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Tyrosine Hydroxylase as a Target for Deltamethrin in the Nigrostriatal Dopaminergic Pathway[1]

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Objective To study the effects of deltamethrin on tyrosine hydroxylase in nigrostriatum of male rats. **Methods** Sprague-Dawley rats were daily treated with deltamethrin at 6.25 or 12.5 mg/kg body weight by gavage for 10 days. Then HPLC-fluorescence detection was used to analyze the contents of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homoranillic acid (HVA) in substantial nigra and striatum. The activities of tyrosine hydroxylase (TH) were also detected by HPLC-fluorescence detection. TH mRNA or TH protein levels were measured by RT-PCR and immunohistochemistry method. **Results** The content of DA in striatum was significantly decreased by the treatments, suggesting an inhibition of DA synthesis by deltamethrin. The contents of DA metabolites DOPAC and HVA increased, indicating increased dopamine turnover. Furthermore, deltamethrin significantly decreased the activity, as well as the mRNA and protein levels of TH. **Conclusions** These findings reveal a novel aspect of deltamethrin neurotoxicity and suggest tyrosine hydroxylase as a molecular target of deltamethin on dopamine metabolism in the nigrostriatal pathway.

Key words: Deltamethrin; Nigrostriatum; Dopamine; Tyrosine hydroxylase; Parkinson's disease

INTRODUCTION

Degeneration of the nigrostriatal pathway is a primary component of Parkinson's disease (PD), a late-onset, progressive neurodegenerative disease[1-2]. Although cases of familial PD, which are rare, have been linked to mutations in α-synuclein or parkin, the cause of the more commonly encountered sporadic PD remains unknown[3-4]. A genetic twin study has failed to show a difference in concordance for PD in monozygotic twins compared with dizygotic twins, suggesting that idiopathic PD is not inherited[5]. On the other hand, epidemiological studies have suggested several possible causative environmental factors for PD[4,6]. These associations include exposure to heavy metals, drinking well water, rural living, farming, and exposure to pesticides[7-11]. In addition, increased prevalence of PD in industrialized countries and its geographic heterogeneity also suggest a linkage to greater use of environmental chemicals[12-15]. Moreover, recent evidence reveals that a combination of pesticide exposure and poor metabolizer polymorphisms of CYP2D6, a P450 enzyme that metabolizes MPTP and certain pesticides, increases the risk of PD in a population with high levels of exposure to pesticides[16]. In experimental animals, several chemicals have been shown to produce PD-like lesions including neurotoxicant MPTP[6,17-18], or to cause damages in the nigrostriatal pathway, such as pesticides rotenone[19], paraquat[20], and heptachlor[21].

Pyrethroid pesticides, the major class of insecticides, are commonly used in agriculture and urban settings due to their high potency and selectivity as nerve poisons and low persistent residues compared with other classes of insecticides[22]. Human exposure to pyrethroids is widespread. Deltamethrin, one of the most potent pyrethroid insecticides with a α-cyano substitute, produces the prototypical type II neurological syndrome (also

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Abbreviations: PD, Parkinson's disease; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; L-DOPA, L-3,4-dihydroxyphenylalanine; TH, tyrosine hydroxylase; ROS, reactive oxygen species; HPLC-FD, high performance liquid chromatography with fluorescence detector.

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named as the "choreoathetosis with salivation" or "CS" syndrome) of pyrethroids, which is characterized by salivation without lacrimation followed by jerking leg movements and progressive writhing convulsions (choreoathetosis)[22-23]. Several mechanisms have been suggested for the neural toxicity of deltamethrin. In acute exposure, deltamethrin blocks nerve impulse by modifying the kinetics of sodium channels[24]. Voltage-sensitive sodium channels appear to be the principal site of acute insecticidal action of pyrithroids, which is supported by genetic analysis, in which a mutation in the *Vssc1* gene (encoding the voltage-sensitive sodium channel in houseflies) is associated with the *kdr* (knockdown resistant) trait that confers resistance to pyrethroids[25]. In mammals, deltamethrin has been shown to produce multiple neurotoxicities in a dose- and route-dependent manner, implicating multiple targets in its neuronal effects. Voltage-sensitive sodium channels, GABA receptors, nicotinic acetylcholine receptors, and excitatory glutamate receptors have been implicated in certain neurotoxic effects of the pesticide in vertibrates[23]. We have previously shown that deltametherin increases apoptosis in brain accompanied with increased expression of p53 and Bax, which are pro-apoptotic, and decreased expression of Bcl-2, which is anti-apoptotic[26-28]. Recently, Bloomquist *et al.*[29] showed that deltamethrin selectively increases dopamine release and uptake in the dopaminergic nerve terminals of the striatum in mice. Data on the health effects and exposure levels of pyrethoid pesticides in humans are currently lacking. However, because of the wide use of deltamethrin and related pesticides, the possibility that long term exposure to the pesticides causes lesions in the dopaminergic neurons in CNS and contributes to developing PD is of both occupational and public health concerns.

In this study, we investigated the effect of deltamethrin on the neurochemistry of dopamine in the nigrostriatal pathway in rats. The data reveal, for the first time, that chronic treatment with deltamethrin selectively inhibits the synthesis of dopamine while increasing the turnover of dopamine in the striatum. Moreover, deltamethrin inhibits the activity and the mRNA/protein expression of tyrosine TH in striatum, suggesting TH as a molecular target of the pesticide in the nigrostriatal pathway.

MATERIALS AND METHODS

Chemicals

Deltamethrin (97.3%) was obtained from Roussel-Uclaf Corp (Romainville Cedex, France). Anti-rabbit tyrosine hydroxylase polyclonal antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). HPLC-grade methanol was purchased from Fisher Scientific, Inc (Pittsburgh, PA). DA, DOPAC, HVA, L-3,4-dihydroxyphenylalanine (L-DOPA) and m-hydroxy-benzylhydrazine (NSD-1015) were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest commercial grades.

Animals and Treatments

Male Sprague-Dawley rats weighing 180-220 g were provided by the Animal Experimental Center of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, Hubei, China). Rats were housed in polypropylene cages (32 cm× 40 cm×18 cm) under controlled temperature (22℃-24℃) and humidity in a 12-hour light/dark cycle with free access to food and water.

In order to examine whether deltamethrin could selectively affect dopamine neurochemistry in the nigrastriatal pathway, we chose doses of 6.25 and 12.5 mg/kg body weight, being well below the doses for acute toxicity in rats. The LD_{50} value of deltamethrin in male SD rats was about 95 mg/kg[23]. Deltamethrin was dissolved in corn oil and administered to rats by gavage. Treatment was given once a day for 10 days. Control rats received corn oil. Twenty-four hours after the last treatment, the rats were sacrificed by decapitation. In preliminary studies, this treatment produced changes in dopamine metabolism in brain without obvious acute toxicity. Brain tissues of substantia nigra (SN) and striatum were collected separately, frozen immediately in liquid nitrogen, and then stored at -80℃ for later use.

Determination of Contents of Dopamine and Its Metabolites

Brain tissues were homogenized in 0.1 mol/L perchloric acid and centrifuged at 40 000×g for 20 min at 4℃. The supernatant was used to determine the contents of DA and its metabolites DOPAC and HVA by high performance liquid chromatography with a fluorescence detector (HPLC–FD). The stationary phase used was an ODS 5 μm phase 2 column (4.5×150 mm; Varian, Inc., Walnut Creek, CA, USA). The mobile phase was a buffered solution containing 0.02 mol/L sodium citrate/0.05 mol/L sodium dihydrate phosphate (pH 4.5), and 5% methanol (v/v). The mobile phase was circulated at a flow rate of 1 mL/min. Signals were detected by fluorescence measurement using a PROSTAR fluorometer with detection wavelength (Ex 280 nm;

Em 320 nm) (Varian, Inc., Palo Alto, CA). Quantification of the contents of DA, DOPAC and HVA was carried out by measuring the chromatographic peak areas using external standards. Results were expressed as μg metabolite/g wet weight tissue for striatum (Table 1) or ng metabolite/g wet weight tissue for substantia nigra (Table 2).

TABLE 1

Effects of Deltamethrin on Contents of DA and Metabolites in Striatum ($\bar{x} \pm s$)

Treatment	DA	DOPAC	HVA	(DOPAC+HVA)/DA
Control	9.46±0.73	0.17±0.03	0.14±0.02	0.03±0.004
D1	7.96±0.40	0.37±0.03**	0.29±0.04**	0.08±0.004**
D2	6.59±0.80*	0.38±0.04**	0.29±0.04**	0.10±0.005**

Note. *P<0.05; **P<0.01.

TABLE 2

Effects of Deltamethrin on Contents of DA and Metabolites in Substantia Nigra ($\bar{x} \pm s$)

Treatment	DA	DOPAC	HVA
Controls	48.55±9.61	57.21±12.62	-
D1	44.35±11.36	55.14±11.38	-
D2	40.12±9.55	62.65±9.63	-

Tyrosine Hydroxylase Immunohistochemistry

One day after the last treatment, the oil and deltamethrin-treated rats were deeply anesthetized with pentobarbital. The rats were perfused through the transcardiac route with 100 mL of 0.9% saline, followed by 400 mL of ice-cold 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PB, pH 7.4) for over 30 min[30]. The brains were postfixed for 2 h and then cryoprotected overnight in 30% sucrose/0.1 mol/L phosphate buffer. Serial coronal sections were cut on a freezing microtome at a 20 μm thickness. Sections were processed for TH immunohistochemistry using the SP-kit (Zhongshan Biology Technology Inc., Beijing, China) according to instructions from the manufacturer. The anti-TH antibodies were used at a dilution of 1:100. DAB (Diaminobenzidine, Zhongshan Biology Technology Inc.) was used for visualization. Quantification was performed using software BioCaptMW V.10 (Vilber Lourmat, Marne-La-Vallee Cedex 1, France) following suggestions from the software provider.

Assay of TH Activity

TH activity was determined by the method of Carlsson *et al*.[31], which measures the accumulation of L-DOPA following administration of an inhibitor of decarboxylase. Rats were treated with corn oil or deltamethrin once daily for 10 days as described above. Twenty four hours later, the rats were given an intraperitoneal injection of the aromatic-L-amino acid decarboxylase inhibitor NSD-1015 (100 mg/kg body weight, dissolved in 0.9% sodium chloride). Thirty minutes after the NSD-1015 injection, the rats were killed and brain tissues were dissected and assayed for the accumulation of L-DOPA. The contents of L-DOPA in substantia nigra and striatum were measured as described above for the contents of DA and its metabolites.

TH mRNA Level Measured by Reverse Transcription-polymerase Chain Reaction

Total RNA was isolated using Trizol™ following instructions from the manufacturer (Invitrogen Life Technologies, Carlsbad, CA, USA). Briefly, three micrograms of total RNA was reverse-transcribed using 0.5 U of AMV reverse transcriptase in 25 μL of total reaction volume containing reverse transcriptase buffer, random primers, dNTPs, and RNase inhibitor. One microliter of the cDNA was then amplified with Taq polymerase and specific primers for TH or β-actin in a thermal cycler (T-Gradient Thermoblock, Biometra, Goettinggen, Germany). β-actin was used as an internal control for loading variations.

The cDNA was mixed with 0.4 pmol/L of each of two primers for TH, 0.4 mmol/L of each of four deoxynucleoside triphosphates, and 2 units of Taq DNA polymerase. PCR was carried out in a total volume of 50 μL containing a PCR buffer (10 mmol/L Tris-HCl, pH 8.3, 1.5 mmol/L $MgCl_2$, and 50 mmol/L KCl) in thin wall PCR tubes. The PCR cycles were as the following. (1) The reaction mixture was heated at 94℃ for 5 min followed by a touch-down protocol of denaturing at 94℃ for 60

seconds, annealing from 68℃ down to 60℃ in 8 cycles for 60 seconds, and extension at 72℃ for 60 seconds. (2) The reaction was followed by 34 cycles of a 3-step PCR similar to the above protocol with an annealing temperature at 59℃. (3) The reaction was terminated following a 10 min extension at 72℃.

The PCR reaction for β-actin was performed as follows. One μL of single-strand cDNAs was mixed with 0.2 pmol/L of each primer for β-actin and other reagents as described for TH. PCR amplification consisted of 30 cycles at 94℃ for 1 min, 55℃ for 40 seconds, and at 72℃ for 40 seconds, and a 10 min extension at 72℃ at the end of the PCR reaction.

The primers for TH were 5-TCGCCACAGC-CCAAGGGCTTCAGAA-3 (sense) and 5-CCTCG-AAGCGCACAAAATAC-3 (anti-sense). The prim-ers for β-actin were 5-CATCACTATCGGCAAT-GAGC-3 (sense) and 5-GACAGCACTGTG-TTGGCATA-3 (anti-sense). The PCR products were separated on 1.5% agarose gels and stained with ethidium bromide. The cDNA bands were visualized under UV transillumination and quantitated using software BioCaptMW V.10 (Vilber Lourmat, Marne-La-Vallee Cedex 1, France).

Statistical Analysis

Data were presented as $\bar{x} \pm s$ Differ-ences between groups were analyzed by one-way ANOVA, followed by the Tukey–Kramer test. A probability value of less than 0.05 was considered statistically significant.

RESULTS

Deltamethrin Altered Dopamine Metabolism in Striatum

To analyze the effects of deltamethrin on the nigrostriatal dopaminergic pathway, we first examined dopamine metabolism in striatum containing dopaminergic nerves. As shown in Table 1, treatment with deltamethrin decreased the content of dopamine in striatum at both low and high doses. The reduction was statistically significant at the dose of 12.5 mg/kg body weight (P<0.05, compared with the control). On the contrary, the treatment significantly increased the contents of DOPAC and HVA, two major metabolites of dopamine, at both doses of 6.25 and 12.5 mg/kg body weight (P<0.01, compared with the controls). The percentage of increases over the controls for DOPAC and HVA was 117.65% and 107.14% at the dose of 6.25 mg/kg body weight or 125.13% and 107.14% at the dose of 12.5 mg/kg body weight, respectively. The (DOPAC+HVA)/DA ratio reflecting the turnover rate of dopamine, was increased by 2.7- and 3.3-fold at the low or high doses, respectively.

Alterations in the contents of DA, DOPAC, and HVA in striatum after deltamethrin treatment could reflect the pesticide's effect on the neuron bodies located in the substantia nigra. Therefore, we analyzed dopamine metabolism in the substantia nigra region. Table 2 shows that treatment with deltamethrin caused a marginal decrease in dopamine content and a slight increase in DOPAC at the dose of 12.5 mg/kg body weight in substantia nigra (P>0.05, compared with the controls). HVA was not detectable by the HPLC-FD method used in the study. The observation that deltamethrin produced more profound effects in striatum than in substantia nigra suggested that the dopaminergic nerves of striatum were more susceptible to deltamethrin than its nigral cell bodies.

As expected, the rats treated with deltamethrin did not show overt acute toxication of deltamethrin since the doses were well below those for acute toxicity. However, upon close examination, we observed that the rats receiving deltamethrin treatment at 12.5 mg/kg body weight exhibited mild hidopoiesis and slightly increased locomotor activity and aggression upon dosing, followed by a lack of locomotor activity at a later stage.

Deltamethrin Inhibited TH Activity

Deltamethrin decreased the content and increased the turnover of dopamine in striatum. However, the increase in DOPAC and HVA formation accounted for only a small fraction of dopamine reduction by deltamethrin (Table 1). For instance, at a dose of 12.5 mg/kg body weight, deltamethrin decreased dopamine by 2.87 μg/g wet weight and increased DOPAC+HVA by only 0.36 μg/g wet weight. Therefore, reduction in dopamine content by the pesticide was largely due to inhibition of dopamine synthesis. TH catalyzes the hydroxylation of tyrosine to form L-DOPA, a rate limiting step in the synthesis of dopamine. We studied the effect of deltamethrin on the activity of TH in nigrostriatum. TH activity was measured *in vivo* using NSD-1015, which blocked the conversion of L-DOPA to dopamine by inhibiting L-amino acid aromatic decarboxylase. Thus an increase in the rate of formation of L-DOPA could reflect an increased activity of TH. As shown in Fig. 1, deltamethrin decreased the activity of TH (expressed as rate of formation of L-DOPA) in both striatum (P<0.01) and substantia nigra (P<0.05). In striatum, the activity was decreased by 31.42% (from 2.2515 μg/g wet

weight/30 min in the control to 1.5441 μg/g wet weight/30 min in treated rats) at a dose of 6.25 mg/kg body weight; whereas in substantia nigra, the activity was reduced by 36.72% (from 0.5852 μg/g wet weight/30 min in the control to 0.3703±0.0806 μg/g wet weight/30 min in the treated group). Deltamethrin at a higher dose (12.5 mg/kg body weight) further decreased the activities of TH in both striatum (by 76.35%) and substantia nigra (by 44.34%) in comparison to the controls. The data revealed that deltamethrin inhibited dopamine synthesis by blocking the activity of TH.

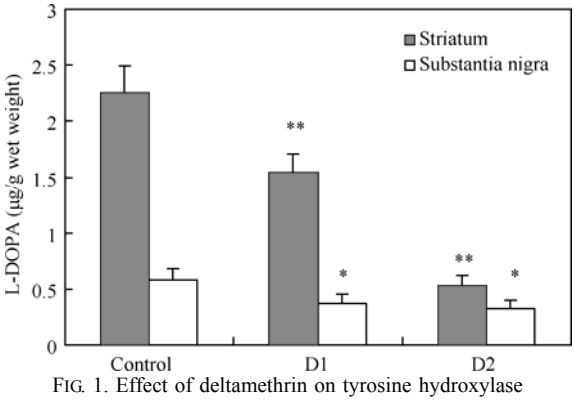

FIG. 1. Effect of deltamethrin on tyrosine hydroxylase activity in striatum and substantia nigra. *P<0.05; **P<0.01.

Deltamethrin Inhibited the Expression of TH Messenger RNA and Protein

Reduction of TH activity could result from decreased expressions of the mRNA and/or protein of TH. The messenger RNA level of TH was analyzed by RT-PCR. Figs. 2 and 3 shows that TH mRNA was detected in both striatum and substantia nigra. Treatment with deltamethrin significantly reduced the mRNA levels of TH in striatum (Fig. 2) and substantia nigra (Fig. 3) at both low (P<0.05) and high (P<0.01) doses. Reduction was 20.65% and 23.46% at the low dose and 61.87% and 59.75% at the high dose in striatum and substantia nigra, respectively. Immunohistochemical analyses using specific antibodies against TH revealed that deltamethrin reduced the protein level of TH in striatum at 12.5 mg/kg body weight (P<0.01) (Fig. 4). However, the differences in the protein levels of TH between the treatment and control groups were not significant in substantia nigra. The smaller effect of deltamethrin on the protein level of TH compared with its mRNA level might be due to a lower sensitivity and larger variation of the immunohistochemistry method compared with those of the RT-PCR method.

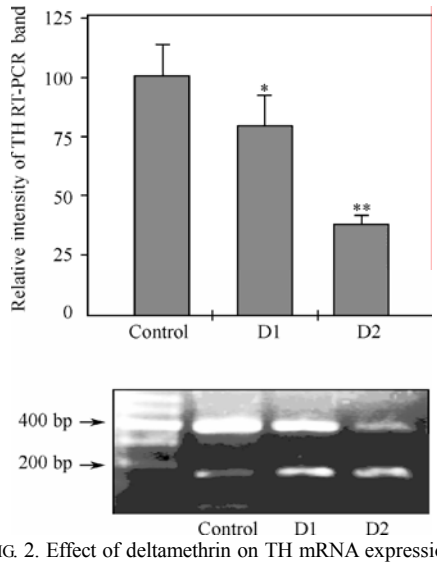

FIG. 2. Effect of deltamethrin on TH mRNA expression in striatum. *P<0.05; **P<0.01.

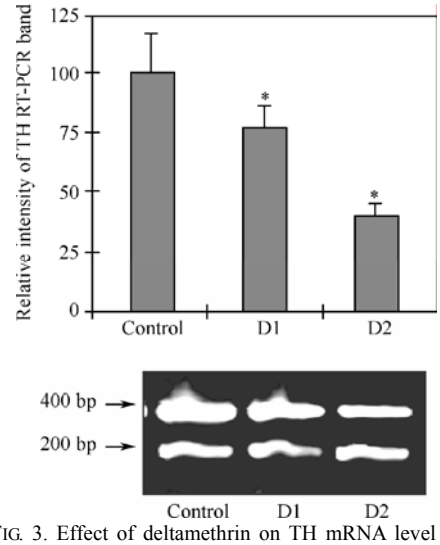

FIG. 3. Effect of deltamethrin on TH mRNA level in substantia nigra. *P<0.05.

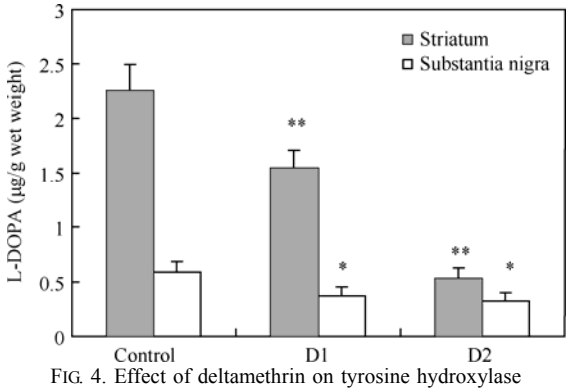

FIG. 4. Effect of deltamethrin on tyrosine hydroxylase protein level. *P<0.05; **P<0.01.

DISCUSSION

The sporadic form of Parkinson's disease, accounting for the majority of the disease, is associated with increased exposure to pesticides[1,6]. Many pesticides are neurotoxins of insects and mammals. However, information on the effect and mechanism of action of pesticides on the dopaminergic nigrostriatal pathway, a primary target of Parkinson's disease, is sparse. Deltamethrin, a prototype of type II pyrethroid pesticides commonly used in farming and urban settings, has been shown to affect multiple targets in CNS including voltage-sensitive sodium channels, chloride channels, GABA receptors, nicotinic acetylcholine receptors, and excitatory glutamate receptors. In addition, treatment with deltamethrin in rats causes neuronal apoptosis in the brain[26]. These targets of deltamethrin may contribute to its acute toxicity, i.e., the type II or CS syndrome characterized by salivation followed by jerking leg movements and progressive choreoathetosis[22-23]. A recent study showed that deltamethrin can increase the release and uptake of dopamine in synapses of the nigrostriatal pathway in mice. Moreover, dopaminergic nerve terminals of the striatum are more sensitive to pyrethroid than those of other neurotransmitters such as serotonin and glutamine[29]. In this study, we found that continuous daily treatment of rats with deltamethrin for 10 days decreased the content of dopamine in striatum by selectively inhibiting dopamine synthesis and increasing the turnover of dopamine. Furthermore, the data revealed for the first time, that the treatment inhibited the activity and the mRNA and protein expressions of TH, the rate-limiting enzyme in the synthesis of dopamine in the nigrostriatal pathway. Since the effect is observed at doses well below the dose for its acute toxicity, the effect appears not to be the result of its acute toxicity but to be caused by a selective effect(s) of deltamethrin on the dopamine neurochemistry in the nigrostriatal pathway. Together, these findings provide evidence supporting the hypothesis that environmental/occupational exposure to pyrethroid pesticides may produce specific damage to the nigrostriatal pathway, thereby contributing to the development of sporadic Parkinson's disease in humans.

The mechanism by which deltamethrin inhibits TH can be two-fold: inhibition of the TH catalytic activity and suppression of TH expression. Since the decrease in TH activity is greater than that in TH protein (Figs. 1 vs 4), it is assumed that inhibition of TH activity by deltamethrin represents a major mechanism of action in the inhibition of TH. Several possibilities exist to explain TH inhibition by deltamethrin. Deltamethrin may directly inhibit TH by interacting with the TH protein, altering its phosphorylation status, or affecting its coenzyme (tetrahydrobiopterin) function. Alternatively, metabolites of deltamethrin, such as derivatives of its cyano moiety, may produce the inhibition. Biochemical analyses of interactions of the pesticide and metabolites with TH may distinguish the possibilities in future studies. The third possible mechanism of TH inhibition involves oxidative damage and energy metabolism. It is known that dopamine neurons in the nigrostriatal pathway are susceptible to damage by reactive oxygen species (ROS) due to their high oxygen consumption, high lipid content, relatively low level of endogenous oxidant scavengers, and high concentration of iron required for the activity of key enzymes of dopamine synthesis[32-35]. We have previously shown that treatment with deltamethrin causes oxidative stress in the brain, including increased levels of lipid peroxidation products and decreased activities of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) (unpublished data). Other pyrethroids, such as cypermethrin, have been reported to produce oxidative stress in tissues as well[36]. In addition, studies have shown that oral administration of deltamethrin down-regulates several membrane-bound ATPases (Na^+-K^+-ATPase, Mg^{2+}-ATPase, Ca^{2+}-ATPase, and Ca^{2+}-Mg^{2+}-ATPase) in the cerebral cortex and hippocampus tissues. Inhibition of ATPases reduces energy supply in neurons, contributing to enzyme inhibition and neuronal damage. Thus, both ROS production and inhibition of ATPases by deltamethrin can inhibit TH in dopaminergic neurons.

The expression of TH is regulated through an intricate regulatory scheme involving many different mechanisms[37]. The reduction in mRNA and protein levels of TH can be transcriptional, in which deltamethrin reduces the transcriptional, or post-transcriptional rate of TH, in which the pesticide affects the translation of TH mRNA or the stabilities of the mRNA and protein of TH. Analyses of these possibilities may provide insights into the molecular targets of deltamethrin in the regulation of TH in dopaminergic neurons. Alternatively, the reduction of TH function by deltamethrin can be due to a loss of the dopaminergic neurons or nerves. It was reported that treatment with deltamethrin increases cell apoptosis in the hippocampus and cortex areas[26].

We found that treatment with deltamethrin increased the levels of DOPAC and HVA, two major metabolites of dopamine, and the (DOPAC+

HVA)/DA ratio, reflecting the turnover rate of dopamine in striatum. These data suggest that deltamethrin increases the catabolism of dopamine in nigrostriatum. Bloomquist *et al*.[29] recently reported that striatal dopamine uptake is increased by 70% by intraperitoneal injection of deltamethrin three times over a two-week period at a dose of 6 mg/kg body weight in mice, and the increased uptake is specific for dopamine since uptake of other neurotransmitters did not increase. The increase is consistent with increased dopamine outflow *in vivo* suggesting an up-regulation in dopamine transporter expression. These findings indicate that deltamethrin selectively increases catabolism, axonal transport, and release of dopamine in striatum. The molecular target(s) of deltamethrin in the induction of catabolism and axonal release of dopamine will be analyzed in future.

In the current study, we did not observe overt behavior changes associated with Parkinson's disease such as akinesia and bradykinesia under the treatment with deltamethrin. Upon close examination, we found that, at the high dose (12.5 mg/kg body weight), treated rats showed mild hidopoiesis and slightly increased locomotor activity and aggression upon dosing, followed by a lack of locomotor activity at a later stage. The discrepancy between a large decrease in dopamine content in striatum and the lack of Parkinsonian-like symptoms can be explained by clinical observations that the symptoms of PD are not manifested until >90% of the nigrostriatal dopaminergic neurons and >80% of dopamine are depleted[38]. A recent report showed that chronic systemic exposure to a lipophilic pesticide rotenone causes highly selective nigrostriatal dopaminergic degeneration associated with hypokinesia, rigidity, and formation of fibriliar cytoplasmic inclusions in substantia nigra neurons. The mechanism of dopamine neuropathy by rotenone includes inhibition of mitochondrial complex I and stimulation of ROS production[19]. In a separate study, exposure to pesticides paraquat and maneb during the critical periods of postnatal days can produce permanent and progressive lesions of the nigrostriatal dopamine system and enhance adult susceptibility to the pesticides[20]. It has been shown that pesticides and metals can produce synergistic effects on the fibrillation of α-synuclein, a critical molecular event in the formation of Lewy bodies[39]. In addition, there is evidence that expression of inflammatory cytokines such as TNF α is an early event in the MPTP model of PD and may promote the development of dopaminergic neurotoxicity of MPTP. Lack of TNF α receptors is associated with protection against such toxicity[18]. Thus, it remains possible that long term and/or neonatal exposure to deltamethrin or co-exposure to deltamethrin may produce profound damage in the nigrostriatal dopaminergic pathway contributing to the development of Parkinson's disease. These possibilities can be tested in future studies.

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