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Enhancement of serotonergic neural activity contributes to cyclosporine-induced tremors in mice

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Abstract

A single cyclosporine injection (50 mg/kg, i.p.) significantly enhanced harmine- but not oxotremorine-induced tremors in mice. This potentiation became more apparent when cyclosporine (50 mg/kg, i.p.) was administered once a day for seven days. These findings suggest an involvement of monoaminergic mechanisms in cyclosporine-induced tremors. The effects of cyclosporine were examined on the dynamics of noradrenaline, dopamine and serotonin in the mouse brain. Both single and repeated treatment with cyclosporine significantly facilitated the serotonin turnover as estimated from the probenecid-induced accumulation of 5-hydroxyindoleacetic acid, but either mode of treatment failed to change the contents of monoamines and their metabolites or the turnover of noradrenaline and dopamine. Therefore, the cyclosporine-enhanced activity of serotonin neurons may be interpreted as producing adverse central effects, including tremors. © 1998 Elsevier Science B.V.

Keywords: Cyclosporine; Tremor; Harmine; Central nervous system; 5-HT (5-hydroxytryptamine, serotonin) turnover

1. Introduction

Cyclosporine, a powerful immunosuppressant with a specific effect on T-lymphocytes, is one of a family of cyclic peptides consisting of 11 amino acid residues. It is given orally or intravenously for the prophylaxis of graft rejection in organ and tissue transplantations, and is currently being investigated in clinical trials for a wide range of autoimmune diseases. Adverse effects of cyclosporine include nephrotoxicity, hypertension, hepatotoxicity, neurotoxicity, hirsutism, gingival hyperplasia, and gastrointestinal toxicity such as nausea, vomiting, diarrhea, anorexia, and abdominal pain. Increasing attention is being paid to the central adverse effects of cyclosporine; these symptoms include tremors, ataxia, confusion, agitation, mental depression, flushing, headache, sleep disturbances, lethargy, coma, convulsions, leukoencephalopathy, cortical blindness, and spasticity or paralysis of limbs (Durrant et al., 1982; Thompson et al., 1984; Berden et al., 1985; Nordal et al., 1985; Sloane et al., 1985; De Groen et al., 1987; Kunzendorf et al., 1988; Hughes, 1990; Gottrand et al., 1991; Hinchey et al., 1996; Teshima et al., 1996). Hypocholesterolemia (De Groen et al., 1987), hypomagnesemia (Thompson et al., 1984), aluminum overload (Nordal et al., 1985), high-dose methylprednisolone treatment (Durrant et al., 1982), and high serum levels of cyclosporine and its metabolite (Kunzendorf et al., 1988; Gottrand et al., 1991) may induce cyclosporine neurotoxicity. However, adverse neurologic events have been documented in patients with none of these risk factors; these events might be associated with dysfunction of the blood–brain barrier (Sloane et al., 1985; Gottrand et al., 1991). Among these central adverse effects, tremors have been reported to occur in 39% of patients treated with cyclosporine alone (European Multicentre Trial Group, 1983). Cyclosporine-induced tremors are manifested in general as a fine hand tremor with mild severity, and occasionally develop into seizures and/or encephalopathy (Hughes, 1990; Hinchey et al., 1996; Teshima et al., 1996). The mechanism by which cyclosporine produces these tremors has not yet been clarified.

In the present study, we first examined the effect of cyclosporine on harmine- and oxotremorine-induced tremors in mice to elucidate whether monoaminergic and/or cholinergic mechanisms were related to cyclosporine-induced tremors. Based on the behavioral re-

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sults obtained, we further examined the effect of cyclosporine on the mouse brain content and turnover of monoamines including dopamine, noradrenaline and serotonin (5-hydroxytryptamine).

The present study yielded the first evidence that single and repeated administration of cyclosporine enhances serotonergic neural activity in the brain. This enhancement may be involved in mediating the tremorogenic action of cyclosporine.

2. Materials and methods

2.1. Animals

Male day mice weighing 25–30 g (Seiwa Experimental Animals, Fukuoka) were used. The animals were housed in a room at a temperature of 22 ± 2°C under an alternating 12-h light/dark cycle (lights on at 06:00 h). Food and water were given ad libitum. All experiments were performed between 13:00 and 17:00 h. This experiment was approved by the Committee on the Ethics of Animal Experiments of the Faculty of Medicine, Kyushu University, and was carried out in accordance with the Guidelines for Animal Experiments of the Faculty of Medicine, Kyushu University, and The Law (No. 105) and Notification (No. 6) of the Japanese Government.

2.2. Drugs

Cyclosporine (Sandimmun injection) obtained from Sandoz Pharmaceutical (Osaka) was diluted in the vehicle solution consisting of polyoxyethylene castor oil and ethanol (the same mixture as the vehicle of the Sandimmun injection) and used within 3 h. Harmine hydrochloride, oxotremorine, α-methyl-*p*-tyrosine methyl ester hydrochloride (α-MT) and probenecid were purchased from Sigma Chemical (St. Louis, MO). Harmine hydrochloride, oxotremorine and α-MT were dissolved in 0.9% saline. Probenecid was first dissolved in 0.15 M NaOH and the pH was adjusted to 8.5 with 0.2 M HCl. The doses of the salt-form drugs are expressed as the weight of the bases. All drugs were injected intraperitoneally in a volume of 0.1 ml/10 g body weight.

2.3. Behavioral experiment

The mice received a single cyclosporine injection of 20 or 50 mg/kg, i.p. or chronic treatment (50 mg/kg, i.p. daily for seven days). For observation, each mouse was placed in a screened cage ($10 \times 20 \times 15$ cm) at least 30 min before the injection of the tremorogenic agent to allow adaptation to the new environment. Harmine (10 mg/kg) or oxotremorine (0.3 mg/kg) was injected i.p. 55 and 50 min after cyclosporine administration, respectively. Based on the data of preliminary experiments, these doses were selected as inducing a reproducible minimal tremor response. The summed duration of harmine-induced tremors was measured during the 15 min period from 5 to 20 min after harmine administration. Since dose-dependent oxotremorine-induced tremors were short but strong in the preliminary study, they were scored as follows: 0, no tremor; 1, intermittent mild tremor; 2, continuous mild tremor and intermittent moderate tremor; 3, continuous moderate tremor; 4, continuous severe tremor. Assessments were made at 5 min intervals for 30 min (0–30 min after the oxotremorine administration) by the same observer blinded to the pretreatment with cyclosporine or vehicle. Tremor values are expressed as the total of the scores obtained for each 5-min period.

2.4. Determination of monoamines and their metabolites

The mice received single or repeated treatment (once a day for seven days) with 50 mg/kg, i.p. of cyclosporine and were decapitated 1 h after the single or seventh injection. The brain, excluding the cerebellum, was immediately removed, rapidly frozen on dry ice and stored at -80°C until use. The frozen tissue was homogenized in 4 ml of 0.1 M perchloric acid containing 0.1% L-cysteine and 300 ng of deoxyepinephrine as an internal standard. After centrifugation, the supernatant was filtered through a membrane filter (0.45 μm), and a 20-μl aliquot of the filtrate was injected into a high-performance liquid chromatography (HPLC) system with an electrochemical detector for the determination of noradrenaline, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin and 5-hydroxyindoleacetic acid (5-HIAA). The HPLC system consisted of a pump (LC-3A, Shimadzu, Kyoto), a reversed phase column (Eicompak MA-5ODS, 4.6×150 mm, Eicom, Kyoto), and an electrochemical detector (ECD-100, Eicom). The electrode potential was set at $+0.65$ V versus the Ag/AgCl reference electrode. The mobile phase used for the assay was 0.08 M phosphate buffer containing 1.5 mM sodium octanesulfonate, 8% acetonitrile, and 10 μM EDTA, pH 4.0.

2.5. Estimation of monoamine turnover in the brain

The turnovers of noradrenaline and dopamine were estimated from the decreases in their levels induced by the treatment with α-MT (315 mg/kg, i.p.) (Brodie et al., 1966). The turnover of serotonin was estimated from the accumulation of 5-HIAA induced by the treatment with probenecid (200 mg/kg, i.p.) (Neff et al., 1967). α-MT or probenecid was injected immediately after the single treatment or 1 h after the seventh treatment with cyclosporine. The mice were then killed 90 and 180 min after the α-MT injection or 60 and 120 min after the probenecid injection.

2.6. Statistics

All data are presented as means $\pm$ S.E.M. The behavioral data were analyzed by Kruskal–Wallis rank test, and

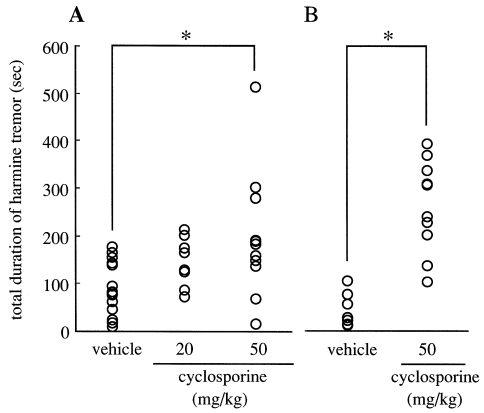

Fig. 1. (A) Effects of single cyclosporine administration on harmine-induced tremors. Mice were injected i.p. with harmine hydrochloride (10 mg/kg) 55 min after treatment with vehicle or cyclosporine (20 and 50 mg/kg) ($n = 10$–15 per group). (B) Effects of repeated cyclosporine administration on harmine-induced tremors. Mice were treated with vehicle or cyclosporine (50 mg/kg) once a day for 7 d and injected i.p. with harmine hydrochloride (10 mg/kg) 55 min after the seventh treatment ($n = 10$ per group). $^{*}P < 0.05$ compared with the vehicle-treated group.

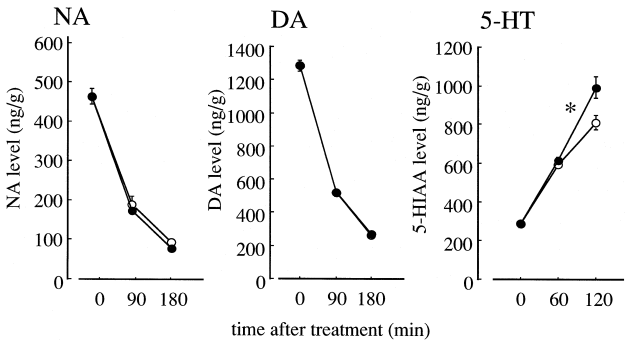

Fig. 2. Effects of single cyclosporine administration on monoamine turnover in mouse brain. Mice were injected i.p. with α-MT (315 mg/kg) or probenecid (200 mg/kg) immediately after the treatment with vehicle (open circles) or cyclosporine (50 mg/kg; closed circles). Each result represents the mean ± S.E.M. for eight animals. $^{*}P < 0.05$ compared with the vehicle-treated group.

individual comparisons were performed by means of the Mann–Whitney *U*-test if a significant difference was shown in the Kruskal–Wallis rank test. The neurochemical data were analyzed by one-way or two-way analysis of variance (ANOVA) followed by post-hoc Sheffe's *F*-test. Differences were regarded as significant at $P < 0.05$.

3. Results

3.1. Effects of cyclosporine on harmine- and oxotremorine-induced tremors

Mild tremors occurred at 5 min, peaked at 10–15 min and disappeared 20 min after the injection of harmine (10 mg/kg, i.p.). The total duration of the harmine-induced tremors in the mice pretreated with vehicle, cyclosporine 20 mg/kg and 50 mg/kg were 80 ± 15, 117 ± 24 and 199 ± 40 s/15 min ($P < 0.05$), respectively (Fig. 1A). When vehicle and cyclosporine 50 mg/kg were administered to mice once a day for 7 d, the total duration of the harmine-induced tremors was 33 ± 11 and 262 ± 31 s ($P < 0.05$), respectively (Fig. 1B). The chronic treatment with cyclosporine produced more marked prolongation of the duration of harmine-induced tremors than did the single cyclosporine treatment.

Oxotremorine (0.3 mg/kg, i.p.) evoked mild tremors which occurred at 2–3 min, peaked at 5–15 min and disappeared 30 min after the injection. The total scores for intensity in the oxotremorine-induced tremors in the mice pretreated with vehicle, cyclosporine 20 mg/kg and 50 mg/kg were 7.8 ± 0.8, 8.4 ± 0.6 and 8.9 ± 0.7/30 min, respectively. Cyclosporine had no significant effect on the intensity of the oxotremorine-induced tremors (data not shown).

3.2. Effects of cyclosporine on the levels of monoamines and their metabolites and the turnovers of monoamines in the mouse brain

Neither the single nor the repeated administration of cyclosporine had any significant effect on the brain levels

Table 1
Effects of single and repeated cyclosporine administration on the levels of monoamines and their metabolites in the mouse brain

Treatment	Dose (mg/kg, i.p.)	Brain levels (ng/g)				
		NA	DA	DOPAC	5-HT	5-HIAA
Single administration						
Vehicle		490 ± 5	1264 ± 26	134 ± 8	715 ± 18	297 ± 12
Cyclosporine	50	487 ± 6	1247 ± 28	131 ± 9	759 ± 31	335 ± 28
Repeated administration						
Vehicle		448 ± 19	1309 ± 40	117 ± 8	725 ± 14	327 ± 13
Cyclosporine	50	448 ± 13	1266 ± 28	118 ± 4	722 ± 19	332 ± 12

Mice were killed 1 h after the single or seventh treatment with cyclosporine (50 mg/kg, i.p.) or vehicle. Each value represents the mean ± S.E.M. for eight animals.
NA; noradrenaline, DA; dopamine, DOPAC; 3,4-dihydroxyphenylacetic acid, 5-HT; 5-hydroxytryptamine, 5-HIAA; 5-hydroxyindoleacetic acid.

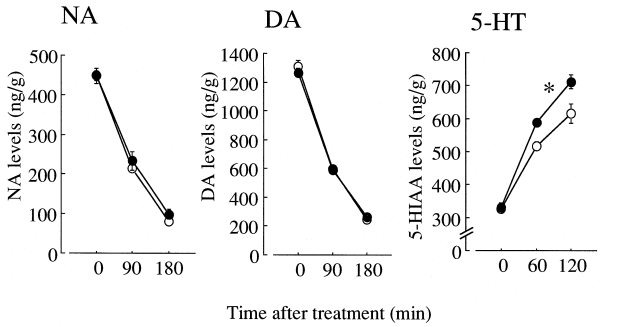

Fig. 3. Effects of repeated cyclosporine administration on monoamine turnover in mouse brain. Mice were injected i.p. with α-MT (315 mg/kg) or probenecid (200 mg/kg) 1 h after the seventh treatment with vehicle (open circles) or cyclosporine (50 mg/kg; closed circles). Each result represents the mean ± S.E.M. for eight animals. $^{*}P < 0.05$ compared with the vehicle-treated group.

of noradrenaline, dopamine, DOPAC, serotonin and 5-HIAA, compared with those in the vehicle-treated group (Table 1). Both single and repeated cyclosporine administration failed to change the α-MT-induced decreases of noradrenaline and dopamine, but significantly increased the probenecid-induced accumulation of 5-HIAA (Figs. 2 and 3).

4. Discussion

The present study demonstrated for the first time that cyclosporine enhanced harmine-induced tremors and serotonergic neural activity in mice. This stimulatory action of cyclosporine on serotonin neurons in the brain may be closely related to the occurrence of adverse central effects such as tremor, convulsion and encephalopathy during cyclosporine therapy.

In the behavioral experiment, both single and repeated treatments with cyclosporine significantly enhanced the harmine-induced tremors but not the oxotremorine-induced tremors in mice. Oxotremorine- and harmine-induced tremors in animals may arise through modulation of the central cholinergic system (Frances et al., 1980; Kawanishi et al., 1981) and of the serotonin, catecholamine and γ-aminobutyric acid neurons, respectively (Cox and Potkonjak, 1971; Kelly and Naylor, 1974; Costall et al., 1976; Kawanishi et al., 1981). Kawanishi et al. (1981) showed that harmine-induced tremors in mice were augmented by a serotonin precursor (5-hydroxytryptophan) and inhibited by serotonin synthesis inhibitors (*p*-chlorphenylalanine and *p*-chloramphetamine) and dopaminergic activators (L-DOPA, apomorphine and amantadine). It is therefore likely that harmine induces tremors by activating serotonergic neurons and inhibiting dopaminergic neurons. Our present behavioral findings thus suggest that monoaminergic mechanisms are involved in the mediation of cyclosporine-induced tremors. We therefore examined the effects of cyclosporine on the dynamics of noradrenaline, dopamine and serotonin in the mouse brain. Both single and repeated cyclosporine administration significantly increased serotonin turnover but not noradrenaline or dopamine turnover in the mouse brain, suggesting that cyclosporine enhances serotonergic neural activity. These neurochemical results are support for the present behavioral observations. A study of neurotransmitter release in PC12 and rat striatal synaptosomes revealed that immunosuppressants facilitate and/or inhibit the release of neurotransmitters, depending on the stimulation of tissues through immunophilins, by influencing the phosphorylation state of synaptic vesicle-associated proteins and nitric oxide formation (Steiner et al., 1996). Further studies are required to clarify the precise mechanism by which cyclosporine stimulates serotonergic neural activity.

Consistent with the abnormal behavior in animals, including scratching, head twitch and tremor due to activation of serotonergic neurotransmission, the serotonin syndrome is most commonly seen in patients receiving a combination of serotonin-potentiating agents (Sporer, 1995). The serotonin syndrome is characterized by a constellation of at least 3 of the following symptoms: mental status change, agitation, myoclonus, hyperreflexia, fever, shivering, tremor, diaphoresis, ataxia and diarrhea (Sternbach, 1991). These symptoms also appear in some patients who are undergoing cyclosporine therapy. Organ transplant recipients often have clinical problems due to depression and anxiety disorders, thereby leading to combined treatment with serotonergic agents and cyclosporine. In these patients, the possible interaction between cyclosporine and serotonin-potentiating agents, such as monoamine oxidase inhibitors, tricyclic antidepressants, lithium and selective serotonin reuptake inhibitors, on serotonin neurons presents an increased risk of serotonin syndrome.

In addition, serotonin is one of the most powerful vasoactive amines that produce vasoconstriction in cerebral vessels and increase microvascular permeability leading to edema. Elevation of serotonin levels has been documented in the cerebrospinal fluid, brain and spinal cord after traumatic, ischemic and metabolic insults to the central nervous system (Sharma et al., 1990; Globus et al., 1992; Olsson et al., 1992). It has become apparent in recent years that cyclosporine administration can cause encephalopathy (Hinchey et al., 1996; Teshima et al., 1996). The most common abnormality detected by neuroimaging in patients was edema in the brain, predominantly in the posterior portions of the cerebral white matter. This cerebrotoxicity of cyclosporine may reflect a direct induction of brain cell death, especially that of oligodendrocytes and neurons (McDonald et al., 1996). However, taking the findings described above into consideration, the possible involvement of an excitatory action of cyclosporine on serotonin neurons in this event cannot be excluded. The accumulated evidence lends further support to the notion that enhancement of serotonergic activity by cyclosporine contributes to the occurrence of central adverse effects.

In conclusion, the cyclosporine-enhanced activity of serotonin neurons may be interpreted as producing adverse central effects, including tremors. The present findings may help in the quest to alleviate adverse central effects and to improve the quality of life in patients receiving cyclosporine therapy.

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