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Acute Effects of Pyrethroids on Serotonin Release in the Striatum of Awake Rats: An In Vivo Microdialysis Study

Muhammad M. Hossain^{1,2}, Tadahiko Suzuki², Jason R. Richardson¹, and Haruo Kobayashi²

¹Department of Environmental and Occupational Medicine and Environmental and Occupational Health Sciences Institute, UMDNJ–Robert Wood Johnson Medical School, Piscataway NJ 08854, USA

²Department of Veterinary Pharmacology, Faculty of Agriculture, Iwate University, Morioka 020-8550, Japan

Abstract

The present study examined the acute neurotoxic effects of three different pyrethroids, allethrin, cyhalothrin, and deltamethrin on the release of serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the striatum of conscious rats using microdialysis. Allethrin 10 mg/kg reduced extracellular levels of 5-HT to 46%, whereas 20 and 60 mg/kg increased the release to 177% and 243% of baseline, respectively. Cyhalothrin increased 5-HT release to 145–204% and deltamethrin decreased to 58–32% of baseline in a dose-dependent manner. None of the pyrethroids tested altered extracellular levels of 5-HIAA. Local infusion of the voltage-gated sodium channel antagonist tetrodotoxin (TTX) into striatum completely prevented the effects of allethrin, cyhalothrin, and deltamethrin (10 and 20 mg/kg) on 5-HT release. The effect of deltamethrin at 60 mg/kg was completely abolished by striatal infusion of nimodipine (L-type Ca^{++} channel antagonist) with TTX. These findings suggest that pyrethroids disrupt the serotonergic neurotransmission in striatum in a dose-related manner with Na^+ and Ca^{2+} channel-dependent mechanisms.

Keywords

Pyrethroids; Microdialysis; Striatum; Serotonin; Tetrodotoxin; Nimodipine

INTRODUCTION

Pyrethroids are synthetic insecticidal compounds mostwidely used for control of a broad range of insectpests in agriculture, public health, veterinary medicine, and residential settings [1]. The use of pyrethroids has tremendously been increasing over recent years and accounts for 30% of the worldwide insecticide market since restrictions have been placed on many of the organophosphorus insecticides [2, 3]. Based on chemical structure and

symptomology after acute toxicity, pyrethroids are classified into type I and type II groups. Type I (e.g., allethrin, permethrin) noncyano compounds cause a “T syndrome” characterized by hyperexcitation, incoordination, and whole-body tremor [4, 5]. Type II (e.g., cyhalothrin, deltamethrin) cyanocompounds cause a “CS syndrome” characterized by profuse salivation, choreoathetosis, hypersensitivity, and paralysis [4,5]. However, it has been suggested that the classification of pyrethroids into two groups does not account for all the observed effects. For example, rats exhibit choreoathetosis following acute intoxication by deltamethrin but not with cyhalothrin [6–8].

The mechanism by which pyrethroids are thought to exert their neurotoxic effects involves delaying the closing of voltage-gated Na^+ channels [9]. Classically, the type II compounds keep the channels open for longer periods than the type I compounds [10]. Prolonged opening of voltage-gated Na^+ channels results in persistent depolarization of neurons, leading to repetitive firing of action potentials, which can result in seizures, paralysis, and even death [11]. Pyrethroids also increase neurotransmitter release primarily by acting on voltage-gated Na^+ channel [7], and our recent studies demonstrated that deltamethrin enhances Ca^{++} -dependent neurotransmitter release in vivo [6, 8]. Similarly, other investigators reported that deltamethrin induced Ca^{++} -dependent glutamate release in the isolated presynaptic nerve terminal in vitro [12, 13].

Serotonin (5-HT) is a monoamine neurotransmitter that has been implicated in the regulation of control of numerous important behavioral and physiological functions such as mood, sleep, thermoregulation, appetite, aggression, and sexual behavior that might be sensitive to the neurotoxic effects of pyrethroids [14, 15]. 5-HT is broken down to its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) by monoamine oxidase [15,16]. Although 5-HT projections are widespread throughout the central nervous system, the striatum is one region with high-density 5-HT innervation [15, 16]. Sublethal exposure of rats to permethrin caused aggressive behavior [17] and disrupted feeding behavior [18], which may have a serotonergic component. However, there are few data available on the effects of pyrethroids on serotonergic neurotransmission. Martinez-Larranaga and co-workers found that high-dose exposure to deltamethrin, cyfluthrin, or cyhalothrin for 6 days decreased total striatal 5-HT and the primary metabolite 5-HIAA [19]. However, the mechanism of this decrease is not clear.

The present study was designed to examine whether pyrethroids have a neuromodulatory role on striatal 5-HT release in rats. Therefore, the effects of the type I pyrethroid allethrin was studied along with the type II pyrethroids cyhalothrin and deltamethrin for their ability to alter extracellular levels of 5-HT and 5-HIAA in the striatum by in vivo microdialysis.

MATERIALS AND METHODS

Animals

Experiments were carried out on male Sprague–Dawley rats weighing 200–250 g (SLC Japan, Hamamatsu, Japan). The animals were housed in an animal room one per cage under the standard laboratory conditions ($23 \pm 1^\circ\text{C}$, $55 \pm 5\%$ humidity) with a 12 h light/dark cycle (dark from 8 PM) and had free access to food and drinking water. Animal handling and

procedures were conducted in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals and approved by the Animal Experiment Committee in Iwate University, Japan.

Chemicals

Allethrin and deltamethrin were purchased from Wako Pure (Osaka, Japan), cyhalothrin was a gift from Zeneca (Tokyo, Japan). Sodium acetate (CH_3COONa), citric acid monohydrate ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$), and sodium 1-octanesulfonate were purchased from Nacalai Tesque (Kyoto, Japan). $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, ethanol, dimethyl sulfoxide (DMSO), and Tween 20 were from Kanto (Tokyo, Japan), and 5-HT, 5-HIAA, tetrodotoxin (TTX), and EDTA were from Sigma (St. Louis, MO).

Stereotaxic Surgery and Cannulation

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic apparatus (Kopf Instruments, Tokyo, Japan). The guide cannula (AG-8; Eicom, Kyoto, Japan) was implanted into the left striatum (A + 0.2 mm; L + 3.0 mm and V – 3.5 mm from the bregma) and fixed to the skull with dental cement [20]. Following surgery, the animals were returned to their home cage and allowed to recover for 3 days before the beginning of the experiment.

Microdialysis Procedure and HPLC Analysis

The extracellular concentration of 5-HT and 5-HIAA was measured using the microdialysis technique as previously described [7]. Briefly, the microdialysis probe (A-1-8-03; Eicom) was inserted into the striatum through the guide cannula. The inlet of the microdialysis probe was connected to a 2.5-mL gas-tight syringe (Hamilton Co., Reno, NV) and perfused with Ringer's solution (NaCl 147 mM, KCl 4.0 mM, and CaCl_2 2.3 mM) at a constant flow of 2 $\mu\text{L}/\text{min}$ using a microperfusion pump (ESP-32; Eicom) devised to allow the rats to move freely in a cubic Plexiglas[®] box (30 × 30 × 40 cm). The dialysate collected for the first 60 min was discarded to ensure a stable baseline of 5-HT release. Samples were collected every 20 min, and 20 μL was then injected directly into a high-performance liquid chromatograph equipped with an electrochemical detector (HPLC-ECD; Eicom). 5-HT and 5-HIAA were separated on a cation exchange column (EICOMPAK SC-5 ODS; 3.0 mm × 150 mm; Eicom) with sodium lauryl sulfate (0.5 mg/mL) using an isocratic mobile phase (0.05 M sodium acetate, 0.05 M citric acid monohydrate, 0.74 mM sodium 1-octanesulfonate, 0.013 mM EDTA 2Na, and 15% methanol; pH 4.1) pumped at a flow rate of 0.5 mL/min. The compounds were quantified by electrochemical detection using a graphite electrode set at 700 mV against an Ag/AgCl reference electrode. The basal efflux was defined as the average output of three samples prior to drug administration, and results were calculated as a percentage of the baseline output of 5-HT and 5-HIAA.

In Vitro Recovery Experiment

The recovery of 5-HT and 5-HIAA by the probes was calibrated at room temperature. The dialysis probes were immersed in known concentrations (100 pg) of 5-HT and 5-HIAA solutions. The probes were perfused with Ringer's solution at 2 $\mu\text{L}/\text{min}$, and 20 min samples

of dialysate were collected. The peak height of the resulting dialysate divided by the peak height of the standard solution was used to determine the recovery. The recovery of 5-HT and 5-HIAA was $22.5 \pm 0.2\%$ and $16.5 \pm 1.3\%$ ($n = 5$), respectively.

Location of the Probe Verification

At the end of microdialysis procedure, rats were euthanized with chloroform, decapitated, and the brains removed and frozen on dry ice. The probe location in the striatum was verified by visual examination after sectioning the brain into 20 μm sections on a cryostat (Microm HM 550; Thermo Scientific, Franklin, MA).

Treatment

Doses (10–60 mg/kg) of allethrin, cyhalothrin, and deltamethrin used in this study are at least 2.25 times below the oral LD50 and were selected based on our previous work [6–8]. Pyrethroid compounds were dissolved in 50 μL ethanol or DMSO with 20 μL Tween 20 and then mixed with isotonic sodium chloride solution and were administered to the rats by i.p. injection. Control animals were injected same amount of ethanol or DMSO containing isotonic sodium chloride. For TTX (1 μM) and nimodipine (10 μM), these were applied locally into the striatum simultaneously with pyrethroid administration to examine involvement of voltage-gated sodium and calcium channels, respectively. The concentration of TTX and nimodipine was selected on the basis of our previous study [6,8]. Neither TTX nor nimodipine altered extracellular 5-HT overflow in control rats.

Data Analysis

All values are presented as mean $\pm$ SEM ($n = 5$ rats per group). Basal extracellular levels of 5-HT and 5-HIAA from individual rats were calculated as the mean of three fractions collected before pyrethroid or saline administration and were set to 100%. Change from baseline was used to present the data for 5-HT and 5-HIAA releases in the figures as well as for tests of significant differences over time using two-way analysis of variance (ANOVA) with repeated measurements (treatment vs. time) followed by Dunnett's post hoc test. A level of $p < 0.05$ was considered statistically significant.

RESULTS

Clinical Signs and Symptoms

Rats exhibited no clinical signs and symptoms of toxicity at doses of 10 and 20 mg/kg with any of the pyrethroids tested. However, signs and symptoms of toxicity were observed in rats with doses of 60 mg/kg. Allethrin (60 mg/kg) produced signs of toxicity characterized by tremor, diarrhea, hypoactivity, and drowsiness. Treatment with 60 mg/kg cyhalothrin produced signs of toxicity including salivation, tremor, tiptoe gait, diarrhea, and hypoactivity. Animals treated with 60 mg/kg deltamethrin exhibited hyperactivity, aggression, vocalization, choreoathetosis, salivation, and lacrimation. All rats recovered completely within 12 h of pyrethroid administration.

Basal Levels of Extracellular 5-HT and 5-HAA in the Striatum

As mentioned above, the average of three intrastriatal 20 min samples prior to injection of pyrethroid or vehicle was used to calculate the baseline release (100%) of 5-HT and 5-HIAA. Percentage changes of release of 5-HT and 5-HIAA were then calculated. The basal rates of efflux from the striatum of untreated rats were 13.06 ± 0.63 pg 5-HT/20 μ L/10 min and 727.18 ± 41.41 pg 5-HIAA/20 μ L/10 min ($n = 15$ individual rats). Vehicle treatment produced no significant changes in striatal 5-HT and 5-HIAA release in the experiment.

Effects of Allethrin on Extracellular Levels of 5-HT and 5-HAA in the Striatum of Awake Rats

The effects of allethrin on changes in extracellular levels of 5-HT are shown in Figure 1A. Allethrin (20 mg/kg) caused a significant increase of extracellular 5-HT level from 40 to 90 min, with the maximum increase of basal release (177%) reached 90 min after injection. The 5-HT level thereafter decreased progressively to baseline levels by the end of the experiment. Conversely, allethrin at the higher dose (60 mg/kg) produced a long-lasting (up to 3 h) elevation in 5-HT release by 242% of baseline at 90 min after injection. Since both 20 and 60 mg/kg of allethrin showed an increase in 5-HT release, a lower dose (10 mg/kg) was also examined. This treatment decreased the release of 5-HT to 46% of basal release at 90 min after administration, and thereafter progressively returned to control values. Local application of the Na^+ channel blocker TTX via the dialysis probe completely prevented the effects of allethrin at all doses on 5-HT release (Figure 1B). Allethrin treatment had no significant effect on 5-HIAA levels (Figure 1C).

Effects of Cyhalothrin on Extracellular 5-HT and 5-HAA Levels in the Striatum of Awake Rats

Extracellular 5-HT levels were dose dependently increased in the striatum after cyhalothrin administration (Figure 2A). Administration of 10, 20, and 60 mg/kg cyhalothrin caused maximum increases of basal 5HT release by 145%, 161%, and 204% of baseline, with a peak time of 60 min after administration. In the two lower doses, this effect disappeared by 90–120 min after the injection. In contrast, the dose of 60 mg/kg cyhalothrin led to a sustained increase in 5HT release that remained significantly elevated throughout the 3 h experiment. Striatal perfusion of TTX completely prevented the cyhalothrin evoked 5-HT release as shown in Figure 2B. Cyhalothrin had no effect on the outflow of 5-HIAA in the striatum when compared with time course vehicle control (Figure 2C).

Effects of Deltamethrin on Extracellular 5-HT and 5-HAA Levels in the Striatum of Awake Rats

The effects of deltamethrin on changes in extracellular levels of 5-HT are shown in Figure 3A. Deltamethrin decreased the extracellular 5-HT levels in a dose-dependent manner. Deltamethrin, at the dose of 20 mg/kg, decreased the efflux of 5-HT in the striatum for about 1.5 h. The efflux was reduced to 44% of basal release 60 min after administration, which subsequently returned to control values by the end of experiment. In contrast, a dose of 60 mg/kg decreased the basal 5-HT release throughout the 3 h experiment. A maximum inhibition to 32% of baseline was observed 60 min after drug administration. Since the

above two doses of deltamethrin caused a decrease in 5-HT release, a lower dose of 10 mg/kg was also tested. This treatment decreased the release to 58% of basal level at 60 min after administration, and thereafter progressively returned it to control values. The local application of TTX into striatum completely abolished the effects of 10 and 20 mg/kg of deltamethrin on 5-HT but only partly prevented the effects of deltamethrin at 60 mg/kg (Figure 3B). However, the effect of 60 mg/kg deltamethrin on striatal 5-HT release was completely abolished by a local infusion of 10 μ M nimodipine with TTX (Figure 4). Deltamethrin also had no effect on striatal 5-HIAA levels as comparisons with controls were not significantly different (Figure 3C).

DISCUSSION

This study demonstrates that three different pyrethroids affect the basal release of striatal 5-HT in different ways. Allethrin at the lowest dose decreased extracellular 5-HT release, whereas at higher doses it increased release of extracellular 5-HT. Despite the fact cyhalothrin and deltamethrin are type II pyrethroids, cyhalothrin increased whereas deltamethrin decreased 5-HT release in a dose-dependent manner. Although no behavioral or clinical signs of toxicity were noted with lower doses of each compound, the highest dose tested produced behavioral and clinical symptoms that were correlated with changes in 5-HT release. Allethrin and cyhalothrin displayed hypoactivity, whereas deltamethrin caused aggressive behavior and hyperactivity. These syndromes are similar to those previously observed with permethrin and cypermethrin [17]. A substantial body of literature demonstrates that reduced function and depletion of 5-HT produces aggressive behavior and increases locomotor activity, whereas high 5-HT levels are associated with opposite behavior [14, 21].

Pyrethroids act axonally on voltage-gated Na^+ channels, slowing their inactivation, prolonging membrane depolarization, and facilitating the release of neurotransmitters [6–10, 22]. It is conceivable that increased release of 5-HT is mediated by prolonged action of sodium channels on serotonergic nerve membranes in the striatum. The decrease in 5-HT release may be attributed to increased gamma-aminobutyric acid (GABA) release either in the striatum or the dorsal raphe nucleus. Striatum in particular contains an abundance of GABAergic spiny neurons, which project to different nerve terminals [23–25]. Therefore, low doses of allethrin and deltamethrin might increase GABA release by acting on sodium channels of GABAergic interneurons, which in turn may suppress the release of 5-HT from serotonergic nerve terminals in the striatum. This result is supported by previous findings that allethrin and cyhalothrin indirectly inhibited glutamate release in the hippocampus by increasing GABA release as a result of interactions with Na^+ channels on GABAergic interneurons [6]. Alternatively, it is possible that low doses of allethrin cause a decrease in 5-HT release by activating autoreceptors. Indeed, the activation of 5-HT_{1A} autoreceptors inhibits 5-HT synthesis and release in the striatum and frontal cortex [26–28].

Local infusion of TTX into the striatum completely prevented the effects of allethrin, and cyhalothrin on the release of 5-HT, indicating the involvement of TTX-sensitive sodium channels. TTX also completely abolished the effects of lower doses of deltamethrin, but only partially blocked the effects of deltamethrin at 60 mg/kg, suggesting other potential

targets may be involved or that this high dose also affected TTX-resistant channels. Striatal infusion of nimodipine with TTX completely prevented the effects of deltamethrin at 60 mg/kg on extracellular level of 5-HT, suggesting both Na⁺ and Ca⁺⁺ channels are involved in the effects of deltamethrin on 5HT-levels at 60 mg/kg. There is evidence that voltage-sensitive Ca⁺⁺ channels may also be targets of some pyrethroid compounds [5, 29–31]. Indeed, Cao et al. [32] showed that deltamethrin to be the most potent than other pyrethroids to stimulate calcium influx in cortical neurons and that secondary calcium influx may be mediated by L-type calcium channels, reversal of the Na⁺/Ca⁺⁺ exchanger, or by *N*-methyl-D-aspartic acid receptors.

In conclusion, these findings offer the first in vivo evidence that the pyrethroid insecticides allethrin, cyhalothrin, and deltamethrin acutely alter the function of serotonergic neurotransmission primarily through Na⁺ channels and secondary effects through Ca⁺⁺ channels. These results may provide insight into the different presentation of acute toxic effects following pyrethroid poisoning.

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FIGURE 1.

Effects of allethrin on the release of striatal 5-HT and 5HIAA in conscious rats. The control animals were treated with same volume of vehicle containing isotonic sodium chloride. The effects of allethrin on the release of 5-HT in the absence (A) and presence (B) of 1 μ M TTX, and 5-HIAA (C) are expressed as the percentage changes from respective baseline, i.e., the mean of three fractions collected before pyrethroid or saline administration. Each value represents the mean $\pm$ SEM of five individual rats in each group. Statistical analysis of the data was carried out using two-way ANOVA with repeated measurements (treatment vs. time) followed by Dunnett's post hoc test. Asterisks indicate significantly different from time course vehicle control (* p < 0.05, ** p < 0.01).

FIGURE 2.

Effects of cyhalothrin on the release of striatal 5-HT and 5HIAA in conscious rats. The control animals were treated with same volume of vehicle containing isotonic sodium chloride. The effects of cyhalothrin on the release of 5-HT in the absence (A) and presence (B) of 1 μ M TTX, and 5-HIAA (C) are expressed as the percentage changes from respective baseline, i.e., the mean of three fractions collected before pyrethroid or saline administration. Each value represents the mean $\pm$ SEM of five different rats in each group. Statistical analysis of the data was carried out using two-way ANOVA with repeated measurements (treatment vs. time) followed by Dunnett's post hoc test. Asterisks indicate significantly different from time course vehicle control (* p < 0.05, ** p < 0.01).

FIGURE 3.

Effects of deltamethrin on the release of striatal 5-HT and 5HIAA in conscious rats. The control animals were treated with same volume of vehicle containing isotonic sodium chloride. The effects of deltamethrin on the release of 5-HT in the absence (A) and presence (B) of 1 μ M TTX, and 5-HIAA (C) are expressed as the percentage changes from respective baseline, i.e., the mean of three fractions collected before pyrethroid or saline administration. Each value represents the mean $\pm$ SEM of five different rats in each group. Statistical analysis of the data was carried out using two-way ANOVA with repeated measurements (treatment vs. time) followed by Dunnett's post hoc test. Asterisks indicate significantly different from time course vehicle control (* p < 0.05, ** p < 0.01).

FIGURE 4.

Effects of deltamethrin on the release of striatal 5-HT in the presence of nimodipine and TTX. Blockade of Na^+ and Ca^{++} channels together completely prevents the effects of deltamethrin at a higher dose on basal release of 5-HT. Data are expressed as the percentage changes from respective baseline, i.e., the mean of three fractions collected before pyrethroid or saline administration. Each value represents the mean $\pm$ SEM of five different rats in each group. Statistical analysis of the data was carried out using two-way ANOVA with repeated measurements (treatment vs. time) followed by Dunnett's post hoc test.