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Modification of 2,2′,2″-Tripyridine-Induced Tremor in Mice by Serotonergic Agonists and Antagonists and Benzodiazepines

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LIN-SHIAU, S.-Y. AND K.-S. HSU. *Modification of 2,2′,2″-tripyridine-induced tremor in mice by serotonergic agonists and antagonists and benzodiazepines.* PHARMACOL BIOCHEM BEHAV **48**(3) 665–670, 1994.—The administration of 2,2′,2″-tripyridine produced generalized tremor, myoclonus, and hindlimb abduction, similar to the "5-hydroxytryptamine (5-HT) syndrome," in mice. Pretreatment with mianserin, cyproheptadine, methysergide, or metergoline ameliorated, whereas 5-hydroxytryptophan (5-HTP), 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT), or 8-hydroxy-2-[di-*n*-propylamino]tetraline hydrobromide (8-OH-DPAT) augmented the 2,2′,2″-tripyridine-induced tremor. Furthermore, diazepam and flunitrazepam exhibited a dose-dependent protection against 2,2′,2″-tripyridine-induced tremor in mice, but pentobarbital only had a slightly protective effect. The inhibitory effects of diazepam and flunitrazepam on the 2,2′,2″-tripyridine-induced tremor were potentiated in mice pretreated with *p*-chlorophenylalanine (PCPA). These observations suggest a serotonin-mediated action of 2,2′,2″-tripyridine in its tremor action and that the benzodiazepine agonist attenuation of the 2,2′,2″-tripyridine-induced tremor is probably mediated through the GABAergic inhibition of serotonergic neurons.

2,2′,2″-Tripyridine Tremor Serotonergic system Diazepam Flunitrazepam

2,2′,2″-TRIPYRIDINE is a pyridine derivative that has been found to be a synthetic by-product of the herbicide paraquat (9). We have identified 2,2′,2″-tripyridine in the crude products of paraquat and found it to be a strong mutagen (9,12). In addition, 2,2′,2″-tripyridine was more potent than paraquat in producing carcinogenic action and DNA damage (6,12), and it possessed a curare-like action in the mouse phrenic nerve diaphragm (14). Further characterization of the pharmacological action of 2,2′,2″-tripyridine demonstrated that it exerted generalized tremor, myoclonus, and hindlimb abduction, similar to the 5-hydroxytryptamine (5-HT) syndrome, in mice (13). In the previous study (13), we have shown that 2,2′,2″-tripyridine-induced tremor could be suppressed by diazepam, an agonist of the benzodiazepine receptor, but not by haloperidol, phenoxybenzamine, or atropine. In addition to the noradrenergic neurons innervating the dorsal raphe nuclei for maintained firing of 5-HT neurons (4), neurochemical evidence has suggested that GABAergic neurons exert tonic control over ascending 5-HT neurons in the median raphe region (4). Because activation of the central serotonergic system has been shown to produce behavioral syndromes including tremor (18), and that 2,2′,2″-tripyridine-induced behavior change is similar to serotonergic hyperfunction syndrome in mice, we attempted to test whether or not an alternation in central 5-HT might be responsible for tremorogenesis by 2,2′,2″-tripyridine and also to characterize whether the modification by diazepam of 2,2′,2″-tripyridine-induced tremor involves the serotonergic system.

METHOD

Animals

Male ICR mice, weighing 20–25 g, were used throughout the experiment. Animals were housed at a room temperature of 22–24°C, with humidity at 60–70% and lighting 0600–2000 h.

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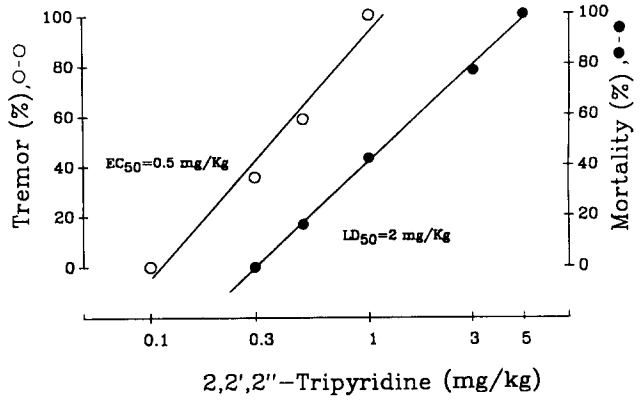

FIG. 1. Dose-dependent-induced tremor and toxicity by 2,2′,2″-tripyridine in mice. 2,2′,2″-Tripyridine was injected IP to 10 mice per each dose and percentage of tremor (○) and mortality (●) was recorded within 24 h after the injection.

Drug Preparation and Administration

2,2′,2″-Tripyridine was administered at doses of 0.3 and 1.0 mg/kg. It was dissolved in 0.1% alcohol (1 mg/ml) and was diluted with 0.9% isotonic saline solution in concentrations that permitted injections at a volume of 10 ml/kg b.wt. The final alcohol concentrations of the injections were 0.003% and 0.01%, respectively, and these concentrations of alcohol have no effects on the behaviors of the mice. Mianserin, metergoline, cyprohepadine, methysergide, 5-hydroxytryptophan (5-HTP), 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT), and 8-hydroxy-2-[di-*n*-propylamino]tetraline hydrobromide (8-OH-DPAT) were dissolved in 0.9% saline solution. The 8-OH-DPAT was injected SC, and the other drugs were administered IP. Methysergide, metergoline, mianserin, and cyprohepadine were given 60 min, but diazepam, flunitrazepam, and pentobarbital were given 30 min prior to IP administration of 2,2′,2″-tripyridine in a dose of 1 mg/kg, equivalent to the ED_{99} for production of tremor. Diazepam and flunitrazepam were administered at doses of 10 and 20 mg/kg. They were dissolved in saline to which polyoxyethylene sorbitan monooleate (Tween-80, Sigma) had been added (1%). Pentobarbital was prepared in 0.9% saline and

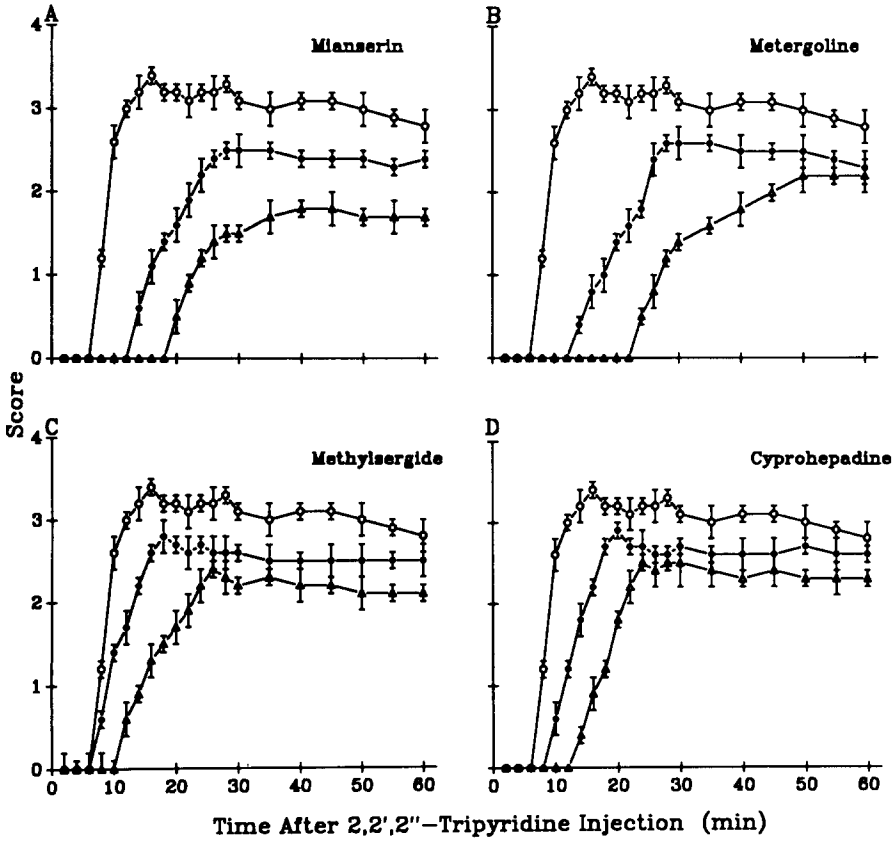

FIG. 2. Effects of serotonergic antagonists on 2,2′,2″-tripyridine-induced tremor in mice. Mice were treated IP with (A) mianserin (● 10 or △ 20 mg/kg), (B) metergoline (● 10 or △ 20 mg/kg), (C) methysergide (● 10 or △ 20 mg/kg), or (D) cyproheptadine (● 10 or △ 20 mg/kg) for 60 min, prior to administration of 2,2′,2″-tripyridine (1 mg/kg). All pretreatments had significant effects by one-way ANOVA, with $p < 0.01$ for metergoline (10–20 mg/kg), mianserin (10–20 mg/kg), methysergide (20 mg/kg), and cyproheptadine (20 mg/kg) and $p < 0.05$ for methysergide (10 mg/kg) and cyproheptadine (10 mg/kg) compared with the control (○). Each point represents a mean score of eight animals. Data are presented as mean ± SEM.

was administered at doses of 10 and 30 mg/kg. 5-HTP, 5-MeODMT, or 8-OH-DPAT was injected 5 min befor the administration of 2,2′,2″-tripyridine in a dose of 0.3 mg/kg, corresponding to the ED_{40} for production of tremor. The effect of 2,2′,2″-tripyridine, diazepam, and flunitrazepam was also observed in mice pretreated with PCPA (100 mg/kg) for 3 days daily, the treatment that produced 50–60% depletion of brain 5-HT (7,8). All drugs were freshly prepared before administration and the drug concentrations were adjusted so that each mouse received no more than 10 ml/kg b.wt. of the drug solution. Control animals received an equivalent volume of the 0.9% saline only.

Toxicity Test

2,2′,2″-Tripyridine (1 mg/ml, 0.1% alcohol) was diluted with 0.9% saline solution to various concentrations and administrated IP to 10 mice per each dose. Mortality within 24 h after injection was recorded. The LD_{50} (dose for 50% lethality) was obtained from the dose-lethality curve. The slope function and 95% confidence limit of LD_{50} were calculated according to the method described by Litchfield and Wilcoxon (15).

Behavioral Observation

Behavioral experiments were performed between 09:00 and 18:00 h by one observer. Mice were examined for their behavior in an individual plastic cage. After injection of 2,2′,2″-tripyridine, tremor was scorded by the modified method of Dickinson and Curzon (3), and that was recorded individually every 2 min for 60 min. Tremor was scored on a 0–4 rating scale: 0, absent; 1, equivocal or present a few times; 2, weak or several times; 3, moderate or frequent; and 4, marked or continuous.

Chemicals

Methysergide maleate was from Sandoz, Ltd (Basel); 5-MeODMT oxalate, L-5-HTP, metergoline, cyproheptadine HCl, mianserin HCl, PCPA methylester, 8-OH-DPAT HBr, 2,2′,2″-tripyridine, diazepam, and flunitrazepam were purchased from Sigma Chemical Co. (St. Louis, MO); and pentobarbital-Na was from Aldrich Chemical Co. (London). The doses of the chemicals were calculated as the total salt form.

Statistical Analysis

Statistical analysis of the difference between groups in tremor studies was done by one-way analysis of variance (ANOVA) followed by the Student's *t*-test.

RESULTS

Toxicity in Mice

2,2′,2″-Tripyridine was injected IP into mice and their mortality was recorded 24 h after injection. The mice injected with 2,2′,2″-tripyridine produced tremor, myoclonus, and hindlimb adduction. 2,2′,2″-Tripyridine at a low dose of 0.3 mg/kg induced tremor lasting for more than 3 h. From the dose-lethality curve (Fig. 1), the LD_{50} value estimated for 2,2′,2″-tripyridine was 2 mg/kg and the ED_{50} value (dose for producing 50% tremor) was 0.4 mg/kg.

Effects of 5-HT Antagonists and Agonists on 2,2′,2″-Tripyridine-Induced Tremor

As shown in Fig. 2, all 5-HT antagonists tested significantly suppressed tremor induced by the ED_{99} of 2,2′,2″-tripyridine (1 mg/kg), although the magnitude of suppression was not equal for all these compounds. For example, metergoline, a nonselective 5-HT antagonist, and mianserin, a 5-HT_2 antagonist, markedly decreased the score of tremor in a dose-dependent fashion, whereas methysergide or cyproheptadine, both nonselective 5-HT antagonists, only moderately ameliorated tremor. Prior administration of 5-HT precusor, 5-HTP, or 5-HT agonists, however, had enhanced effects on the 2,2′,2″-tripyridine-induced tremor in mice (Fig. 3). 5-HTP, a precursor of 5-HT, clearly intensified tremor produced by an

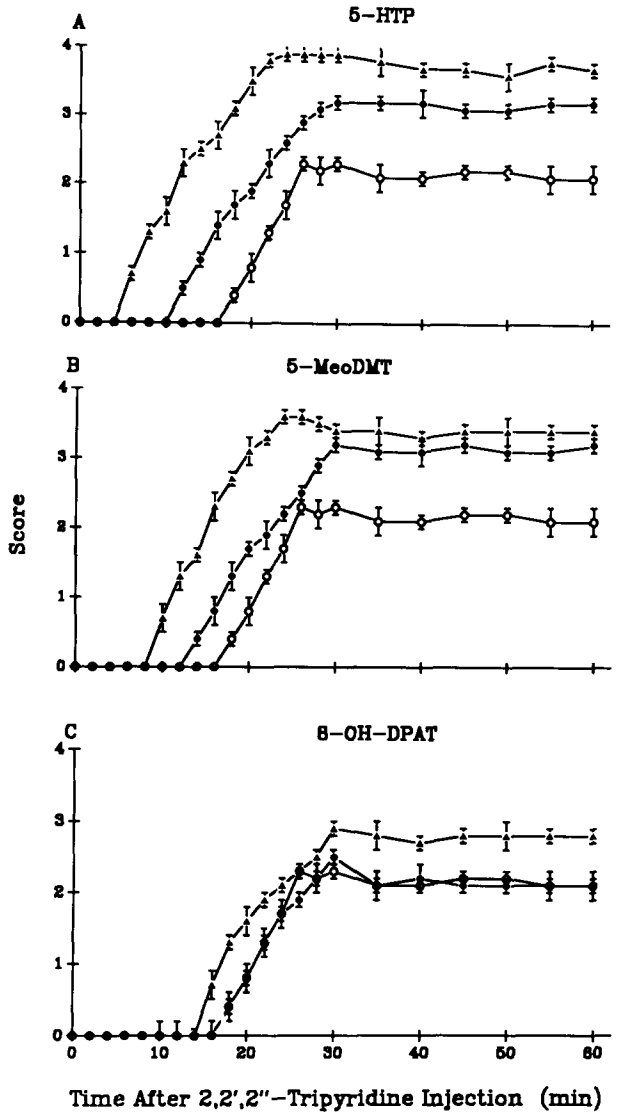

FIG. 3. Effects of serotonergic precursor (5-HTP) and agonists (5-MeODMT and 8-OH-DPAT) on 2,2′,2″-tripyridine-induced tremor in mice. Mice were treated IP with (A) 5-HTP (● 50 or △ 100 mg/kg), (B) 5-MeODMT (● 5 or △ 10 mg/kg), or (C) 8-OH-DPAT (● 5 or △10 mg/kg) for 5 min prior to administration of 2,2′,2″-tripyridine (0.3 mg/kg). A difference ($p < 0.01$) between the groups treated with 2,2′,2″-tripyridine with a pretreatment was only obtained with 5-HTP (50–100 mg/kg) and 5-MeODMT (10 mg/kg) and for 8-OHDPAT (10 mg/kg) ($p < 0.05$) compared with the control (○). Each point represents a mean score of 10 animals. Data are presented as mean ± SEM.

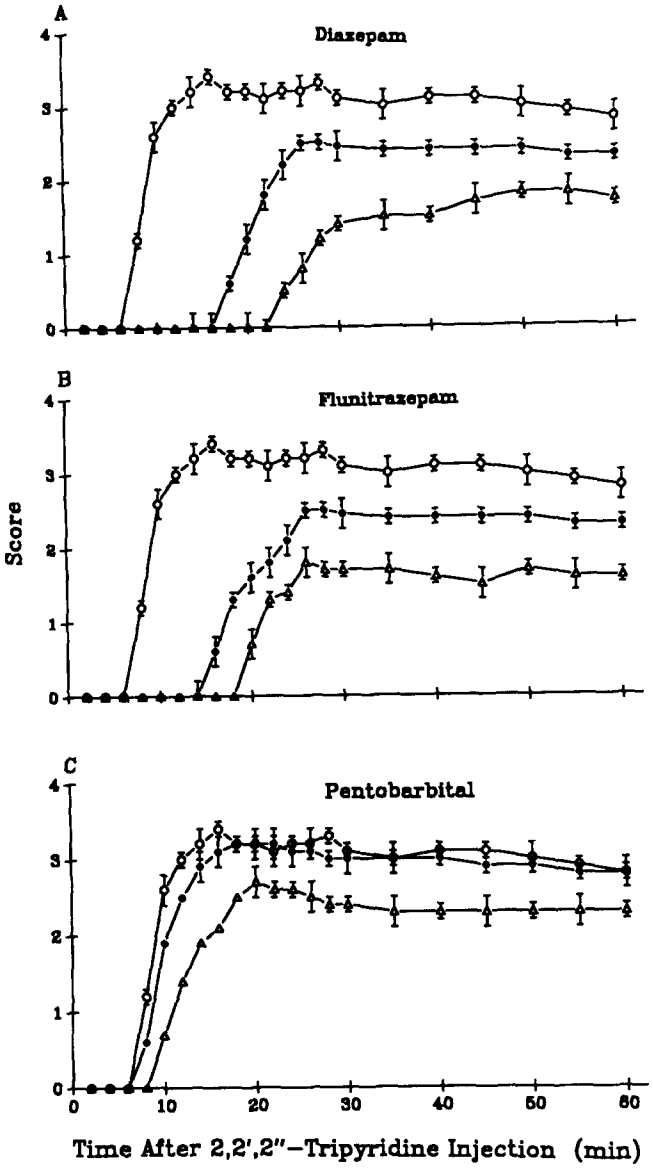

FIG. 4. Effects of diazepam, flunitrazepam, and pentobarital on 2,2',2"-tripyridine-induced tremor in mice. Mice were treated IP with vehicle (○), (A) diazepam (● 10 or △ 20 mg/kg), (B) flunitrazepam (● 10 or △ 20 mg/kg), or (C) pentobarbital (● 10, or △ 30 mg/kg) for 30 min prior to administration of 2,2',2"-tripyridine (1 mg/kg). All pretreatments, except pentobarbital (10 mg/kg), had significant effects by one-way ANOVA, with $p < 0.01$ for diazepam (10–20 mg/kg) and flunitrazepam (10–20 mg/kg) and $p < 0.05$ for pentobarbital (30 mg/kg) compared with the control (○). Each point represents a mean score of egith animals. Data are presented as mean ± SEM.

ED_{40} of 2,2',2"-tripyridine (0.3 mg/kg). 5-MeODMT, a nonselective 5-HT agonist, markedly ehanced the 2,2',2"-tripyridine (0.3 mg/kg)-induced tremor, and 8-OH-DPAT, a 5-HT_{1A} agonist, slightly potentiated the tremor intensity.

Effects of Diazepam, Flunitrazepam, and Pentobarbital on 2,2',2"-Tripyridine-Induced Tremor

The benzodiazepine agonists, diazepam and flunitrazepam, significantly supressed tremor induced by the ED_{99} of 2,2',2"-tripyridine (1 mg/kg), and pentobarbital only ameliorated tremor slightly (Fig. 4). To ascertain whether diazepam acts on presynaptic or postsynaptic 5-HT neurons to modulate the 2,2',2"-tripyridine-induced tremor, we examined tremor to 2,2',2"-tripyridine in mice with PCPA-induced depletion of brain 5-HT. As shown in Fig. 5, there was a significant attenuation in the 2,2',2"-tripyridine-induced tremor intensity in PCPA-pretreated (100 mg/kg, for 3 days) mice, a treatment regimen reported to produce 50–60% depletion of brain 5-HT in mice (7,8). In addition, the inhibitory effect of diazepam and flunitrazepam in PCPA-pretreated mice was more significant than that of untreated control mice.

DISCUSSION

The present behavioral study revealed that pretreatment with 5-HT antagonists, mianserin, cyproheptadine, methysergide, or metergoline, attenuated 2,2',2"-tripyridine-induced

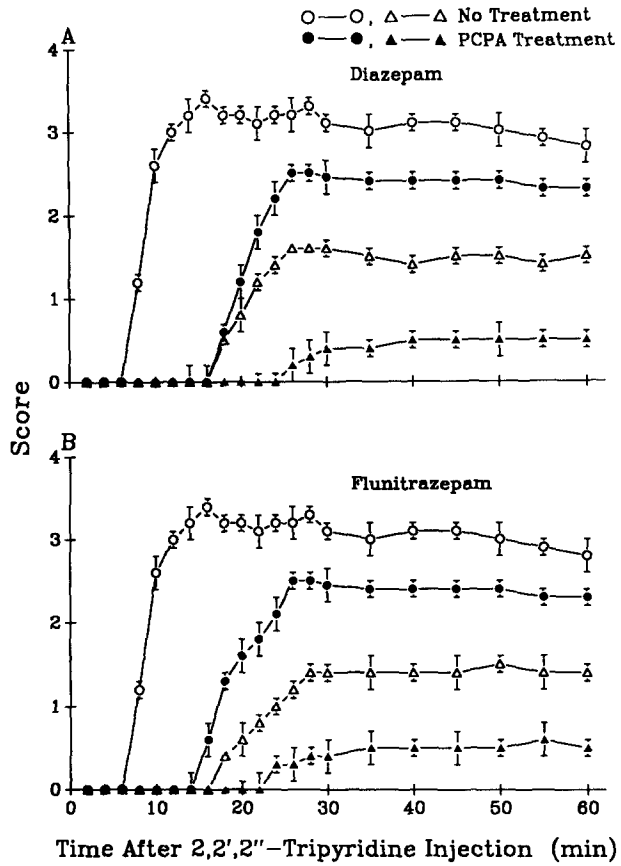

FIG. 5. Effect of PCPA pretreatment on the action of diazepam and flunitrazepam against 2,2',2"-tripyridine-induced tremor in mice. Mice were pretreated with PCPA (100 mg/kg) daily for 3 days (filled symbols) compared with the 0.9% saline control (open symbols). (A) Diazepam (△ ▲ 10 mg/kg) and (B) flunitrazepam (△ ▲ 10 mg/kg) were given IP 30 min prior to administration of 2,2',2"-tripyridine (1 mg/kg). Note that there was a significant attenuation in the 2,2',2"-tripyridine-induced tremor intensity in PCPA-treated mice with (▲) or without (●) diazepam (A) and flunitrazepam (B) pretreatment. There was a difference ($p < 0.01$) between the groups with (▲) and without (△) PCPA treatment during the diazepam and flunitrazepam treatment. Each point represents a mean score of eight animals. Data are presented as mean ± SEM.

tremor in mice, whereas the 5-HT precursor, 5-HTP, or 5-HT agonists (5-MeODMT or 8-OH-DPAT) augmented the effect. In addition, diazepam and flunitrazepam exhibited a dose-dependent protection against 2,2′,2″-tripyridine-induced tremor.

The fact that 2,2′,2″-tripyridine-induced tremor was augmented by 5-MeODMT and 8-OH-DPAT was compatible with the proposed functional correlates of 5-HT receptor subtypes in which tremor might be 5-HT_{1A} receptor mediated (16). From the present study, we considered that 2,2′,2″-tripyridine-induced tremor might also be linked to 5-HT_2 receptor, because the tremor was greatly inhibited by metergoline and mianserin. Methysergide and cyproheptadine had weaker effects than metergoline or mianserin against tremor. These compounds have been shown to be 5-HT_2 antagonists with the properties of 5-HT_1-like partial agonists (11). Therefore, the integrated effect, which is mild against tremor, may be attributable to the possible interaction of neuronal effects at different receptors. 5-HTP has been found to produce both central excitation, demonstrated by the hyperactivity syndrome (5), and depression, evidenced by inhibition of pentylenetetrazol-induced convulsions (2), amphetamine-induced hyperactivity syndrome (16), electroconvulsive seizures (1), and myoclonus induced by urea (10). In the present study, 5-HTP (50 and 100 mg/kg) dose-dependently augmented 2,2′,2″-tripyridine-induced tremor, suggesting that at this dose level 5-HTP can increase 5-HT levels in the brain and increase 5-HT activity.

Evidence for a benzodiazepine receptor interaction with 2,2′,2″-tripyridine-induced tremor is provided by the antagonism study. Diazepam (10 and 20 mg/kg) and flunitrazepam (10 and 20 mg/kg) attenuated 2,2′,2″-tripyridine-induced tremor in a dose-dependent fashion. This is consistent with the notion of an antagonism of 2,2′,2″-tripyridine-induced tremor by diazepam and flunitrazepam in vivo. However, at 10 mg/kg, the sedative and myorelaxant activity of diazepam and flunitrazepam might hamper observation of tremor. To exclude the possibility of a physiological antagonism, which could be due to different mechanisms of action of these drugs, a second experiment was performed with pentobarbital. Pentobarbital is a barbiturate receptor agonist, with sedative and hypnotic effects. In our study, it only had slight inhibitory effects on the 2,2′,2″-tripyridine-induced tremor at 30 mg/kg. Thus, we suggested that the inhibitions of 2,2′,2″-tripyridine-induced tremor by diazepam and flunitrazepam were specific, and benzodiazepine receptors might be involved in these antagonistic effects. In addition, there was a more significant attenuation in the 2,2′,2″-tripyridine-induced tremor intensity by diazepam and flunitrazepam in PCPA-treated mice. Administration of PCPA (100 mg/kg) daily for 3 days was found to produce about 50–60% depletion of the brain content of 5-HT in mice (7,8). In this study, mice that were treated with PCPA (100 mg/kg) daily for 3 days showed potentiation with the inhibitory effects of diazepam (10 mg/kg) and flunitrazepam (10 mg/kg). This finding suggests that the mechanisms involved in the inhibition of tremors by diazepam and flunitrazepam may be associated with central inhibitory action of 5-HT.

It has been reported that GABAergic neuron exert tonic control over ascending 5-HT neuron in the median raphe region (4). In addition, Shukla et al. (17) have demonstrated that the GABAergic inputs in the raphe nucleus are inhibitory in nature and that GABAergic agents, by acting on these receptors located on 5-HT neurons, show a protective action against harmine-induced tremors in mice. The present findings also support an interaction between GABA-benzodiazepine receptors and the 5-HT system in the brain.

In conclusion, this study provided evidence for a serotonin-mediated action of 2,2′,2″-tripyridine in its tremor action, and indicated the benzodiazepines' attenuation of the 2,2′,2″-tripyridine-induced tremor is possibily mediated through the GABAergic inhibition of serotonergic neurons.

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