Pharmacology Biochemistry & Behavior, Vol. 34, pp. 817–821. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Nicotine-Induced Tail-Tremor and Drug Effects

YUTAKA GOMITA, KATSUYA SUEMARU, KATSUSHI FURUNO AND YASUNORI ARAKI

Department of Hospital Pharmacy, Okayama University Medical School Shikata-cho, Okayama 700, Japan

Received 20 October 1988

GOMITA, Y., K. SUEMARU, K. FURUNO AND Y. ARAKI. *Nicotine-induced tail-tremor and drug effects*. PHARMACOL BIOCHEM BEHAV **34**(4) 817–821, 1989.—Tail-tremor induced by repeated and daily administration (0.5 mg/kg SC × 6 times/day) of nicotine as well as effects of various drugs on this response were investigated in Wistar strain male rats. Daily administration of nicotine in doses of 0.5 mg/kg SC caused tail-tremors to appear beginning on the 3rd day. Tail-tremor induced by the first injection of each day gradually increased with the daily injections, however, the heightened effect of this first injection at the beginning of each day decreased during the day upon repeated administration of 6 times/day at 2-hr intervals. Basically, tail-tremor appeared about 5 min after SC administration of nicotine and reached a peak approximately 7–9 min after injection, declining to zero afterwards. Different drugs showed various effects on this response. While mecamylamine (0.5 and 1.0 mg/kg IP) abolished nicotine-induced tail-tremor, arecoline (0.5 and 1.0 mg/kg IP), atropine (2.5 and 5.0 mg/kg IP), scopolamine (1.0 and 2.0 mg/kg IP) and hexamethonium (0.5 and 1.0 mg/kg IP) showed no such effects. Furthermore, physostigmine (0.1 mg/kg IP) actually potentiated this action. These results suggest that tail-tremor induced by nicotine may be mediated through central nicotinic receptor system.

Nicotine Tail-tremor Nicotinic receptor Mecamylamine Hexamethonium Rat

IT is well known that nicotine, one of the main constituents of tobacco, is capable of causing tremors throughout the body, followed by convulsions if doses are high enough (20). Intracerebroventricular administration of nicotine, in addition to peripheral administration, produces convulsions (3). Furthermore, vomiting, ear twitching, salivation, and panting have all been observed (4). Regarding the mechanisms of these phenomena, it has been demonstrated that convulsions produced by nicotine are mediated through a central nicotinic receptor system (3). In addition, it has been found that vomiting and ear twitching are also mediated through central nicotinic receptor systems (4).

Repeated systemic administration of nicotine appears to generate two phenomena, i.e., both suppression (tolerance) and enhancement of the nicotine effect. Tolerance, for example, has been observed in the effects of nicotine in decreasing locomotor activity (21), in suppressing operant behavior with respect to FR scheduling of water or milk reinforcement (9,10), and in decreasing the heightened corticosterone and serotonin levels often induced by stress (6). Furthermore, acute tolerance, i.e., tachyphylaxis, has been observed in the effects of nicotine in decreasing locomotor activity and rearing (22), and in nicotine's analgesic action (23). Enhancement of the effects of nicotine after daily administration has been observed in increases in locomotor activity (12,17). There have been no reports, however, regarding tremor appearing only in the tail (tail-tremor) as induced through the daily systemic administration of nicotine.

In the present paper, we report on tail-tremor induced by systemic daily administration of nicotine, as well as the effects of repeated short-time interval administration on the tremor, and, in addition, the effects of various drugs with respect to this action.

METHOD

Animals

Male Wistar strain rats (supplied by Charles River Lab., Japan) weighing 400–440 g were used as subjects in the experiment. Rats were kept in groups of 4–5 animals each in plastic walled cages (26 × 36 × 25 cm) in a room with a 12-hr light-dark cycle at a temperature of 22 ± 1°C and a relative humidity of 60%. Animals were allowed free access to water and food during the experiment.

Drugs

Drugs used in the present experiment consisted of nicotine free base (donated by the Council for the Study of Smoking and Health, Japan), physostigmine salicylate powder (Sigma), arecoline hydrobromide powder (Sigma), atropine sulfate powder (Merck), scopolamine hydrochloride powder (Sigma), hexamethonium bromide injection (Yamanouchi) and mecamylamine hydrochloride powder (Sigma). All were dissolved in 0.9% sodium chloride (saline).

Tail-Tremor Observation

The behavioral observation of nicotine-induced tail-tremor and

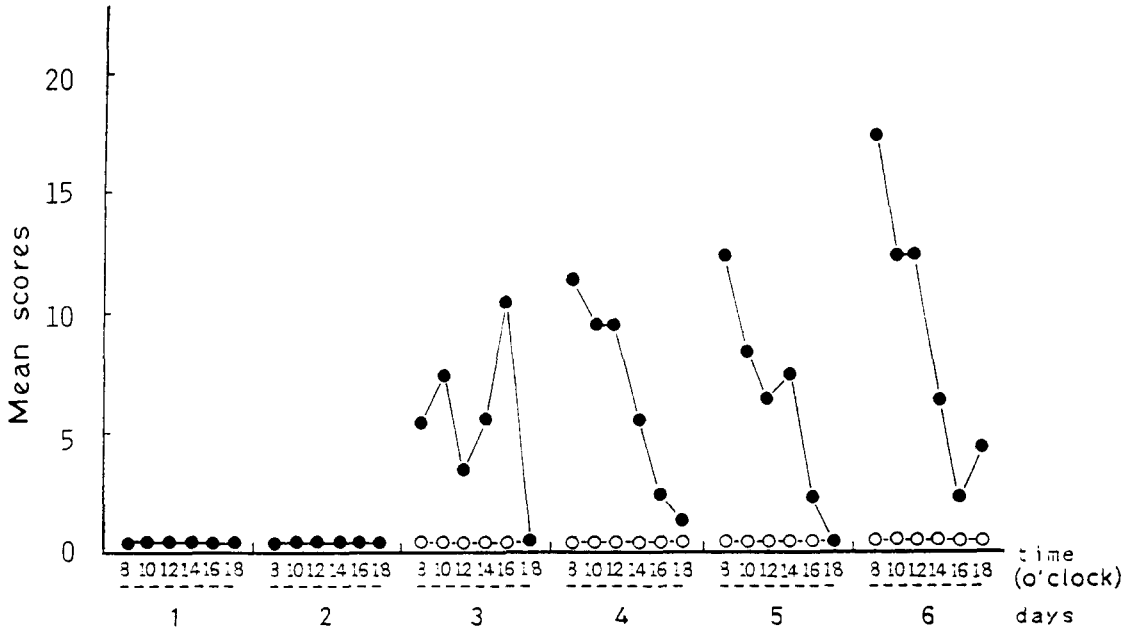

FIG. 1. Tail-tremor induced by daily and repeated administration of nicotine. Nicotine, at a dose of 0.5 mg/kg, or saline was subcutaneously administered 6 times per day at 2-hr intervals. Each point indicates the mean score for 15 min. ○: Saline 1 ml/kg × 6 times/day (N=4); ●: nicotine 0.5 mg/kg × 6 times/days (N=5).

the effects of drugs on it were performed after each animal was transported to the individual wire mesh cage (10×10×10 cm) from the plastic home cages 10 min before the testing. Behavioral observation of tail-tremor after nicotine administration was carried out using the video camera and display apparatus (Type NV-M3, National). The degree of tail-tremor was determined by scoring through the display in another days after the experiments. The scoring indices were as follows: tail-tremor continued less than 1 sec, score 0.5; continued for 1 to 2 sec, score 1; for 2 to 3 sec, score 2; and continued for over 3 sec, score 3. Actually, there was no animal that exhibited the tail-tremor continuously more than 5 sec. The scores of tail-tremor were measured by one investigator under the blind situation through the display apparatus. The numbers of tail-tremor induced by nicotine are measured for every min after nicotine administration in chronological changes, and for 15 min immediately after the administration in the development of tail-tremor when administered daily and repeatedly and in the drug effects.

Drug Administration and Procedure

For testing the development of nicotine-induced tail-tremor, nicotine at a dose of 0.5 mg/kg or saline was subcutaneously administered 6 times per day at 2-hr intervals (i.e., at 0800, 1000, 1200, 1400, 1600 and 1800) every day for at least 6 days. As for the effects of various drugs on tail-tremor, animals exhibiting scores of greater than 10 with 10% variation during 15 min for two successive days were used for testing. All animals for drug effects were tested at almost the same times (i.e., 1800–1900) during the day. Drugs or saline were intraperitoneally administered at specific times with respect to nicotine, subcutaneously injected at a dose of 0.5 mg/kg. The doses of drugs and the time period were chosen by referring to references (8, 11, 14, 15, 24), i.e., arecoline, immediately before nicotine administration; scopolamine, 30 min before; atropine, physostigmine, hexamethonium and mecamylamine, 60 min before. The effects of each drug on tail-tremor were observed for 15 min after nicotine administration. Animals rested for at least 10 days between drug administrations.

Statistical Analysis

The appearance and development of tail-tremor induced by repeated and daily administrations of nicotine were analyzed by a repeated-measures analysis of variance (ANOVA) as two factors, and effects of various drugs on nicotine-induced tail-tremor were assessed by one-way ANOVA as one factor.

RESULTS

Development of Tail-Tremor Induced by Nicotine

The appearance and development of tail-tremor as caused by bihourly and daily administration of nicotine are shown in Fig. 1. Administration of nicotine six times per day at a dose of 0.5 mg/kg began to elicit tail-tremor responses beginning the 3rd day after initial administration. The effect of the initial nicotine injection of the day gradually increased with the number of days up to a maximum at 6 days. There were significant differences in values to initial injection on daily administered day between the nicotine-administered group and the saline-administered group, $F(1,48) = 141.1$, $p<0.01$, and also in values of intragroup to daily administration, $F(5,48) = 18.2$, $p<0.01$. Further, in the development of tail-tremor elicited by bihourly administration of nicotine, there are significances in values between the two groups [$F(1,48) = 43.9$, $F(1,48) = 59.5$, $F(1,48) = 55.7$, $F(1,48) = 73.9$, on the 3rd, 4th, 5th and 6th day, respectively, all $p<0.01$]. On the other hand, the effects of the first injection decreased throughout the drug upon repeated administration at 2-hr intervals as shown in Fig. 1. Significant differences were observed in values of intragroup for

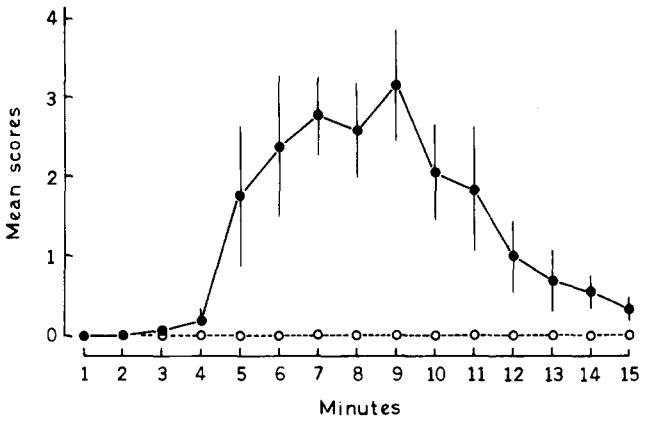

FIG. 2. Chronological changes in nicotine-induced tail-tremor. This data is the result of the first nicotine injection on day 6. Nicotine was subcutaneously administered at a dose 0.5 mg/kg. Each point indicates the mean score per one min with S.E.M. ○: Saline 1 ml/kg (N=4); ●: nicotine 0.5 mg/kg (N=5).

the response to bihourly administration [$F(5,48)=3.4$, $p<0.05$ on the 3rd day, $F(5,48)=4.4$, $p<0.01$ on the 4th day, $F(5,48)=5.1$, $p<0.01$ on the 5th day, and $F(5,48)=5.2$, $p<0.01$ on the 6th day]. Figure 2 illustrates the time-response profile of tail-tremor for the first nicotine administration of the day on day 6. Tail-tremor began 5 min after the nicotine injection and reached a peak at approximately 7–9 min after injection, after which the response declined over the next 6–8 min after injection, after which the response declined over the next 6–8 min. Tail-tremors almost disappeared about 15 min after the injection. There is a significant difference in values on the 5th–10th day between the two groups, $F(1,48)=84.6$, $p<0.01$.

Drug Effects on Tail-Tremor

Figures 3 to 5 show the effects of various drugs on tail-tremor induced by 0.5 mg/kg of nicotine. As shown in Fig. 3, physostigmine (0.05 and 0.1 mg/kg), an anti-cholinesterase inhibitor, increased nicotine-induced tail-tremor in a dose-dependent manner. Furthermore, there was a significant difference between the scores of the 0.1 mg/kg physostigmine-administered group and those of the saline-administered group, $F(1,16)=17.4$, $p<0.01$. With arecoline (0.5 and 1.0 mg/kg), however, a muscarinic receptor agonist, no such effect was observed. Furthermore, atropine (2.5 and 5.0 mg/kg) and scopolamine (1.0 and 2.0 mg/kg), both muscarinic receptor antagonists, also showed no effects (Fig. 4). While none of the muscarinic receptor antagonists seemed to exert any effects on nicotine-induced tail-tremor, different effects were noted when central and peripheral ganglionic blocking agents were administered. Hexamethonium (0.5 and 1.0 mg/kg), noted for its peripheral action, demonstrated no effects on tail-tremor, while mecamylamine (0.5 and 1.0 mg/kg), possessing both central and peripheral effects, abolished tail-tremor completely as shown in Fig. 5. The differences between the 0.5 and 1.0 mg/kg mecamylamine-administered groups and the saline-administered group were significant [$F(1,21)=17.7$ and $F(1,20)=15.4$, $p<0.01$ in both cases].

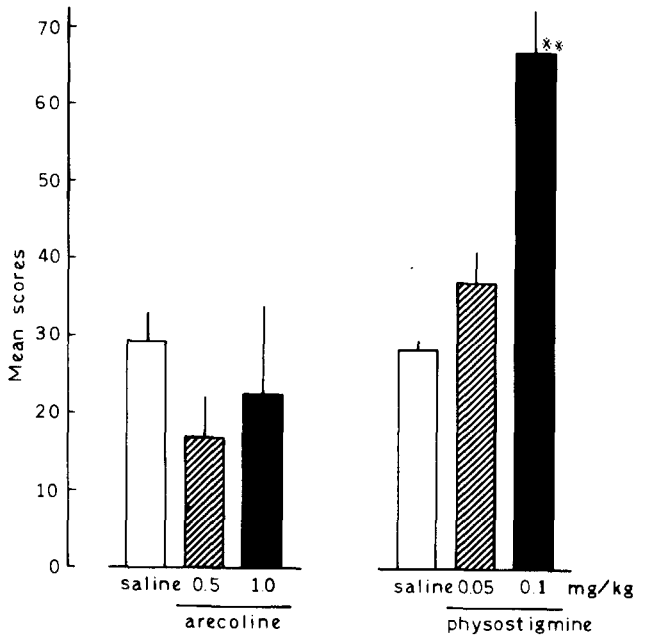

FIG. 3. Effects of arecoline and physostigmine on nicotine-induced tail-tremor. Nicotine was subcutaneously administered at a dose of 0.5 mg/kg immediately after arecoline, at doses of 0.5 and 1.0 mg/kg (N=5 for each), and 60 min after physostigmine, at doses of 0.05 and 0.1 mg/kg (N=6 for each), had been intraperitoneally injected. Each value indicates the mean score for 15 min. The saline administered control group for comparison with arecoline-administered animals and that for comparison with physostigmine-administered animals consisted of 10 and 12 rats, respectively. $^{**}p<0.01$.

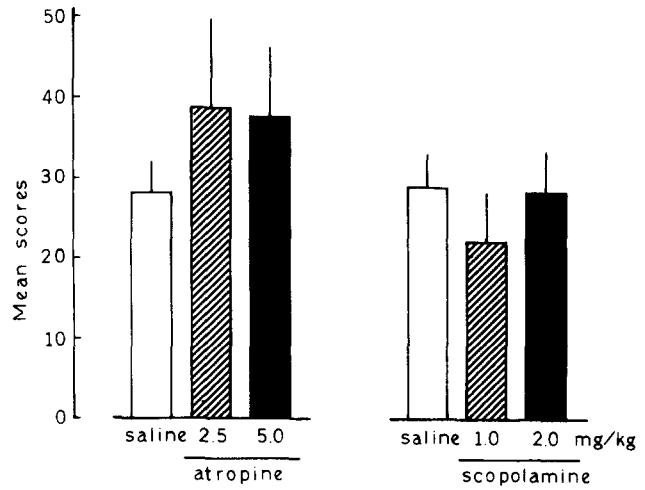

FIG. 4. Effects of atropine and scopolamine in nicotine-induced tail-tremor. Nicotine was subcutaneously administered at a dose of 0.5 mg/kg 60 min after atropine, at doses of 2.5 and 5.0 mg/kg (N=4 for each) and scopolamine, at doses of 1.0 and 2.0 mg/kg (N=4 for each), had been intraperitoneally injected. Each value indicates the mean score for 15 min. The saline-administered control group for comparison with atropine-administered animals and that for comparison with scopolamine-administered animals each consisted of 8 rats.

DISCUSSION

The tremor is a characteristic effect of nicotine. It is said that the first effect of appropriate doses of intravenously administered

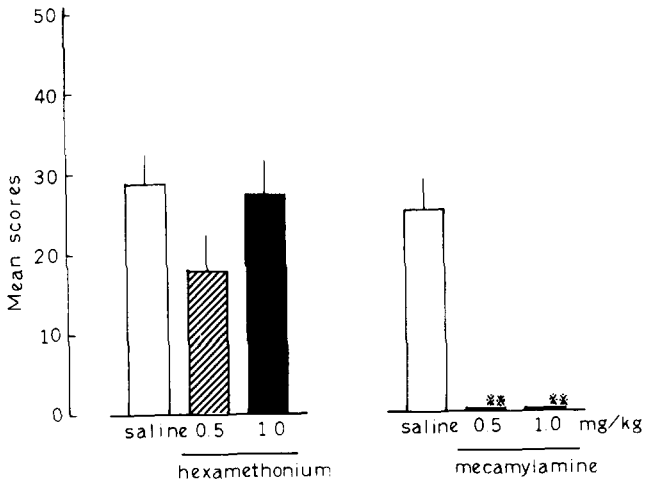

FIG. 5. Effects of hexamethonium and mecamylamine on nicotine-induced tail-tremor. Nicotine was subcutaneously administered 60 min after hexamethonium, at doses of 0.5 and 1.0 mg/kg (N=5 and 7, respectively), and mecamylamine, at doses of 0.5 and 1.0 mg/kg (N=8 for each) had been intraperitoneally injected. Each value indicates the mean score for 15 min. The saline control group for comparison with hexamethonium-administered animals and that for comparison with mecamylamine-administered animals consisted of 10 and 15 rats, respectively. $^{**}p<0.01$.

nicotine in mice is, in fact, tremor (22). Tremor is generally caused throughout the whole body of animals. A dosage of 0.5 mg/kg of nicotine, however, used in the present experiment, did not produce such a whole body effect. Instead, when nicotine was administered daily, a tremor located only in the tail was observed 3 days after initiation of the injections and gradually increased with the number of days. In addition, repeated administration of nicotine during the day at 2-hr intervals resulted in a decreasing trend in a tremor activity with increasing injections during the day. It has been claimed that nicotine not only augments its own stimulant effect upon repeated administration, but also exerts a tolerance effect (11, 12, 17, 18). As for the former, Ksir *et al.* (11) observed that daily subcutaneous administration of nicotine at doses of 0.1 to 0.4 mg/kg resulted in enhancement of the stimulant effect of nicotine on locomotor activity, as well as an 18–26% increase in cortical nicotinic receptors. Accordingly, the enhancement of nicotine-induced tail-tremor by daily administration observed in the present experiment may also be related to an increase in nicotinic receptors. Further study, however, may be required to determine whether or not these two phenomena are directly and functionally linked.

As for the tachyphylaxis effect of nicotine, Stolerman *et al.* (21) observed that the increased exploratory and rearing behavior of rats induced by nicotine would decrease within 2 hr upon repeated administration, indicating a tachyphylactic effect. The decreasing effect of nicotine-induced tail-tremor upon repeated administration at 2-hr intervals observed in the present experiment may be related to a tachyphylaxis effect in the same manner. The reasons, however, why no decreasing effects on nicotine-induced tail-tremor were observed after long-term repeated administration remain unclear.

According to pharmacological studies on the central effects of nicotine, whole body tremor induced by high doses of nicotine is said to be potentiated by drugs such as amphetamines, epinephrine, norepinephrine and so on, but, regarding the suppressive effects of drugs against nicotine tremor, uniform results have not been obtained (20). On the other hand, when sufficient doses of nicotine are used, tremor is often followed by convulsions. Convulsions evoked by intracerebroventricular nicotine injection can be abolished by intracerebroventricular administration of nicotine receptor antagonists such as mecamylamine and hexamethonium, but not by drugs such as atropine, scopolamine, phenoxybenzamine, practol, chlorpromazine, haloperidol and methysergide. Further, reserpine, 5,6-dihydroxytryptamine, and hemicholinium abolished the convulsion (3). These indicate that nicotine-induced convulsions may be mediated through the central nervous system associated with nicotine receptors.

Other central effects of nicotine, besides convulsion and tremor, include vomiting, ear twitching and so on. Beleslin *et al.* (4) showed that the vomiting and ear twitching are abolished by the intracerebroventricular administration of the nicotine blocker hexamethonium, indicating that they are also mediated through central nicotinic receptor system. Furthermore, an enhancement of the stimulant effect of nicotine repeated administration on locomotor activity is antagonized by the peripheral administration of mecamylamine, but not by that of hexamethonium (7,11), indicating that this behavioral effect may be mainly attributed to an action on central nicotinic receptor system.

In the present experiment on the effects of various drugs on tail-tremor, mecamylamine, a drug possessing central and peripheral nicotinic blocking properties, completely abolished all tail-tremor. Hexamethonium, however, having peripheral nicotinic blocking action, arecoline, having muscarinic action, and atropine and scopolamine, having anti-muscarinic action, all showed no effects on tail-tremor. Physostigmine, having cholinesterase inhibitory action in the central and peripheral nervous systems, potentiated nicotine-induced tail-tremor. From these results, the tail-tremor induced only by repeated administrations of nicotine may be mediated through the central nervous system related to nicotine receptor.

As for brain nicotinic receptor subtype, it is known that the brain has two binding sites, i.e., one that can be bound with [^{3}H]-nicotine or [^{3}H]-acetylcholine and one that can be bound with [^{125}I]-α-bungarotoxin (13,19). Nicotine may elicit behavioral effects by interacting at brain [^{3}H] nicotine and [^{125}I]-α-bungarotoxin binding sites (8). Some investigators demonstrated that classical nicotinic blockers such as hexamethonium and mecamylamine inhibit brain nicotine or α-bugarotoxin binding poorly (1, 13, 16). However, mecamylamine prevents many of the behavioral effect of nicotine (7, 16, 23), even if there is no evidence to support mecamylamine as receptor antagonist. In the present experiment, the tail-tremor induced by nicotine was also inhibited by mecamylamine. So, this behavioral effect may not be directly attributable to brain nicotinic receptors. It may act indirectly by blocking the ion channel of nicotinic receptor complex (2). Further investigations are necessary for verifying a mode of action of nicotine for it.

ACKNOWLEDGEMENTS

This work was supported by a grant from Smoking Research Foundation. The authors are indebted to Dr. Tomoji Yanagita of Central Institute for Experimental Animals for his valuable advice.

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