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Involvement of nitric oxide in development of tail-tremor induced by repeated nicotine administration in rats

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Abstract

Daily administration of nicotine (0.5 mg/kg per day s.c.) to rats caused a tremor that appeared only in the tail (tail-tremor) and which became more marked over 8 days. Nitric oxide (NO) synthase inhibitors, N_w-nitro-L-arginine (10 mg/kg per day i.p.) or N_w-nitro-L-arginine methyl ester (20 and 40 mg/kg per day i.p.), administered each day before nicotine attenuated the development of the tail-tremor. However, neither N_w-nitro-L-arginine (2–10 mg/kg i.p.) nor N_w-nitro-L-arginine methyl ester (10–40 mg/kg i.p.) affected the tail-tremor that developed after 14 days of repeated nicotine administration. The noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, MK-801 ((+)-5-methyl-10,11,-dihydro-5*H*-dibenzo[*a*,*b*]cyclohepten-5,10-imine hydrogen maleate) at 0.2 mg/kg per day (i.p.), or competitive antagonist, CPP (3-[(±)-2-carboxypiperazin-4-yl] propyl-1-phosphonic acid) at 2 mg/kg per day (i.p.), administered each day before nicotine attenuated the development of the tail-tremor. MK-801 (0.01–0.2 mg/kg i.p.) but not CPP (0.5–4 mg/kg i.p.) suppressed the tail-tremor that developed after 14 days of repeated nicotine administration. These results suggest that NO formation mediated by NMDA receptors is involved in the mechanisms underlying the tail-tremor induced by the repeated administration of nicotine. © 1997 Elsevier Science B.V.

Keywords: Nicotine; Tail-tremor; Chronic administration; Nitric oxide (NO); NMDA receptor

1. Introduction

Chronic smoking induces not only the addictive properties of tobacco use but also hand tremor (Shiffman et al., 1983). In behavioral studies with rats, repeated exposure to cigarette smoke or nicotine causes a tremor only in the tail (tail-tremor) (Gomita et al., 1988, 1991). Oxotremorine- and harmine-induced whole body tremors are accompanied by rigidity or immobility of the whole body in rodents. However, nicotine-induced tail-tremor is associated with locomotor hyperactivity without rigidity or immobility of the whole body, suggesting that the nicotine-induced tail-tremor model is useful for studying the mechanism underlying tremor associated with movement (Suemaru et al., 1994).

Nitric oxide (NO), which is synthesized from L-arginine by NO synthase, is released from neurons following stimulation of the excitatory NMDA receptor and may act as a novel intercellular messenger in the central nervous system (Bruhwyler et al., 1993; Snyder and Bredt, 1991). The NO synthase inhibitors, N_w-nitro-L-arginine methyl ester (L-NAME) and N_w-nitro-L-arginine (L-NA), attenuate sensitization to the locomotor-stimulating effects of cocaine (Pudiak and Bozarth, 1993) and methamphetamine (Ohno and Watanabe, 1995), suggesting the involvement of NO formation in the behavioral sensitization induced by central stimulation.

We showed that the nicotine-induced tail-tremor becomes more marked in intensity with repeated administration (Suemaru et al., 1994) and that central nicotinic receptors (Gomita et al., 1988) and β-adrenoceptors (Suemaru et al., 1993) are associated with the mechanisms. However, the mechanisms underlying sensitization of the tremorogenic effect remain unclear. Thus, we investigated the effects of NO synthase inhibitors and NMDA receptor antagonists on the tail-tremor induced by daily administration of nicotine.

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2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River Lab., Atsugi, Japan) weighing 200–230 g were housed in the experimental animal center of Okayama University School of Medicine at an ambient room temperature (22 ± 2°C) with a 12 h light/dark cycle (lights on at 6:00 a.m.). Food and water were provided ad libitum.

2.2. Tail-tremor observation

Tail-tremor begins 3 min after nicotine injection and reaches a peak at approximately 7–9 min after the injection. Thereafter, the tail-tremor disappears about 15 min after the injection. Nicotine-induced tail-tremor was observed in rats kept in individual cages ($20 \times 15 \times 15$ cm) for 15 min immediately after the subcutaneous administration of nicotine (Gomita et al., 1988). The degree of tail-tremor was scored every minute as follows: no tremor, 0; occasional slight tremor, 1; moderate intermittent tremor, 2; gross tremor with occasional quiescent periods, 3 and gross intense continuous tremor, 4. All observations were made by the same observer, who was unaware of the treatment schedule. Tremor intensity is expressed as the total sum of the score per min for 15 min.

2.3. Drugs

We used (−)-nicotine tartrate (Sigma, St. Louis, MO, USA), mecamylamine hydrochloride (Sigma), N_w-nitro-L-arginine (L-NA; Sigma), N_w-nitro-L-arginine methyl ester hydrochloride (L-NAME; Sigma), N_w-nitro-D-arginine methyl ester hydrochloride (D-NAME; Sigma), (+)-5-methyl-10,11,-dihydro-5*H*-dibenzo[*a*,*b*]cyclohepten-5,10-imine hydrogen maleate (MK-801; Research Biochemicals International, Natick, MA, USA) and 3-[(±)-2-carboxypiperazin-4-yl] propyl-1-phosphonic acid (CPP; Research Biochemicals International). All drugs were dissolved in 0.9% saline and injected in a volume of 0.1 ml per 100 g body weight. The pH of the nicotine solution was adjusted to 7 with NaOH. Drug doses were expressed in terms of the free base.

2.4. Drug administration

We reported that the intensity of the nicotine-induced tail-tremor is increased by daily administration of nicotine, and that nicotine at doses of 0.25–0.75 mg/kg causes a dose-dependent increase in tail-tremor (Suemaru et al., 1994). In the present study, we investigated the effects of NO synthase inhibitors and NMDA receptor antagonists on the development and appearance of tail-tremor induced by daily administration of nicotine (0.5 mg/kg per day s.c.). For studying the development of tail-tremor, rats were administered nicotine for 12 days and NO synthase inhibitors and NMDA receptor antagonists were intraperitoneally administered 30 min before the daily injection of nicotine on Days 1 to 8. To investigate the effects on the appearance of tail-tremor, drugs were administered 30 min before the 14th nicotine injection.

2.5. Statistical analysis

The development of tail-tremor induced by daily administration of nicotine was analyzed by a two-way repeated measures analysis of variance (ANOVA). Individual comparisons were made by Student's *t*-test or Dunnett's test. Probability values less than 0.05 were considered to show a significant difference.

3. Results

3.1. Effects of NO synthase inhibitors on nicotine-induced tail-tremor

Figs. 1 and 2 show the effects of NO synthase inhibitors on the development of tail-tremor induced by daily injections of nicotine at a dose of 0.5 mg/kg. In the control (saline plus nicotine-treated) group, the tail-tremor score increased gradually with repeated nicotine exposure for 8 days. The NO synthase inhibitors, L-NAME (40 mg/kg i.p.) and L-NA (10 mg/kg i.p.), administered 30 min before nicotine for 8 days significantly suppressed the development of tail-tremor ($P < 0.001$, respectively, two-way ANOVA). When rats were challenged with nicotine (0.5 mg/kg s.c.) without NO synthase inhibitors on the 9th day, L-NAME (20 and 40 mg/kg i.p.) and L-NA (10 mg/kg i.p.)-treated groups exhibited significantly lower tail-tremor scores than those of the saline-treated control

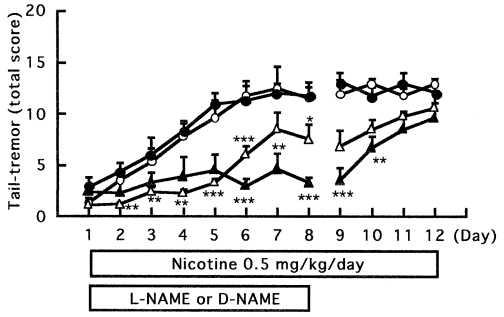

Fig. 1. Effects of NO synthase inhibitors, L-NAME, and an inactive isomer, D-NAME, on tail-tremor induced by repeated administration of nicotine to rats. L-NAME, D-NAME and saline were intraperitoneally administered 30 min before daily injection of nicotine (0.5 mg/kg s.c.) from Days 1 to 8. (○), saline; (△), L-NAME 20 mg/kg; (▲), L-NAME 40 mg/kg; (●), D-NAME 40 mg/kg. Each point represents the mean total score of tail-tremor for 15 min with S.E.M. ($n = 6$ for each). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the saline control (Dunnett's test).

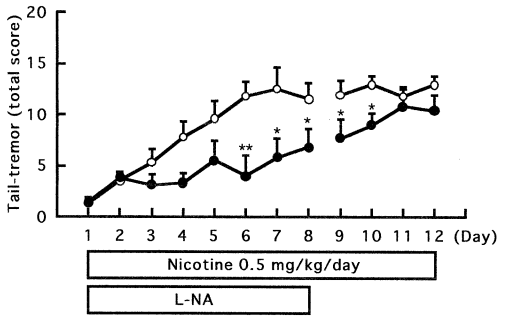

Fig. 2. Effect of the NO synthase inhibitor, L-NA, on tail-tremor induced by repeated administration of nicotine to rats. L-NA and saline were intraperitoneally administered 30 min before daily injection of nicotine (0.5 mg/kg s.c.) from Days 1 to 8. (○), saline; (●), L-NA 10 mg/kg. Each point represents the mean total score of tail-tremor for 15 min with S.E.M. ($n = 6$ for each). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the saline control (Student's *t*-test).

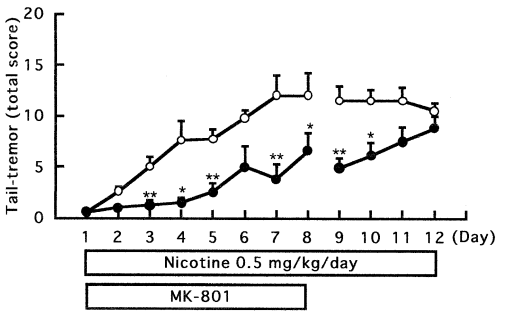

Fig. 3. Effect of the NMDA receptor antagonist, MK-801, on tail-tremor induced by repeated administration of nicotine to rats. MK-801 and saline were intraperitoneally administered 30 min before daily injection of nicotine (0.5 mg/kg s.c.) from Days 1 to 8. (○), saline; (●), MK-801 0.2 mg/kg. Each point represents the mean total score of tail-tremor for 15 min with S.E.M. ($n = 6$ for each). $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the saline control (Student's *t*-test).

group, indicating attenuation of the sensitization to the tremorogenic effect of nicotine. Thereafter, these tail-tremor scores in the groups given an NO synthase inhibitor increased gradually with subsequent nicotine exposure. However, D-NAME (40 mg/kg i.p.), an inactive isomer, did not affect the development of tail-tremor.

Table 1 shows the effects of a single administration of NO synthase inhibitors on the tail-tremor induced by repeated exposure to nicotine (0.5 mg/kg per day s.c.) for 14 days. L-NA (2–10 mg/kg i.p.), L-NAME (10–40 mg/kg i.p.) or D-NAME (20–40 mg/kg i.p.) 30 min before the 14th nicotine injection did not affect the mean tail-tremor score.

3.2. Effects of NMDA receptor antagonists on nicotine-induced tail-tremor

As shown in Figs. 3 and 4, the NMDA receptor antagonists, MK-801 (0.2 mg/kg i.p.) and CPP (2 mg/kg i.p.), given 30 min before the daily nicotine injections for 8 days significantly suppressed the development of tail-tremor

Table 1
Effects of NO synthase inhibitors on nicotine-induced tail-tremor in rats

Drug	Dose	*n*	Total tail tremor score (mean ± S.E.M.)
Saline	1 ml/kg	5	12.1 ± 1.1
L-NA	2 mg/kg	5	10.7 ± 1.4
	5 mg/kg	5	12.1 ± 0.5
	10 mg/kg	5	10.4 ± 2.6
L-NAME	10 mg/kg	5	10.5 ± 2.1
	20 mg/kg	5	9.2 ± 2.5
	40 mg/kg	5	8.1 ± 1.0
D-NAME	20 mg/kg	5	11.6 ± 0.6
	40 mg/kg	5	11.8 ± 0.8
	60 mg/kg	5	13.8 ± 1.1

NO synthase inhibitors were intraperitoneally administered 30 min before the 14th nicotine (0.5 mg/kg s.c.) injection. Each value represents the mean total score of tail-tremor for 15 min.

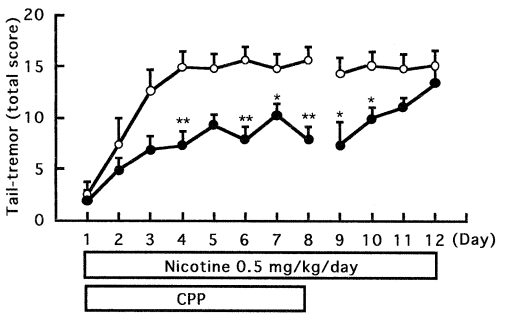

Fig. 4. Effect of the NMDA receptor antagonist, CPP, on tail-tremor induced by repeated administration of nicotine to rats. CPP and saline were intraperitoneally administered 30 min before daily injection of nicotine (0.5 mg/kg s.c.) from Days 1 to 8. (○), saline; (●), CPP 2 mg/kg. Each point represents the mean total score of tail-tremor for 15 min with S.E.M. ($n = 6$ for each). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the saline control (Student's *t*-test).

Table 2
Effects of NMDA receptor antagonists and mecamylamine on nicotine-induced tail-tremor in rats

Drug	Dose	*n*	Total tail-tremor score (mean ± S.E.M.)
Saline	1 ml/kg	6	12.0 ± 1.9
MK-801	0.01 mg/kg	6	10.5 ± 1.1
	0.1 mg/kg	6	7.8 ± 1.3 [a]
	0.2 mg/kg	6	2.8 ± 0.9 [b]
CPP	0.5 mg/kg	5	11.8 ± 1.9
	1 mg/kg	5	12.1 ± 1.4
	2 mg/kg	5	10.3 ± 1.6
	4 mg/kg	5	10.5 ± 2.7
Mecamylamine	0.1 mg/kg	5	10.7 ± 0.9
	0.2 mg/kg	5	3.2 ± 1.5 [b]
	0.5 mg/kg	5	0 [c]
	1 mg/kg	5	0 [c]

NMDA receptor antagonists and mecamylamine were intraperitoneally administered 30 min before the 14th nicotine (0.5 mg/kg s.c.) injection. Each value represents the mean total score of tail-tremor for 15 min.
[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ compared with the saline-treated control group (Dunnett's test).

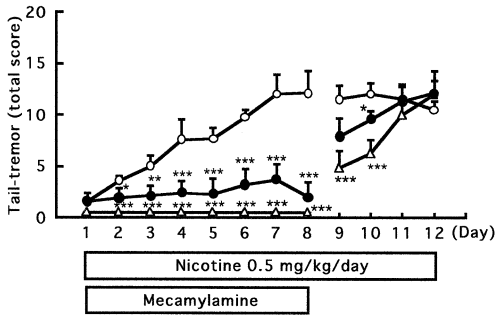

Fig. 5. Effect of the nicotinic receptor antagonist, mecamylamine, on tail-tremor induced by repeated administration of nicotine to rats. Mecamylamine and saline were intraperitoneally administered 30 min before daily injection of nicotine (0.5 mg/kg s.c.) from Days 1 to 8. (○), saline; (●), mecamylamine 0.2 mg/kg; (△), mecamylamine 0.5 mg/kg. Each point represents the mean total score of tail-tremor for 15 min with S.E.M. ($n = 5$ for each). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the saline control (Dunnett's test).

($P < 0.001$, respectively, two-way ANOVA). When rats were challenged with nicotine (0.5 mg/kg s.c.) alone on the 9th day, those given MK-801 or CPP showed significantly lower tail-tremor scores than those given saline. The tail-tremor score after the 14th nicotine injection was dose dependently decreased by a single prior administration of MK-801 (0.01–02 mg/kg i.p.) but not CPP (0.5–4 mg/kg i.p.) (Table 2).

3.3. Effect of mecamylamine on nicotine-induced tail-tremor

Mecamylamine (0.2 and 0.5 mg/kg i.p.) administered 30 min before nicotine for 8 days significantly suppressed the development of tail-tremor ($P < 0.001$, respectively, two-way ANOVA) in a dose-dependent manner, and 0.5 mg/kg of mecamylamine abolished it. When rats were challenged with nicotine (0.5 mg/kg s.c.) without mecamylamine on the 9th day, the mecamylamine (0.5 mg/kg i.p.)-treated animals had a significantly lower tail-tremor score than the saline-treated control group (Fig. 5). A single prior administration of mecamylamine (0.01–1 mg/kg i.p.) 30 min before the 14th nicotine injection dose dependently decreased the tail-tremor score, and 0.5 and 1 mg/kg of mecamylamine abolished the tail-tremor (Table 2).

4. Discussion

In the present study, the NO synthase inhibitor, L-NAME or L-NA, given daily before nicotine suppressed the development of the tail-tremor induced by nicotine in rats. However, the inactive isomer, D-NAME, did not inhibit the development of tail-tremor. The NO synthase inhibitors had no effect on the tail-tremor induced by 14 days of nicotine. These findings suggest that NO formation is involved in the mechanisms underlying the development of tail-tremor induced by repeated nicotine administration.

L-NAME not only inhibits NO synthase but also has an antagonistic action at muscarinic receptors (Buxton et al., 1993). However, it is unlikely that the muscarinic system is involved in the development of tail-tremor, since nicotine-induced tail-tremor is not antagonized by the muscarinic receptor antagonists, atropine and scopolamine (Gomita et al., 1988). This notion is supported by the present finding that the NO synthase inhibitor, L-NA, which is devoid of anti-muscarinic activity (Buxton et al., 1993), attenuated the development of tail-tremor.

The repeated administration of nicotine to rats causes increased sensitization to the locomotor-stimulating effect of nicotine (Clarke and Kumar, 1983; Suemaru et al., 1992). This action of nicotine is considered to be due largely to dopaminergic activation of the mesolimbic system (Clarke et al., 1988; Imperato et al., 1986), and nicotinic (Ksir et al., 1985; Fung and Lau, 1988) as well as NMDA receptors are involved in the behavioral sensitization (Shoaib and Stolerman, 1992; Shoaib et al., 1994).

In the present study, the noncompetitive NMDA receptor antagonist, MK-801, suppressed the development of nicotine-induced tail-tremor. It also inhibited the appearance of tail-tremor induced by repeated exposure to nicotine. MK-801, which blocks the ion channels of NMDA receptors, can also block the ion channel of nicotinic receptors (Ramoa et al., 1990; Amador and Dant, 1991). Therefore, it is likely that MK-801 influences the appearance and development of tail-tremor by blocking nicotinic receptors in a manner similar to mecamylamine. In contrast, the noncompetitive NMDA receptor antagonist, CPP, suppressed the development but not the appearance of tail-tremor. Furthermore, MK-801 and CPP attenuate the development of sensitization to the locomotor-stimulating effect of nicotine (Shoaib and Stolerman, 1992; Shoaib et al., 1994). These findings and those of the present study suggest that NMDA receptors are associated with the development of tail-tremor.

We showed that repeated administration of nicotine but not dimethyl phenyl piperazinium iodide (DMPP), a peripheral nicotinic receptor agonist, causes tail-tremor, and that the tail-tremor is suppressed by mecamylamine, a central nicotinic receptor antagonist, but not by hexamethonium, a peripheral nicotinic receptor antagonist (Suemaru et al., 1994). The development of tail-tremor induced by repeated nicotine administration is suppressed by daily doses of mecamylamine (0.5 mg/kg per day for 8 days), (Suemaru et al., 1997). These findings suggest that central nicotinic receptors are involved in the mechanisms underlying the appearance and development of the tail-tremor. In the present study, we confirmed that daily doses of 0.2 and 0.5 mg/kg of mecamylamine dose dependently suppress the development of nicotine-induced tail-tremor in rats.

There is evidence that NO is released from neurons

following the stimulation of excitatory NMDA receptors (Snyder and Bredt, 1991), and that not only a blockade of NMDA receptors but also the inhibition of NO synthase attenuates the development of sensitization to the locomotor-stimulating effect of cocaine in rats (Pudiak and Bozarth, 1993) and methamphetamine in mice (Ohno and Watanabe, 1995). The findings of the current study, that an NO synthase inhibitor and an NMDA receptor antagonist inhibited the repeated nicotine-induced tail-tremor in rats, agree with these results. In conclusion, the results of this study suggest that NO formation, possibly mediated by NMDA receptors, is involved in the mechanisms underlying the development of tail-tremor induced by repeated nicotine administration and that central nicotinic receptors are essential for the appearance and development of this phenomenon.

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