

BRIEF COMMUNICATION

Tail-Tremor Induced by Exposure to Cigarette Smoke in Rats

YUTAKA GOMITA, KATSUYA SUEMARU, KATSUSHI FURUNO AND YASUNORI ARAKI

Department of Hospital Pharmacy, Okayama University Medical School, Okayama 700, Japan

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GOMITA, Y., K. SUEMARU, K. FURUNO AND Y. ARAKI. *Tail-tremor induced by exposure to cigarette smoke in rats.* PHARMACOL BIOCHEM BEHAV 40(2) 453–455, 1991.—Tremors appearing only in the tail (tail-tremor) induced by cigarette smoke and subcutaneous nicotine were investigated using a smoking machine and Wistar rats. Daily exposure (twice a day) to smokes of two commercial cigarettes (Mild-Seven Select® for the first 7 days and Long-Peace® for the next 6 days) caused the tail-tremor to appear even if it was slight. A single subcutaneous nicotine (0.5 mg/kg) administration to rats exposed to the cigarette smokes for 13 days markedly caused the tail-tremor. On the other hand, daily subcutaneous injection of nicotine (0.5 mg/kg/day) also caused the tail-tremor to appear beginning on the 4th day and the incidence of tremor increased to 100% by the 12th day. These results indicate that tail-tremor can be caused not only by daily subcutaneous administration of nicotine but also by daily exposure to cigarette smoke.

| Cigarette smoke | Nicotine | Tail-tremor | Rat |
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TOBACCO smoke influences not only the peripheral organs such as cardiovascular systems but also the central nervous system. It is well known that nicotine, one of the main constituents of tobacco, induces tremors throughout the body, and the high doses can cause convulsions (1–3, 6–8). It has also been reported that convulsions induced by nicotine are mediated by central nicotinic receptors (1, 9, 10). In addition, we have already observed a tremor appearing only in the tail (tail-tremor) which is induced by daily systemic administration of low dose of nicotine, and is mediated by central nicotinic receptors (5). However, there have been no reports of the cigarette smoke causing tail-tremor. In the present paper, we report that tail-tremors can be induced by daily exposure to cigarette smoke, and we compare cigarette smoke-induced tail-tremor with nicotine-induced tail-tremor.

METHOD

Animals

Male Wistar rats (Charles River Lab., Japan) weighing 175–266 g were used in the experiment. They were kept in groups of 4–5 animals each in a home cage (26 × 36 × 25 cm) in a room with a 12-h light-dark cycle (lights on from 0800 to 2000 h) at 22 ± 1 °C and approximately 60% relative humidity. They were allowed free access to food and water during the experiment.

Cigarettes and Drugs

The cigarettes used in the present experiment were commercial cigarettes (Mild-Seven Select® and Long-Peace®; Japan To-

bacco Co.). Mild-Seven Select® cigarette weighs approximately 0.926 g per cigarette which contains 0.9 mg of nicotine and 13 mg of tar when smoked by a smoking machine until two-thirds (inhalation volume: 35 ml, duration: 2 s and interval: 1 min), and Long-Peace® weighs approximately 1.02 g per cigarette which contains 2.2 mg of nicotine and 23 mg of tar.

Pure nicotine solution was donated from the Smoking Research Foundation of Japan. Nicotine solution was diluted with physiological saline (0.9% NaCl) for administration at 1 ml/kg body weight.

Apparatus for Exposure to Cigarette Smoke

For exposing the animals to cigarettes, the Hamburg II Smoking Machine (Bogwaldt) was used (4). The apparatus consists of the smoking head (up to 30 cigarettes can be attached), the smoking channel (smoke and air can be mixed), the smoking chamber slide piece, the inhalation chamber and animal holders (10 rats can be exposed to the smoke).

In the experiment, 15 cigarettes in the smoking head were lighted initially and the remaining 15 cigarettes were lighted after the first 15 had burned out. The smoke from the lighted cigarettes was pumped into the smoking chamber, mixed with air at a ratio of 1:7 and sent to the inhalation chamber. Animals were exposed to the smoke for 20 min under the conditions: 35 ml inhalation volume, 2 s duration, 15/min frequency.

Experimental Procedures

Concerning the daily cigarette smoking experiment, 8 or 9 animals were exposed to the smoke simultaneously for 20 min

each twice a day (at 0800 and 1700) for 13 days. That is, the animals were exposed to Mild-Seven Select® cigarette smoke for the first 7 days and Long-Peace® cigarette smoke for the next 6 days, and the tail-tremors were observed for 15 min immediately after the 20-min exposure to cigarette smoke at 0800. Control rats for the cigarette smoking experiment were handled the same as the smoke-exposed rats except that they were not exposed to cigarette smoke. At 24 h after the last exposure of cigarette smoke, the smoke-exposed rats as well as the nonexposed control rats were subcutaneously injected with nicotine at a dose of 0.5 mg/kg and the tail-tremors were observed for 15 min immediately after nicotine injection.

On the other hand, concerning the nicotine daily administration experiment, nicotine at a dose of 0.5 mg/kg was subcutaneously injected once a day (at 1700) for 16 days and the tail-tremor was observed for 15 min immediately after nicotine injection. The control rats for the nicotine administration experiment were given saline vehicle.

Tail-Tremor Observation

The tail-tremors were observed for 15 min immediately after smoke exposure or nicotine injection in an individual wire mesh cage (10 × 10 × 10 cm). The degree of tail-tremor was scored as follows; tail-tremor continued less than 1 s, score 0.5; continued for 1 to 2 s, score 1; for 2 to 3 s, score 2; and continued for over 3 s, score 3. Figures in the present paper show the scored data as well as the percent incidence of tail-tremor. The latter is the percent of rats with a tail-tremor score greater than one.

Statistical Analysis

The appearance and development of tail-tremor were analyzed by Fisher's exact probability test (incidence data) and the Mann-Whitney U-test (score data).

RESULTS

The appearance and development of tail-tremor induced by daily exposure to cigarette smoke, and the appearance when nicotine was challenged to rats exposed with cigarette smokes for 13 days are shown in Fig. 1. Daily exposure to cigarette smoke began to elicit tail-tremors on the 4th day of exposure even if it was slight. The mean tail-tremor score stayed between 0.2 and 0.56 for the first 7 days (during the Mild-Seven Select® cigarette smoke exposure), and between 0.75 and 1.31 for the next 6 days (during the Long-Peace® cigarette smoke exposure). In the nonexposed control rats, the tail-tremor was not apparent. It was significantly different from the control rats on the 4th, 8th, 10th and 12th day ($U = 13.5$, $p < 0.05$, 9, 4.5 and 0, $p < 0.02$, respectively). Thirty to 25 percent of the animals had tail-tremors from the 4th to the 6th day (during Mild-Seven Select® cigarette smoke exposure) and 25 to 63 percent for the next 6 days (during Long-Peace® cigarette smoke exposure). There was no significant difference during the exposure period of Mild-Seven Select® cigarette smoke between the cigarette smoke-exposed rats and the control rats. But significant differences were found on the 10th ($p < 0.02$) and 12th day ($p < 0.05$) during the exposure period of Long-Peace® cigarette smoke between both groups. On the other hand, when subcutaneous nicotine was challenged to the smoke-exposed rats and the nonexposed control rats, the mean score and the incidence of tail-tremor in the smoke-exposed rats markedly increased with significant differences ($U = 3.5$, $p < 0.02$, in mean score and $p < 0.02$ in the incidence) in comparison to these in the nonexposed control rats.

As shown in Fig. 2, when nicotine was subcutaneously in-

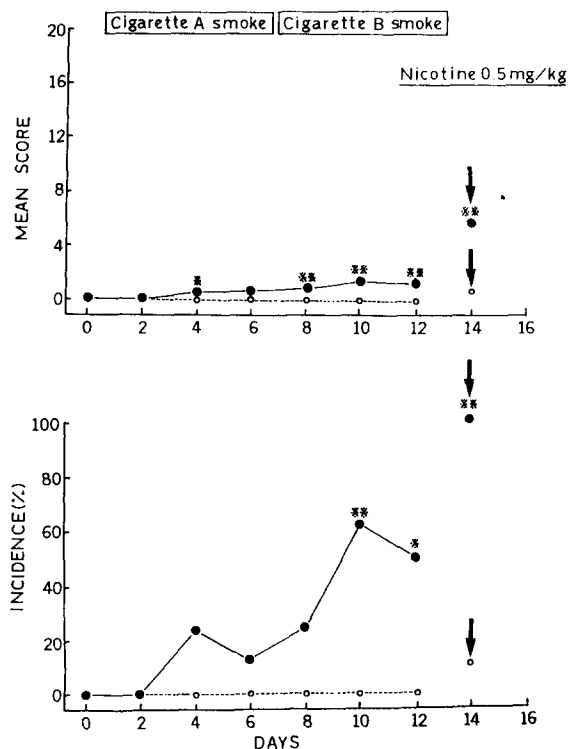


FIG. 1. The appearance and development of tail-tremor induced by daily exposure to two kinds of cigarette smokes. Animals were exposed to the cigarette smokes A (Mild-Seven Select® for 7 days) and B (Long-Peace® for 6 days) for 20 min twice each day (at 0800 and 1700). Moreover, nicotine at a dose of 0.5 mg/kg was subcutaneously injected to rats exposed or nonexposed to the cigarette smoke (indicated by arrows in the figure). The tail-tremor was observed for 15 min immediately after the exposure or nicotine administration. Mean score and incidence data are shown in the upper and lower panels, respectively. ●-●, cigarette smoke-exposed rats (N=8), ○-○, nonexposed control rats (N=9). * $p < 0.05$, ** $p < 0.02$; compared with the control rats.

jected every day, the tail-tremor began to appear on the 4th day. By the 12th day all animals exhibited tail-tremor. The incidence of tail-tremor was significantly different from the vehicle-administered control rats on the 6th ($p < 0.05$), 8th, 12th and 16th day ($p < 0.02$, respectively). The mean tail-tremor score was also significantly different from the control rats from the 4th day onward ($U = 3.5$, 3.5, 0.0 and 0, $p < 0.02$, respectively).

DISCUSSION

The present study confirms that both daily systemic nicotine administration and daily exposure to cigarette smoke can cause tremor in the rat's tail. It is well known that high doses of nicotine, a main constituent of tobacco, can cause tremor throughout the body, followed by the convulsions (1-3, 6-8). In addition, we have previously found that daily administration of nicotine can cause tremor only in the tail, even at doses as low as 0.5 mg/kg. In that study the tail-tremor was observed beginning on the 3rd day and gradually increased with the daily injections (5). On the other hand, in the present experiment, we found that even daily exposure to cigarette smokes can cause tail-tremor, though the incidence and tremor scores were much lower than in the animals given nicotine subcutaneously. As compared between the exposure of Long-Peace® and Mild-Seven Select®

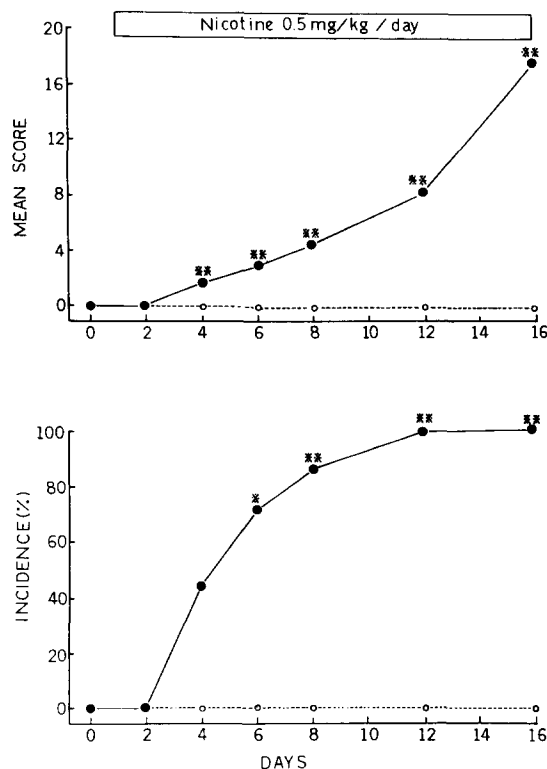


FIG. 2. The appearance and development of tail-tremor induced by daily administration of nicotine. Nicotine at 0.5 mg/kg was given subcutaneously once each day (at 1700) for 16 days. The tail-tremor was observed for 15 min immediately after the injection. ●●, nicotine-injected rats (N = 7) and ○○, vehicle (saline)-injected control rats (N = 7). * $p < 0.05$, ** $p < 0.02$; compared with the control rats.

cigarette smokes, the former cigarette smoke containing high nicotine-tar caused the tail-tremor more than the latter cigarette smoke containing low nicotine-tar. These differences in the incidence and mean score between the tail-tremor induced by daily nicotine injection and cigarette smoke exposure, and between

two cigarette smokes may be attributed, as an anticipation, to the presence and content of nicotine in the cigarette smoke.

For establishing the relationships between the tail-tremor and nicotine, in the present experiment, we had the experiment of nicotine challenge to rats exposed to cigarette smokes for long-term. As the results, when nicotine was administered 24 h after the last exposure to the smoke-exposed rats for 13 days, the incidence of tremors and the tremor scores markedly appeared in comparison to the nonexposed control rats. On the other hand, in the group daily given nicotine subcutaneously, the tail-tremor could not be caused by the first nicotine administration, but thereafter gradually increased with the daily injections and reached to 100% in incidence by the 12th day. That is, a single nicotine administration to nonexposed rats could not cause the tail-tremor, but the same administration to rats exposed to the cigarette smokes for long-term could markedly cause the tail-tremor. These indicate that the appearance of tail-tremor induced by cigarette smoke exposure may be due to nicotine in the cigarette smoke.

Concerning the data in Fig. 2, we have already suggested that the appearance and development of tail-tremor might be mediated by central nicotine receptors (5). The appearance of tail-tremor induced by cigarette smoke may likewise be mediated by central nicotine receptors. Still, there have not yet been any pharmacological studies of this phenomenon. As for gradual increase in the tail-tremor during the smoke exposures, it may be considered to depend on changes in a central neural receptor. That is, we have already observed that hyperlocomotions induced by methamphetamine and apomorphine are potentiated by long-term pretreatment with nicotine, a constituent of tobacco, indicating increases in the susceptibility of monoaminergic neural receptors (11).

It is said that heavy smokers have hand-tremors without whole-body (7). The tail-tremor induced by exposure to cigarette smoke or subcutaneous nicotine administration in the present experiment may be related to the hand-tremor reported in heavy smokers. However, we do not yet have direct evidence of this connection and further investigation is necessary.

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