Effects of Nicotine on Ambulatory Activity in Mice

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Abstract—Nicotine (0.5 and 1.0 mg/kg) administered subcutaneously to mice decreased the ambulatory activity recorded by an ambulo-meter in a dose-dependent manner from 5 to 60 min after the administration, and the higher dose (1.0 mg/kg) caused a long-lasting ataxia. To be noted was the initial increment of ambulation which usually preceded the ataxia-inducing effect with every dose of nicotine, and the lowest dose (0.10 mg/kg) employed herein induced only the increasing effect on ambulation recorded for the first 20 min after its administration. The ataxia-inducing effect of nicotine (1.0 mg/kg) was attenuated by the pretreatment with mecamylamine (0.4–2.0 mg/kg) in a dose-dependent manner, though the attenuating effect waned at a higher dose (4.0 mg/kg). In contrast, pretreatment with either hexamethonium (2.5 and 5.0 mg/kg) or atropine (1.0, 2.5 and 5.0 mg/kg) did not affect the ataxia-inducing effect of nicotine. Atropine when administered alone was found to markedly increase the ambulatory activity at the doses used for the pretreatment. Measurement of the time-dependent change of [3H]-nicotine level in brain tissue after its subcutaneous injection revealed that there is a good correlation between the brain levels of the alkaloid and the intensity of its ataxic effect rather than the initial increasing effect on ambulation. The results obtained herein suggest that nicotine exerts its ataxic effect centrally, but the site and type of the receptor stimulated by nicotine remains to be identified.

There have been many studies on the effects of nicotine on the spontaneous motor activity of drug-naive or drug-tolerant mice and rats (1–4). The commonly observed effect on drug-naive animals has been inhibition of the spontaneous motor activity, which developed in a dose-dependent manner, and high dose levels led to a typical ataxia. In contrast, the studies on drug-tolerant animals have shown that the drug acted as a stimulant on the spontaneous activity (3). Rosecrans and Schechter (5) attempted to correlate the behavioral effects of the alkaloid with the brain levels of nicotine. Clarke and Kumar (3) reported that mecamylamine, a specific antagonist of nicotine in the nicotinic receptor of autonomic ganglion, was able to prevent not only the initial inhibitory effect on the spontaneous motor activity of drug-naive rats but also the stimulating action in the tolerant animals (3). These observations suggest that nicotine exerts its seemingly diverse effects by directly acting on nicotinic acetylcholine receptor within the brain tissues. However, there are some evidences to indicate the possibility that the muscarinic receptor is also involved in the behavioral effects of nicotine. Ebenezer (6) observed that atropine pretreatment is able to reverse the initial inhibitory effect of nicotine on the spontaneous motor activity of drug-naive rats.

In the experiments reported herein, we attempted to investigate how the behavioral effect of nicotine in drug-naive mice would be affected by the pretreatment with mecamylamine, hexamethonium and atropine and to correlate the time course of the behavioral effect with the rise- and fall-pattern of the brain levels of the drug.

Materials and Methods

Animals
Male ddY mice aged 4 weeks (Kiwa Experi-
mental Animals Laboratories, Wakayama, Japan) weighing from 18 to 23 g were purchased. Groups of 10 mice were housed in aluminum cages with dimensions of 22 (W) x 32 (D) x 10 (H) cm, and the animals were given a solid diet (MF: Oriental Yeast Co., Tokyo) and tap water ad libitum under a 12 hr light/dark cycle with dawn and dusk (each over 2 hr) for 3 weeks. The room temperature was kept at 23±2°C, and the humidity was 60±10%. All animals were 7 weeks old and weighed from 28 to 33 g at the start of the experiment.

Drugs

(−)-Nicotine (Maruwaka Chem., Osaka), mecamylamine hydrochloride (Sigma), hexamethonium bromide (Sigma), atropine sulfate monohydrate (Wako Chem., Osaka), (−)-[N-methyl-3H] nicotine (60 Ci/mmol, Amersham, Japan) and saline (Otsuka, Tokyo) were used. The drugs were dissolved in saline and injected subcutaneously in a volume of 0.1 ml/10 g. Doses were expressed in terms of the free base of the drug.

Apparatus

The ambulatory activity was measured with an ambulo-meter (AMB: O'Hara & Co., Ltd., Tokyo). The principles of the device and the method for measurement of ambulatory activity in mice have been reported by Hirabayashi et al. (7). Briefly, each tilting of the mouse is recorded through a microswitch attached to the cage. The apparatuses were set up in the animal room, and the cumulative activity counts during every 5 min segment was printed on paper with an electromagnetic counter (TIDP: O'Hara & Co., Ltd., Tokyo) in the adjacent room. All the experiments were conducted between 9:30 and 13:00.

Procedure

Studies of behavior

All mice were naive to drugs and had no previous experience of the apparatus before the start of the experiment.

Experiment 1: Effects of different doses of nicotine on the ambulatory activity of mice: Each mouse was given an injection of nicotine (0.1, 0.5 and 1.0 mg/kg) and then put into each ambulo-cage. The activity counts were recorded during a 180 min period. The control group was injected with saline.

Experiment 2: Effects of pretreatment with mecamylamine, hexamethonium and atropine on the ataxia-inducing effect of nicotine (1.0 mg/kg) in mice: Mice were first pretreated with mecamylamine (0.04, 0.08, 0.4, 0.8, 2.0 and 4.0 mg/kg), hexamethonium (2.5 and 5.0 mg/kg) or atropine (1.0, 2.5 and 5.0 mg/kg). After placing each mouse in an aluminum cage (the same size as the home cage but a different one) for 20 min, each mouse was injected with nicotine (1.0 mg/kg) or saline and then transferred into an ambulo-cage. The ambulatory activity counts were recorded for a total of 180 min, but for the comparison of the activity between mice, the counts recorded for a 15 min period from 10 to 25 min after nicotine or saline injection were employed, because the ataxia-inducing effect of nicotine was found to reach a plateau.

Measurement of nicotine levels in the brain

The nicotine levels in the brain were measured by the method of Martin et al. (8). Mice were decapitated at 5, 10, 15, 20, 60 and 120 min after injection of 1 mg/kg of (−)-[3H]nicotine (100 µCi/mg nicotine), and each brain was homogenized in 5 ml of 0.05 N hydrochloric acid with 10 strokes of a glass homogenizer fitted with a teflon pestle. Extraction was performed by the method of Huker et al. (9). The brain homogenate was made basic with 3 drops of concentrated ammonium hydroxide and 1.0 ml of 40% K3PO4 (W/V), to which (−)-nicotine (1 mg/200 µl) as a carrier was added. After centrifugation at 1970×g for 5 min, 2 ml of the hexane layer was removed, and the radioactivity was counted by liquid scintillation spectrometry (Packard Model 3255).

Statistical analysis

Statistical analysis was carried out by Student's t-test.

Results

1) Effects of different doses of nicotine on the ambulatory activity of mice: Figure 1A shows the time course of the ambulatory activity recorded for each 20 min period, for a total of 180 min, after placing the animal in the ambulatory cage and the effects of different doses of nicotine. In the saline-treated control group, the activity was the highest in
the first period and thereafter gradually decreased. Nicotine (1.0 mg/kg) persistently depressed the activity from the first period to the third according to the data recorded in this manner. Even in the lower dose (0.5 mg/kg), the drug tended to depress the activity for at least 40 min. The lowest dose (0.1 mg/kg) showed no depressive effect throughout the experiment; and rather, in the first period, the drug was found to significantly stimulate the ambulatory activity.

The biphasic pattern of effects of nicotine on the ambulatory activity of mice became more prominent when the initial two 20 min periods were divided into eight 5 min periods. As shown in Fig. 1B, nicotine in all doses employed herein first increased the activity, and then the ataxic phase of effects of the drug developed, if any, as noted with the high doses of 0.5 or 1.0 mg/kg. The latter phase of the effect was apparently dose-dependent, and the highest dose (1.0 mg/kg) elicited a marked and long-lasting ataxia as seen in Fig. 1A.

2) Effects of pretreatment with mecamylamine, hexamethonium and atropine on the ataxia-inducing effect of nicotine (1.0 mg/kg) in mice: In mice pretreated with varying doses of mecamylamine, the ataxia-inducing effect of nicotine was found to be effectively antagonized by the nicotinic antagonist in a dose range of 0.4–2.0 mg/kg. The antagonism developed in a dose-dependent manner. In a higher dose (4.0 mg/kg), however, the antagonist exhibited its intrinsic inhibitory effect on the ambulatory activity, and no antagonism was discernible (Fig. 2).

In contrast, neither hexamethonium or atropine attenuated the nicotine effect in the doses tested. In the case of atropine, the pretreatment alone markedly increased the ambulatory activity (Table 1).

3) The time course of nicotine levels in the brain tissue after subcutaneous injection to mice: Figure 3 shows the time dependent changes of [3H]nicotine levels in the whole brain. The nicotine content began to increase from 5 min after injection and attained the plateau level at 15 min and thereafter declined quickly, but even 60 min after the injection, a certain level of the drug was detectable in the tissue. The overall time course of the brain level appears to be better correlated with the time course of the ataxic-inducing effect of nicotine (1.0 mg/kg) rather than with the initial phase of the drug effect, namely, increased ambulatory activity (Fig. 1B).

Discussion

The present study reveals that high doses of nicotine (0.5 and 1.0 mg/kg) administered subcutaneously to mice decreased the ambulatory activity in a dose-dependent manner from 5 to 60 min after administration, and the higher dose (1.0 mg/kg) caused a long-lasting ataxia. These findings are consistent with the majority of previous studies. Morrison and Armitage (10) reported
that high doses of nicotine (0.4 and 0.8 mg/kg) administered subcutaneously to mice strongly decreased the spontaneous motor activity (in light beam boxes), leading to no motor activity for the first 10 to 15 min, and the effect lasted at least for the first 60 min. In contrast, a low dose (0.1 mg/kg) of nicotine failed to decrease the ambulatory activity, but rather increased the activity during the first 20 min after the administration. Mansner (11) reported that a low dose (0.125 mg/kg) of nicotine administered subcutaneously to mice increased motor activity (in photoelectric motility boxes) during the first 20 min. However, Clarke and Kumar (3) reported that a low dose (0.1 mg/kg) of nicotine adminis-

![Figure 2](image_url)

**Table 1.** Effects of pretreatment with hexamethonium and atropine on ataxia-inducing effect of nicotine in ambulatory activity in drug naive mice

<table>
<thead>
<tr>
<th>Pretreatment (mg/kg)</th>
<th>Post-treatment (mg/kg)</th>
<th>N</th>
<th>Ambulatory activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (control)</td>
<td>saline</td>
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<td>66±16</td>
</tr>
<tr>
<td>Hexamethonium 5.0</td>
<td>saline</td>
<td>8</td>
<td>94±15 NS</td>
</tr>
<tr>
<td>Atropine 1.0</td>
<td>saline</td>
<td>9</td>
<td>184±45*</td>
</tr>
<tr>
<td>Atropine 2.5</td>
<td>saline</td>
<td>10</td>
<td>225±35**</td>
</tr>
<tr>
<td>Atropine 5.0</td>
<td>saline</td>
<td>9</td>
<td>276±13**</td>
</tr>
<tr>
<td>Saline (control)</td>
<td>nicotine 1.0</td>
<td>8</td>
<td>12±3</td>
</tr>
<tr>
<td>Hexamethonium 2.5</td>
<td>nicotine 1.0</td>
<td>11</td>
<td>20±6 NS sub</td>
</tr>
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<tr>
<td>Atropine 5.0</td>
<td>nicotine 1.0</td>
<td>10</td>
<td>30±10 NS sub</td>
</tr>
</tbody>
</table>

Data were collected for 10 to 25 min after s.c. injection of saline or nicotine. Hexamethonium and atropine were given subcutaneously to mice 20 min before saline or nicotine. Each value represents the mean ± S.E.M. **P<0.01 and *P<0.05: Significantly different from the saline-saline group. NS, not significant as compared with the saline-saline group and the saline-nicotine group, respectively.

N=number of mice.
tered subcutaneously to rats decreased the spontaneous motor activity (in photocell cages) during the first 20 min. So far, there is yet no conclusive explanation for what causes such different effects.

The present study shows that there is a good correlation between the brain level of \([^{3}H]\text{nicotine}\) and the intensity of its ataxia-inducing effect. Similar results have been reported by Rosecrans and Schechter (5) for rats and Mansner (11) for mice, although the brain level after the peak in rats waned more slowly than that in mice. The results taken together suggest that acute-tolerance would not develop to the ataxia-inducing effect of nicotine, at least, within 60 min after the administration.

It is well-known that the quaternary amines such as hexamethonium, generally would not penetrate easily the blood-brain barrier (12). Thus, a possible conclusion would be that nicotine was able to be antagonized beyond the barrier by mecamylamine which could more readily penetrate the barrier than hexamethonium. However, it has been reported that the nicotinic antagonists (hexamethonium, decamethonium and mecamylamine) are poor inhibitors of binding of \([^{3}H]\text{nicotine}\) and \(\alpha-[^{125}I]\text{bungarotoxin}\) to the brain membrane fraction (13, 14). The next step of our study should be identification of the site and type of the receptor at which the antagonism between nicotine and mecamylamine would take place, because many of the behavioral effects of nicotine were in fact blocked by this antagonist, possibly involving the cholinergic receptor in the brain (15).

Ebenzer (6) reported that atropine pretreatment effectively blocks the depressant effect of nicotine on the spontaneous motor activity of drug-naive rats. In the present study, however, atropine failed to affect the ataxic effect of nicotine (1.0 mg/kg) in mice, while the pretreatment alone was found to markedly increase the ambulatory activity. On the other hand, our observations of the latter increasing effect appear to be in accord with the results reported by Kuribara and Tadokoro (16). They reported that scopalamine administered subcutaneously to mice increased the ambulatory activity, suggesting that the increase is caused by the central muscarinic blockade. A similar mechanism would be functioning for the intrinsic action of atropine observed herein.

In conclusion, the results obtained herein suggest that nicotine exerts its ataxic effect centrally and possibly by directly acting on
the nicotinic acetylcholine receptor, but more detailed studies are needed in order to identify the site and type of the receptor that is affected by nicotine to produce the behavioral effect.

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References