Characteristics of Effects of Repeated Scopolamine Administration on Ambulatory Activity in Mice and Methamphetamine Sensitivity in the Scopolamine-Experienced Mice: Comparison among 6 Strains

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Abstract—Effects of scopolamine (0.5 mg/kg, s.c.) on ambulatory activity were investigated in 6 strains of mice (dd, ICR, BALB, C57BL, C3H and DBA). Scopolamine increased ambulatory activity, and the sensitivities were in the order of ICR>C3H>BALB>DBA>C57BL>dd. This was different from that produced by methamphetamine (2 mg/kg, s.c.) where the order was ICR>dd>DBA>C3H>C57BL>BALB. A tolerance to the ambulation-increasing effect of scopolamine was progressively produced in dd, ICR, C57BL and DBA strains, but not in BALB and C3H strains, when the drug was administered 5 times at intervals of 3–4 days. The repeated scopolamine treatment elicited a major enhancement of the sensitivity to the ambulation-increasing effect of methamphetamine (2 mg/kg, s.c.) in BALB and C3H strains and a minor enhancement in the C57BL strain, whereas dd, ICR and DBA strains did not exhibit a marked change in the sensitivity to methamphetamine even after the same treatment with scopolamine. These results suggest that the ambulation-increasing effect of scopolamine is different from that of methamphetamine and that the interaction between scopolamine and methamphetamine varies among different strains of mice.

A number of behavioral studies have demonstrated that a prior drug experience yields complex changes in the sensitivity not only to the analogous drugs but also to other types of drugs (cf., 1–3). In this respect, it is important to study the effects of drugs in both drug-naive and drug-experienced animals.

Previously, we (4) reported that repeated administration of scopolamine, a prototype muscarinic anticholinergic drug, at intervals longer than 1 day, produced a tolerance to its ambulation-increasing effect in the dd strain of mice. However, it is well-known that there are marked differences in the neuronal activity of central cholinergic systems as well as in the sensitivities to cholinergic and/or anticholinergic drugs among mouse strains (5–8). Thus, mouse strain differences should be considered when making a general statement on the effect of repeated administration of scopolamine on ambulatory activity in mice.

Hence, the first purpose of this experiment was to study the change in the scopolamine-induced increase in the ambulatory activity after repeated administration to 6 strains of mice. The second purpose was to study the change in sensitivity to the ambulation-increasing effect of methamphetamine in the scopolamine-experienced mice. This is not only because the ambulation-increasing effect of methamphetamine is sometimes modified by a prior experience with the different types of drugs (3, 9, 10), but also because cholinergic systems interact with catecholaminergic systems in the brain (11–13).

Materials and Methods

Animals: The experimental animals were male mice of the dd, ICR, BALB, C57BL,
C3H and DBA strains. We have reported that these 6 mouse strains exhibit characteristic sensitivities to the ambulation-increasing effects of methamphetamine and morphine (3). The dd strain mice were provided by the Institute of Experimental Animal Research, Gunma University School of Medicine. The other 5 strains of mice were purchased from Charles River Japan, Inc. They were housed in groups of 8–10 in aluminum or acrylfiber cages of 30(D)×20(W)×10(H) cm in a controlled room (temperature: 23±2°C and light period: 6 a.m.–6 p.m.), and they were freely given a solid diet (MF: Oriental Yeast Co.) and tap water except during the times of the experiment. The experiment was started when the mice were 7 weeks old and weighed 28–32 g in the dd and ICR strains, and 23–27 g in the BALB, C57BL, C3H and DBA strains. The numbers of mice used in the experiment were 20–40 of each strain.

Procedure: The apparatus for measurement of ambulatory activity of the mouse was a tilting-type ambulometer with a plexiglas activity cage of 20 cm in diameter (AMB-10, O'hara & Co. Ltd.). Each mouse was put into the activity cage, and after an adaptation period of 30 min, drug was administered.

The drugs used and the doses (shown in the salt forms) were scopolamine HBr (Sigma Chemical Co., 0.5 mg/kg) and methamphetamine HCl (Dainippon Pharm. Co., 2 mg/kg). They were dissolved in physiological saline vehicle and administered subcutaneously (s.c.) in a fixed volume of 1 ml/100 g body weight.

Scopolamine was administered 5 times at intervals of 3–4 days, and the mouse's ambulatory activity was observed for 90 min after each administration. Four days after the 5th administration, the scopolamine-experienced mice were given methamphetamine, and the ambulatory activity was observed for 180 min thereafter. As the control experiment, the effect of methamphetamine in drug-naive mice of the same age as the scopolamine-experienced mice were investigated. The doses of scopolamine (0.5 mg/kg) and methamphetamine (2 mg/kg), and the intervals and number of the repeated administration of scopolamine (3–4 days intervals and 5 times, respectively) were considered to be optimum according to our previous experiments (3, 4, 14, 15).

The measurement of the ambulatory activity was carried out between 10 a.m.–4 p.m.

Statistical analysis: The mean overall ambulatory activity counts for 90 min after scopolamine and for 180 min after methamphetamine were calculated in each strain. The statistical comparisons between the mean values were done by Student's t-test. When P values were equal to or less than 0.05, they were defined to be significantly different.

Results

Effects of scopolamine in the drug-naive mice: Figure 1 shows temporal changes in
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the mean ambulatory activity counts in the drug-naive mice of 6 strains after the administration of scopolamine (0.5 mg/kg, s.c.). The dd and ICR strains exhibited comparatively higher activity counts than the other 4 strains immediately after they were placed in the activity cage. However, there was no marked difference in the activity counts among the 6 strains at the time of the drug administration. In all strains, scopolamine increased the ambulatory activity with the maximum effect at around 20 min after the administration. At this point, the mean activity counts were comparatively higher in the ICR strain, intermediate in the BALB, DBA and C3H strain, and lower in the dd and C57BL strains. Ninety min after the scopolamine administration, the dd, BALB, C57BL and DBA strains exhibited activity counts as high as those of the corresponding pre-drug levels. However, the scopolamine-induced increase in the ambulatory activity lasted for longer than 90 min in the ICR and C3H strains.

Figure 2 shows the mean overall ambulatory activity counts for 90 min after the scopolamine administration in the 6 strains. The activity counts were higher in the order of ICR>C3H>BALB>DBA>C57BL>dd. The mean activity counts of ICR and C3H strains were significantly higher than those of the other 4 strains. The ratio between the highest (ICR strain) and lowest (dd strain) counts was estimated to be 2.5.

Effects of the repeated scopolamine administration: Figure 3 shows the mean overall ambulatory activity counts after the repeated scopolamine administration in the 6 mouse strains. The dd, ICR, C57BL and DBA strains exhibited a progressive decrease in the ambulation-increasing effect of scopolamine, whereas the BALB and C3H strains showed no marked change in the effect throughout the 5 times of administration.

Effects of methamphetamine in the scopolamine-experienced mice: Figure 4 shows the mean overall ambulatory activity counts for 3 hr after the administration of methamphetamine (2 mg/kg, s.c.) in the drug-naive and scopolamine-experienced mice. Methamphetamine increased the ambulatory activity with the maximum effect at 40–60 min after the administration, and the effect lasted for about 3 hr in all the strains. In the drug-naive mice, the BALB and C57BL strains exhibited much lower sensitivity than the other 4 strains to methamphetamine. The scopolamine-experienced mice in the BALB and C3H strains demonstrated a major enhancement of the sensitivity to methamphetamine. A minor enhancement of the sensitivity to methamphetamine was observed in the scopolamine-experienced C57BL strain mice, whereas in the dd, ICR and DBA strains, the repeated scopolamine administration yielded no marked change in the sensitivity to methamphetamine.

Discussion

The present experiment clearly demonstrated that in the drug-naive mice, there is a marked strain difference in the sensitivity to the ambulation-increasing effect of scopolamine. Scopolamine suppresses muscarinic cholinergic systems, and it might indirectly stimulate dopaminergic systems through a blockade of the inhibitory control system of the cholinergic neurons (11–13). It is there-
fore expected that the strain difference observed in this experiment is due to the difference in the neuronal activities of cholinergic and dopaminergic systems as suggested by many researchers (5–8, 16–24), and that there is a positive correlation between the sensitivities to anticholinergic and dopaminergic drugs. However, this concept is insufficient to precisely explain the strain difference. As demonstrated in Fig. 4, methamphetamine increases the ambulatory activity in mice, and the effect is considered to be elicited from a stimulation of the catecholaminergic, in particular dopaminergic, systems. The order of sensitivity to the ambulation-increasing effect of scopolamine was not identical with that to methamphetamine. Thus, there is no definite correlation between the effects of scopolamine and methamphetamine, suggesting that other mechanisms are involved in the strain differences. Scopolamine is mainly metabolized in the liver (25). As can be seen in Fig. 1, there were marked differences in the duration of the ambulation-increasing effect of scopolamine among the mouse strains. However, we did not carry out pharmacokinetic or neurochemical exami-
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The present experiment also demonstrated that repeated scopolamine administration yielded a tolerance to its ambulation-increasing effect in 4 strains: dd, ICR, C57BL and DBA, but no marked change in the sensitivity in 2 strains: BALB and C3H. Thus, it is notable that the repeated administration of scopolamine never produces a reverse tolerance to the ambulation-increasing effect. In addition, the degrees of tolerance do not correlate with the sensitivities to scopolamine in the 1st administration. In contrast, the repeated administration of methamphetamine produces a reverse tolerance to the ambulation-increasing effect in all the strains, and the degrees positively correlate with the drug sensitivities in the 1st administration (3). These results indicate that the characteristics of the ambulation-increasing effect of scopolamine are different from that of methamphetamine. The mechanisms of the tolerance to scopolamine is unclear. Marks et al. (26) reported that the repeated administration of scopolamine (5 mg/kg/day) induced an increase in the QNB binding sites in the C3H strain, suggesting a change in the cholinergic receptors. The dose of scopolamine, however, was 10 times higher than that administered in our experiment, and this mouse strain did not exhibit the tolerance to scopolamine in our experiment.

The present experiment finally demonstrated that the scopolamine-experienced mice in the BALB and C3H strains, which did not exhibit a tolerance to scopolamine, showed a major enhancement of sensitivity to the ambulation-increasing effect of methamphetamine. In contrast, the same treatment with scopolamine failed to produce a marked change in the sensitivity to methamphetamine in dd, ICR and DBA strains, which exhibited a tolerance to scopolamine. The only exception was the result from the C57BL strain which showed a minor enhancement of the sensitivity to methamphetamine in spite of exhibiting a tolerance to scopolamine. This is probably due to a very low sensitivity to methamphetamine in the drug-naive state, and thereby an enhancement of drug sensitivity is easily
induced. In these respects, it is possible to make a general statement on the ambulatory activity in mice that a prior scopolamine experience sometimes, but not always, induces an enhancement of sensitivity to methamphetamine, and that such cross interaction is easily produced in mouse strains that do not exhibit a tolerance to scopolamine. However, mechanisms of the interaction between scopolamine and methamphetamine can not be explained precisely by only the present results. Thus, a further study including a search for dose-related interaction between the two drugs is required.

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