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Full paper

Serotonergic modulation of nicotine-induced kinetic tremor in mice

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ABSTRACT

We previously demonstrated that nicotine elicited kinetic tremor by elevating the neural activity of the inferior olive via α7 nicotinic acetylcholine (nACh) receptors. Since α7 nACh receptors reportedly facilitate synaptic monoamine release, we explored the role of 5-HT receptors in induction and/or modulation of nicotine tremor. Treatment of mice with nicotine induced kinetic tremor that normally appeared during movement. The 5-HT_{1A} agonist, 8-hydroxydipropylaminotetraline (8-OH-DPAT), significantly enhanced nicotine-induced tremor and the action of 8-OH-DPAT was antagonized by WAY-100135 (5-HT_{1A} antagonist). In addition, the cerebral 5-HT depletion by repeated treatment with p-chlorophenylalanine did not reduce, but rather potentiated the facilitatory effects of 8-OH-DPAT. In contrast, the 5-HT_2 agonist, 2,5-dimethoxy-4-iodoamphetamine (DOI), significantly attenuated nicotine tremor, which was antagonized by ritanserin (5-HT_2 antagonist). The 5-HT_3 agonist SR-57227 did not affect nicotine-induced tremor. Furthermore, when testing the direct actions of 5-HT antagonists, nicotine tremor was inhibited by WAY-100135, but was unaffected by ritanserin, ondansetron (5-HT_3 antagonist) or SB-258585 (5-HT_6 antagonist). These results suggest that postsynaptic 5-HT_{1A} receptors are involved in induction of nicotine tremor mediated by α7 nACh receptors. In addition, 5-HT_2 receptors have an inhibitory modulatory role in induction of nicotine tremor.

1. Introduction

Nicotine induces a variety of pharmacological actions such as antidepressant actions, cognitive enhancement, positive reinforcement and motor excitement.[1–8] These actions are mediated by multiple nicotinic acetylcholine (nACh) receptors which are the pentameric cationic channel constructed from various combinations of α-subunits (α2–α10) and β-subunits (β2–β4).[9–11] Specifically, homomeric α7 nACh and heteromeric α4β2 nACh receptors are the most abundant subtypes in the brain and primarily mediate the effects of nicotine in the central nervous system.[9–13]

Nicotine produces diverse motor symptoms in animals, including hyperlocomotion, Straub tail, tremor and convulsive seizures.[1,2,6,7,14–21] We have shown that relatively low doses of nicotine (0.5–1 mg/kg, i.p.) elicited kinetic tremor in rats and mice by activating α7 nACh, but not α4β2 nACh, receptors.[21] Immunohistochemical analysis of Fos expression, a biological marker of neural activities,[22] revealed that nicotine-induced tremor was associated with α7 nACh receptor-mediated neural excitation of the inferior olive (IO). In addition, electric lesioning of the IO suppressed nicotine-induced tremor, illustrating that nicotine elicits kinetic tremor by activating the IO neurons via α7 nACh receptors.[21]

Tremor is the involuntary extrapyramidal movement disorders, which manifests uncontrollable rhythmic movement of a part (e.g., limbs, neck, head and leg) of or whole body.[23] Tremor can be classified into several categories including essential tremor and parkinsonian tremor, the former primarily showing kinetic tremor while the latter resting tremor. In addition, potential causal sites for essential tremor and parkinsonian tremor are considered to be the IO-cerebellar and the basal ganglia (e.g., striatum) regions, respectively.[23–27] Thereby, our findings that activation of the IO neurons is linked to nicotine-induced tremor suggest pharmacological similarities between nicotine tremor and essential tremor.

Regarding the action mechanisms of nicotine, it is known that nicotine enhances synaptic release of monoamines (e.g., 5-HT, noradrenaline and dopamine).[28–33] Specifically, presynaptic α7

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nACh receptors located in the axon terminals increase monoamine release and produce a variety of nicotine actions.[28,31,33] In addition, monoamines (especially 5-HT and noradrenaline) are known to be involved in tremor induction, since harmaline, which inhibits monoamine oxidase A and elevates the levels of 5-HT and noradrenaline,[34] elicits kinetic tremor resembling essential tremor[35–38] and since 5-HT and noradrenaline reportedly induce or facilitate tremor behaviors.[39–41] It is therefore possible that nicotine-induced tremor is mediated and/or modulated by monoamine receptors.

In the present study, we examined the effects of multiple 5-HT receptor ligands on nicotine-induced tremor to elucidate the causal and/or modulatory role of the serotonergic system in nicotine tremor. Since the serotonergic system is known to regulate the induction of parkinsonian symptoms,[27] the effects of 5-HT receptor ligands nicotine-induced tremor are discussed in comparison with those on parkinsonian symptoms.

2. Materials and methods

2.1. Animals

Male ddY mice (Japan SLC, Shizuoka, Japan) at 6–8 weeks of age were used. The animals were kept in air-conditioned rooms (24 ± 2 °C and 50 ± 10% relative humidity) under a 12-h light/dark cycle (light on: 8:00 a.m.) and allowed ad libitum access to food and water. The housing conditions and the animal care methods complied with the Guide for the Care and Use of Laboratory Animals of the Ministry of Education, Science, Sports and Culture of Japan. The experimental protocols were approved by the Experimental Animal Research Committee at Osaka University of Pharmaceutical Sciences.

2.2. Evaluation of nicotine-induced tremor

Animals were intraperitoneally injected with nicotine (1 mg/kg) and placed individually in an observation box (25 × 42 × 20 cm). Tremor duration and intensity were measured in a time-sampling manner over 1–2, 3–4, 5–6, 7–8 and 9–10 min (each 1-min measurement) after the injection of nicotine. The tremor intensity was evaluated using a 4-point ranked scale, 0: none, 1: weak (mild tremor in limited regions including forelimbs, neck, tail and head, usually with Straub tail), 2: moderate (apparent tremor in extended regions including the upper body, trunk and head), 3: marked (intensive tremor over the whole body). The total duration of tremor and the total score of tremor intensity were expressed as the sum of the duration and tremor score at each time point during the 10-min observation period.

2.3. Treatment with 5-HT receptor ligands

To evaluate the role of 5-HT receptors in modulating nicotine-induced tremor, a selective 5-HT_{1A} agonist 8-OH-DPAT (1, 3 mg/kg), 5-HT_2 agonist DOI (0.1–1 mg/kg) or 5-HT_3 agonist SR-57227 (1, 3 mg/kg) was intraperitoneally administered 15 min before the nicotine (1 mg/kg, i.p.) injection. In the combined treatment with 5-HT antagonists, a selective 5-HT_{1A} antagonist WAY-100135 (10 mg/kg, i.p.), 5-HT_2 antagonist ritanserin (3 mg/kg, i.p.) or saline (control) was simultaneously administered with the respective receptor agonist. The dosage of each 5-HT antagonist was set to effectively antagonize each 5-HT receptor according to previous reports.[42–45] In addition, to evaluate the direct actions of the 5-HT antagonists against nicotine-induced tremor, WAY-100135 (10 mg/kg, i.p.), ritanserin (3 mg/kg, i.p.), ondansetron (5-HT_3 antagonist, 1 mg/kg, i.p.) or SB-258585 (5-HT_6 antagonist, 10 mg/kg) per se was also administered 15 min before the nicotine (1 mg/kg, i.p.) injection. Measurement of nicotine-induced tremor was performed as described previously.

2.4. Treatment with p-chlorophenylalanine (PCPA)

To inactivate serotonergic neurons, an irreversible inhibitor of tryptophan hydroxylase PCPA (300 mg/kg) or saline (control) was intraperitoneally administered once daily for 3 consecutive days, as reported previously.[46] On the third day, the effects of 8-OH-DPAT on nicotine-induced tremor were evaluated using PCPA- or saline-treated mice. Animals were first treated with 8-OH-DPAT (3 mg/kg) or saline 105 min after the last injection of PCPA and, 15 min later, they were administered nicotine (1 mg/kg, i.p.). Measurement of nicotine-induced tremor was performed as described previously.

2.5. Drugs

(−)-Nicotine, (±)-8-OH-DPAT hydrobromide, (±)-DOI hydrochloride, ritanserin, ondansetron hydrochloride dehydrate and DL-PCPA methylester hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO), (S)-WAY-100135 dihydrochloride, SR-57227 hydrochloride and SB-258585 hydrochloride were obtained from Tocris (Bristol, UK). Nicotine, DOI, Ondansetron, SR-57227, SB-258585 and PCPA were dissolved in saline. WAY-100135, 8-OH-DPAT and ritanserin were first dissolved in 1% lactate solution and diluted with saline.

2.6. Statistical analysis

Data are expressed as the mean ± S.E.M. Statistical significance of differences among multiple groups was determined by Kruskal–Wallis test followed by the Steel-Dwass post-hoc comparison test. The time-course data was analyzed by two-way ANOVA followed by the Tukey post-hoc comparison test. Comparisons between only two groups were performed by Mann–Whitney's U-test. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of 5-HT agonists on nicotine-induced tremor

As reported previously,[21] nicotine at 1 mg/kg (i.p.) evoked kinetic tremor, which mostly appeared during movements (e.g., locomotion and rearing). We first examined the effects of 5-HT agonists, 8-OH-DPAT (a selective 5-HT_{1A} agonist), DOI (a selective 5-HT_2 agonist) and SR-57227 (a selective 5-HT_3 agonist), on nicotine-induced tremor. Treatment of mice with 8-OH-DPAT (3 mg/kg, i.p.) significantly increased the tremor intensity and duration induced by nicotine (1 mg/kg, i.p.) (Fig. 1A). The total score of nicotine-induced tremor during the 10-min observation period was significantly increased by 8-OH-DPAT ($\chi^2 = 7.5454$, df = 2, $P = 0.0230$) (Fig. 2A). The total duration of nicotine-induced tremor also tended to increase although this did not reach statistical significance. In contrast to 8-OH-DPAT, a 5-HT_2 agonist DOI (1 mg/kg, i.p.) significantly attenuated nicotine-induced tremor (Fig. 1B). DOI significantly reduced the total tremor score and the total duration of tremor in a dose-related manner (total score: $\chi^2 = 10.2614$, df = 3, $P = 0.0165$; total duration: $\chi^2 = 8.9805$, df = 3, $P = 0.0296$) (Fig. 2B). However, the 5-HT_3 agonist SR-57227 did not show significant effects on nicotine-induced tremor (Figs. 1C and 2C).

We next examined the effects of 5-HT antagonists against the actions of 8-OH-DPAT and DOI. A selective 5-HT_{1A} antagonist WAY-