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Effects of β-Blockers and Nicardipine on Oxotremorine-Induced Tremor in Common Marmosets

Minoru Mitsuda, Masahiro Nomoto* and Shin-ichi Iwata

Department of Pharmacology, Faculty of Medicine, Kagoshima University, 8–35–1, Sakuragaoka, Kagoshima 890–8520, Japan

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ABSTRACT—Effects of β-blockers (propranolol, arotinolol and nipradilol) and a Ca^{2+} channel blocker (nicardipine) on oxotremorine-induced tremor were studied in common marmosets. Generalized tremor was elicited by an intraperitoneal administration of 0.25 mg/kg oxotremorine. Intensity of the tremor was classified into 7 degrees, and it was evaluated every 10 min. The total intensity of oxotremorine-induced tremor for each drug was expressed as "points", which were the sum of tremor intensity scores evaluated every 10 min up to 190 min following the administration of oxotremorine. β-Blockers significantly suppressed the tremor. On the other hand, the Ca^{2+} channel blocker exacerbated the tremor.

Keywords: Oxotremorine-induced tremor, β-Blocker, Ca^{2+} channel blocker

β-Blockers have been used for alleviating tremor in patients with essential tremor and Parkinson's disease, and their mechanisms of anti-tremor action have also been investigated using experimental animals. Recently, it has been reported that some Ca^{2+} channel blockers significantly suppressed tremor in patients with essential tremor (1–3). In contrast, Topaktas et al. reported that Ca^{2+} channel blockers were not effective in reducing the tremor (4). Thus, efficacy of Ca^{2+} channel blockers on essential tremor is in dispute. Oxotremorine-induced tremor was suppressed by the same kind of drugs that are effective on essential tremor, i.e., β-blockers, and resembles essential tremor more than harmaline-induced and TRH-induced tremors (5). Therefore, oxotremorine was adopted as a tremologenic agent in the present study. We used β-blockers (propranolol, arotinolol and nipradilol) and a Ca^{2+} channel blocker (nicardipine) in doses equivalent to those used in humans, and investigated their anti-tremor effects on oxotremorine-induced tremor in common marmosets, which are primates small enough to be easily handled and to be kept as a group in a room.

Four common marmosets (male : female = 1 : 1, weighing 270–310 g) were employed in the experiment. Oxotremorine, propranolol and nicardipine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Nipradilol was donated by Kowa Pharmaceutical Co. (Nagoya). Arotinolol was provided by Sumitomo Pharmaceutical Co., Ltd. (Osaka). All drugs were dissolved in 0.9% saline, and the volume of solution was adjusted to 1 ml/kg. β-Blockers and nicardipine were intraperitoneally injected 20 min before administration of oxotremorine. Tremor was chronologically scored by classifying the head tremor into 7 degrees of intensity (Table 1) after the intraperitoneal injection of 0.25 mg/kg oxotremorine. Propranolol (2.5 and 5.0 mg/kg), arotinolol (1.25 and 2.5 mg/kg), nipradilol (0.5 and 1.0 mg/kg) and nicardipine (5.0 and 10 mg/kg) were used. When common marmosets lie down, the head tremor is reduced and it is difficult to evaluate the score of tremor. Therefore, we evaluated head tremor in the uplifted head. The total intensity of the tremor was calculated by adding the score of tremor

Table 1. Scoring of head tremor intensity in common marmosets

Score	Head tremor intensity
0	None
1	Sometimes
2	Often
3	Continuous (weak)
4	Continuous (moderate)
5	Continuous (marked)
6	Continuous (very marked)

Intensity of the head tremor was classified into 7 degrees and evaluated every 10 min after administration of oxotremorine in common marmosets.

* To whom correspondence should be addressed.

every 10 min up to 190 min, and this was expressed as "points."

Statistical analysis was done by the Kruskal-Wallis test; thereafter, the statistical significance of the difference between the control and each of the groups was evaluated by the Mann-Whitney *U*-test. A level of $P<0.05$ was considered statistically significant.

Oxotremorine-induced generalized tremor in common marmosets, which started after 3–5 min, was maximized at 30–60 min, and it continued for approximately 110–120 min after the administration of oxotremorine. The animal also defecated, urinated, produced tears and hypersalivated. The total intensity of the tremor in animals pretreated with saline, propranolol (2.5 mg/kg, 5.0 mg/kg), arotinolol (1.25 mg/kg, 2.5 mg/kg) nipradilol (0.5 mg/kg, 1.0 mg/kg) or nicardipine (5.0 mg/kg, 10 mg/kg) was shown in Fig. 1. All β-blockers significantly suppressed oxotremorine-induced tremor compared with the control. On the other hand, there was no significant difference in the total intensity of the tremor in the group treated with 5.0 mg/kg nicardipine compared with the control. Conversely, 10 mg/kg nicardipine significantly potentiated the tremor. Nicardipine (10 mg/kg) alone evoked no tremor in the animal.

It has been reported that β-blockers suppressed drug-induced tremors in experimental animals. In the present study, all β-blockers suppressed oxotremorine-induced tremor in common marmosets just as we previously reported in mice (5). The reason that β-blockers reduce the tremor is still unknown. However, it seems that β-blockers reduce the tremor mainly by affecting the peripheral nervous system, since β-blockers which cannot cross the blood-brain barrier show anti-tremor action. Moreover, we suggested that features of β-blockers (i.e., β_1- or β_2-selectivity, intrinsic sympathomimetic activity and membrane stabilizing activity) did not primarily contribute to the suppression of the tremor; in other words, that β-blocking activity per se in β-blockers may be important for it (5). A one-fifth dose of arotinolol, compared with the dose of propranolol, could alleviate tremor in patients with essential tremor to the same degree as propranolol (6). It also supports this hypothesis, since the β-blocking activity of orally administered arotinolol on reducing tachycardia induced by isoproterenol is higher than that of propranolol in dogs (7). In the present study, the anti-tremor effect of 2.5 mg/kg arotinolol on oxotremorine-induced tremor was twice as much as that of 2.5 mg/kg propranolol. However, propranolol at a dose of 5 mg/kg showed less anti-tremor effect than a dose of 2.5 mg/kg. The discrepancy in these results may be due to dual effects of propranolol. Low doses, such as 1 μmol/kg (0.295 mg/kg), of propranolol significantly antagonized oxotremorine-induced tremors in mice, while high doses of more than 10 μmol/kg (2.9581 mg/kg), in contrast, facilitated these tremors (8). Propranolol inhibited brain cholinesterase enzyme activity at doses of more than 10 μmol/kg, which enhanced oxotremorine-induced tremor in mice (8). Because oxotremorine may produce tremor in animals through the excitation of the cholinergic system in the brain (9), it seems that propranolol caused more dominant action to block peripheral β-adrenergic functions than that to inhibit brain cholinesterase activity at a dose of 2.5 mg/kg, and vice versa at the dose of 5 mg/kg. In humans, the doses of up to 320 mg/day of propranolol dose-dependently suppressed the tremor in patients with essential tremor, whereas higher doses of more than 320 mg/day caused adverse reaction against the anti-tremor action (10). However, the reason for the adverse reaction of propranolol at the high doses to essential tremor is still unclear.

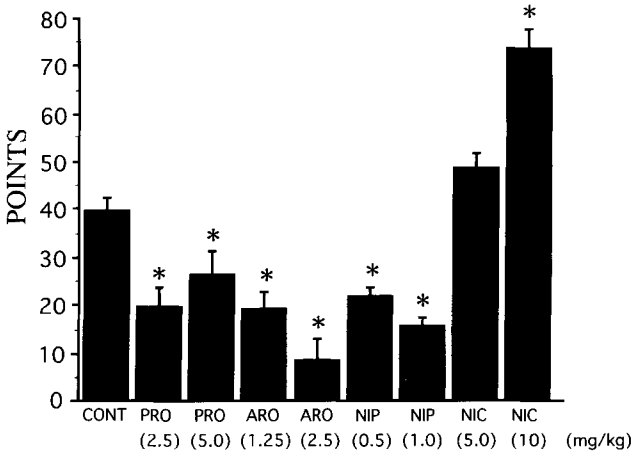

Fig. 1. Effects of β-blockers and nicardipine on oxotremorine-induced tremor in common marmosets. All β-blockers significantly suppressed the tremor compared with the control. There was no significant difference in the total intensity of the tremor in the group treated with 5.0 mg/kg nicardipine compared with the control, but 10 mg/kg nicardipine significantly potentiated the tremor. CONT: control, PRO: propranolol, ARO: arotinolol, NIP: nipradilol, NIC: nicardipine. The ordinate is the sum of tremor intensity scores of the oxotremorine-induced tremors after every 10 min up to 190 min, which is expressed as points. Each column is the mean ± S.E.M. of four experiments. Statistical analysis was done by the Kruskal-Wallis test, followed by the Mann-Whitney *U*-test. (*$P<0.05$ as compared with the control).

Recently, it has been reported that some Ca^{2+} channel blockers are effective for the treatment of patients with essential tremor. These are flunarizine (1), nicardipine (2, 3) and nimodipine. It does not seem that flunarizine is worth prescribing for the patient with essential tremor, since it may cause extrapyramidal disorders such as parkinsonism, akathisia, orofacial dyskinesia, dystonia, and postural tremor because of its D_2-dopamine-receptor—

blocking properties (11). Indeed, its long-term use for essential tremor failed to sustain its anti-tremor effect (12), and it was ineffective for the moderate to severe tremor in patients with essential tremor (13). García Ruiz et al. reported that a single oral dose of 30 mg of nicardipine could ameliorate the amplitude of tremor in patients with essential tremor, but chronic nicardipine administration (60 mg/day) failed to sustain the initial improvement (2). On the contrary, Topaktas et al. reported that a single oral dose of 10 mg of nifedipine, belonging to the same dihydropyridine group as nicardipine, increased the tremor in patients with essential tremor (4). In our study, 5 mg/kg nicardipine showed no significant effect, whereas a high dose (10 mg/kg) of nicardipine enhanced the oxotremorine-induced tremor in marmosets. Mena et al. showed that Ca^{2+} channel blockers involving flunarizine and nicardipine reduce ^{3}H-spiperone binding to bovine striatal membranes, uptake and release of dopamine in catecholamine-rich human neuroblastoma cells NB69, and apomorphine-induced rotation in 6-OH-dopamine-lesioned rats (14). Thus, nicardipine can attenuate the dopaminergic system in the striatum. Nicardipine produced a reduction of apomorphine-induced rotation in rats with 6-OH-dopamine-induced unilateral nigrostriatal lesions, which was not significant at a dose of 10^{-6} mol/kg (0.516 mg/kg) and was significant at doses of more than 10^{-5} mol/kg (5.16 mg/kg) (14). Hence, the doses of more than 10^{-5} mol/kg (5.16 mg/kg) of nicardipine can exert dopamine receptor-blocking properties and enhancing effects on oxotremorine-induced tremor. Consequently, this may be explained by the activation of the acetylcholinergic system through the inactivation of the dopaminergic system in the striatum, because oxotremorine-induced tremor is elicited by the excitation of the cholinergic system in the brain. Ca^{2+} channel blockers reduce the blood pressure, which may cause activation of the sympathetic nervous system to maintain the blood pressure at a normal level and subsequently may increase the tremor. Imai et al. reported that the plasma concentration of catecholamines increased corresponding to the blood pressure reduction induced by nicardipine in rats (15). Accordingly, another possible explanation for the enhancing effect of nicardipine on oxotremorine-induced tremor in marmosets might be a reflex activation of the sympathetic nervous system to maintain the blood pressure. Nicardipine (10 mg/kg) alone, however, evoked no tremor in marmosets, so there may be no significant contribution of the reflex activation on the tremor in the doses of nicardipine applied in this study.

In conclusion, β-blockers alleviated oxotremorine-induced tremor in common marmosets; in contrast, nicardipine enhanced the tremor.

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