzing debrisoquine 4-hydroxylation. Biochem Biophys Res Commun 1987;148: 1144–1150.
[62] Jimenez-Jimenez FJ, Tabernero C, Mena MA, Garcia de Yebenes J, Garcia de Yebenes MJ, Casarejos MJ, Pardo B, Garcia-Agundez JA, Benitez J, Martinez A. Acute effects of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine in a model of rat designated a poor metabolizer of debrisoquine. J Neurochem 1991;57:81–87.
[63] Nasuti C, Gabbianelli R, Falcioni ML, Di Stefano A, Sozio P, Cantalamessa F. Dopaminergic system modulation, behavioral changes and oxidative stress after neonatal administration of pyrethroids. Toxicology 2007;229:194–205.
[64] Dobbing J. Prenatal nutrition and neurological development. UCLA Forum Med Sci 1975;18:401–420.
[65] Lajtha A, Dunlop D. Turnover of protein in the nervous system. Life Sci 1981;29:755–767.

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