Time-Related Facilitation and Suppression of Drinking by Muscarinic Anticholinergic Drugs in Water Non-Deprived Rats

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Abstract—Effects of tertiary anticholinergic drugs, atropine (1.3, 2.5, 5 and 10 mg/kg, s.c.) and scopolamine (0.13, 0.25, 0.5 and 1 mg/kg, s.c.), and a quarternary anticholinergic drug, methylatropine (1.3, 2.5, 5 and 10 mg/kg, s.c.), on the drinking behavior were investigated in water non-deprived rats that were housed in a 12-hr light-dark situation (light period: 6:00–18:00) with a free access to food. Atropine, 5 mg/kg, and scopolamine, 0.13 and 0.25 mg/kg, administered at 12:00, significantly increased the drinking during the 12:00–18:00 period. Furthermore, lower to medium doses of atropine increased the drinking during the 18:00–6:00 period. In contrast, the drinking did not change during the 12:00–18:00 period, but decreased during the 18:00–6:00 period in a dose-dependent manner after administration of methylatropine at 12:00, whereas the drinking during the 18:00–24:00 period decreased in a dose-dependent manner when both the tertiary and quarternary drugs were administered at 18:00. However, the drinking during the 24:00–6:00 period increased in the rats that were administered atropine at the dose of 5 or 10 mg/kg at 18:00, while the drinking still decreased after methylatropine at the same time. The present results suggest that in water non-deprived rats, central muscarinic cholinergic blockade is effective for both increasing and decreasing drinking behavior, depending on the doses, when the drug is administered, and time span between the drug administration and the behavior observation. It is also suggested that peripheral cholinergic blockade monotonously suppresses the drinking behavior.

It has been considered that central muscarinic cholinergic systems are involved in drinking behavior. This concept has come from the following results (see reviews, 1–4). Firstly, administration of muscarinic cholinergic drugs such as muscarine, carbachol and/or physostigmine in the hypothalamic region elicits drinking behavior in water non-deprived rats, and these drinking behaviors are antagonized by anticholinergic drugs. Secondly, drinking behavior induced by water deprivation, hypertonicity, hypovolemia as well as an experimental polydipsia, which is produced by an intermittent food delivery schedule, is suppressed not only by peripheral administration of the tertiary anticholinergic drugs but also by central administration of both tertiary and quarternary anticholinergic drugs. The drinking-suppressing effect of anticholinergic drugs are observed for several hours after the drug administration; and therefore, the behavior observations were carried out only for 2 hr or less in the previous experiments.

In contrast to these results, it has been demonstrated that repeated administration of atropine, a typical central-acting (tertiary) anticholinergic drug, elicits a facilitation of drinking in water non-deprived rats (5, 6) and even in deprived rats (7). In particular, Soulairac (5) and Webb et al. (6) observed that atropine at doses of 40 mg/kg and 10 mg/kg, respectively, transiently suppressed the drinking, and this was followed by a
drinking facilitation when it was administered repeatedly. With respect to this evidence, they suggested that a long-term observation is much more important for investigating the drug effect on the drinking behavior. However, in these experiments, the doses of atropine tested were comparatively higher than those used in the other behavioral investigations, and no dose-effect study was carried out. Furthermore, as yet, no systematic study has been conducted on the effect of anticholinergic drugs on drinking behavior.

The drinking behavior of water non-deprived rats shows a clear diurnal pattern of nocturnal habit with high and low rates of drinking during the dark and light periods, respectively (8). Thus, to evaluate the drug effect on the drinking behavior in water non-deprived rats, time-related as well as dose-effect studies are required. This is because the baseline drinking rate varies depending on time-of-day, and such a rate variation is an important factor in the determination of the drug effects of morphine on feeding and drinking behaviors in rats (9).

Hence, the purpose of this experiment was to investigate the effects of muscarinic anticholinergic drugs on the drinking behavior in water non-deprived rats. The drug administration was carried out at the mid point of the light period (12:00) and at the start of the dark period (18:00) to observe the time-of-day effect. Furthermore, the effects of tertiary and quarternary anticholinergic drugs were studied to compare the role of central and peripheral cholinergic blockades.

**Materials and Methods**

**Animals:** The experimental animals were 30 adult male rats of the Wistar strain that were provided by the Institute of Experimental Animal Research, Gunma University School of Medicine. These animals were individually housed in standard breeding cages of stainless steel wire mesh with dimensions of 45(W) × 25(D) × 20(H) cm and they were allowed free access to food (MF, Oriental Yeast) and tap water.

The breeding room was controlled to a 12-hr light and dark schedule (light period: 6:00–18:00) and temperature of 23±2°C. When the rats were 15 weeks of age and weighed around 400 g, measurement of their drinking behavior was started. These rats were allowed free access to food and water during the experimental period.

**Measurement of drinking behavior:** The apparatus for measurement of rats’ drinking behavior (Model LA-10, O’Hara & Co.) was the same as that used in our previous study (8). This apparatus enabled us to record the drinking behaviors of 10 rats, that were housed under normal breeding conditions, simultaneously and separately by counting water drops of 0.05 ml each, which were made in a specially-made cartridge. In the present experiment, the water drops counted were automatically printed out at intervals of 1 hr, and the water intake was estimated by the drops.

**Drugs and administration schedules:** According to the predrug observation of the rats’ drinking behavior, the 30 rats were assigned into 6 equivalent groups of 5 rats each, and the drug testing was started.

The drugs tested and the doses administered were atropine sulfate (AT, Merck: 1.3, 2.5, 5 and 10 mg/kg, s.c.) and scopolamine hydrobromide (SCP, Sigma Chemical: 0.13, 0.25, 0.5 and 1 mg/kg, s.c.); tertiary drugs, and methylatropine nitrate (Mt-AT, Sigma Chemical: 1.3, 2.5, 5 and 10 mg/kg, s.c.); a quarternary drug. These drugs were dissolved in physiological saline vehicle and the drug solutions were administered at 12:00 (midpoint of the light period) or 18:00 (starting point of the dark period). Each administered volume was fixed to 1 ml/kg.

The 6 groups of 5 rats each were treated with one of 6 conditions of drug administration: drug (AT, SCP and Mt-AT) × time-of-day (12:00 and 18:00). In each drug testing, physiological saline was first administered, and this was followed by the administration of 4 doses of the corresponding drug. The doses (expressed in the salt form) administered progressed from the lower to the higher for the first 3 rats and the reverse order for the last 2 rats. The drug administration was held at intervals of 1 week. One week after the final drug administration, saline was administered again. Therefore, the control value in each animal was the mean of the data obtained from the 2 times saline administration.
Statistical analysis: The mean water intake for every 6 hr was calculated after each drug administration. The mean volumes drunk were compared with the corresponding control values using the paired t-test. When P values were equal to or less than 0.05, they were defined to be significantly different.

Results

Baseline drinking: The total daily water intake of the rats during the no-treatment days was 40-45 ml, which corresponded to 10–12% of the body weight. The 24-hr drinking patterns of the rats well synchronized with the light-dark alteration, and a large proportion (85–95%) of the total daily volume of water was consumed during the dark period of 18:00–6:00. The administration of saline, which was conducted 2 times before and after the drug treatment, produced no marked change in the drinking.

Drug administration at 12:00: Figure 1 shows dose-effect relationships of AT, SCP and Mt-AT, administered at 12:00 (mid light period), for drinking in rats.

AT significantly increased the drinking during the 12:00–18:00 and 18:00–24:00 periods at 5 mg/kg and the 24:00–6:00 period at 1.3–5 mg/kg. However, AT at the dose of 10 mg/kg failed to produce significant change in the water intake throughout the day.

SCP at the doses of 0.13 and 0.25 mg/kg, but not 0.5 and 1 mg/kg, significantly increased the drinking during 12:00–18:00 period. However, this drug did not affect water intake during the 18:00–12:00 period at any doses tested.

Mt-AT at 1.3, 2.5, 5 and 10 mg/kg produced no marked change in the drinking during the 12:00–18:00 period, but dose-dependently decreased water intake during the 18:00–6:00 period.

Drug administration at 18:00: Figure 2 shows dose-effect relationships of AT, SCP and Mt-AT, administered at 18:00 (starting point of the dark period), for drinking in rats in a similar way as in Fig. 1.

AT decreased the drinking during 18:00–24:00 period in a dose-dependent manner while it increased the drinking during the 24:00–6:00 period at 5 and 10 mg/kg. No marked change in the drinking was observed during the 6:00–12:00 and 12:00–18:00 periods at any doses tested.

SCP decreased the water intake during the 18:00–24:00 period and the changes were significantly different at 0.13, 0.5 and 1 mg/kg. However, there was no remarkable change in the amount of drinking during the 24:00–18:00 period except the value during the 6:00–12:00 period after 0.5 mg/kg.

Mt-AT suppressed the drinking during the 18:00–24:00 and 24:00–6:00 periods in a dose-dependent manner. However, no significant change in the water intake was observed during the 6:00–18:00 period.

Discussion

AT and SCP, which are tertiary drugs, easily penetrate the blood-brain barrier and exhibit both central and peripheral effects of muscarinic cholinergic blockade when they are administered peripherally. On the other hand, Mt-AT, which is a quarternary drug, can hardly penetrate the barrier and shows only peripheral effects after its peripheral administration. With respect to these characteristics, it can be expected that the effects observed after the tertiary drugs but not after the quarternary drug are elicited by the central action.

The present experiment demonstrated that after administration of tertiary and quarternary anticholinergic drugs, the rats' drinking behavior changed in a manner closely dependent on the types of drugs as well as on the times-of-day of the drug administration and the time span between the drug administration and behavior observation.

Both AT and SCP, but not Mt-AT, facilitated the drinking during the 12:00–18:00 period when the medium doses were administered at 12:00. This result strongly suggests that AT- and SCP-induced drinking is elicited by the central effect of these drugs.

In general, a central muscarinic cholinergic blockade has been considered to be effective for suppressing drinking behavior which is induced by various treatments, including administration of cholinergic drugs in the hypothalamic region, water-deprivation, administration of hypertonic saline solution, and/or schedule-induction in an intermittent
Fig. 1. Dose-effect relationships of atropine (upper panel), scopolamine (middle panel) and methylatropine (lower panel), administered at 12:00 (mid point of light period), for drinking in water non-deprived rats. The dose=0 indicates the administration of saline vehicle as the control experiment. In this figure, mean water intakes of 5 rats at 6-hr intervals (i.e., 12:00-18:00, 18:00-24:00, 24:00-6:00 and 6:00-12:00 periods) are shown with their S.E.M. *indicates significant difference as compared with the saline-treated control value (P<0.05, paired t-test). Rats were housed in a 12-hr light-dark situation (light period: 6:00–18:00), and stippled bands in the event channel indicate data during the dark period.
Fig. 2. Dose-effect relationships of atropine (upper panel), scopolamine (middle panel) and methylatropine (lower panel), administered at 18:00 (start point of dark period), for drinking in water non-deprived rats. The data are shown in a similar way as in Fig. 1.

Food delivery situation (1–4). Our experimental conditions were markedly different from these situations, but rather similar to those of Soulairac (5) and Webb et al. (6) who used water non-deprived rats in a normal breeding condition. Consistent with our results, Soulairac (5) and Webb et al. (6) also reported a facilitation of drinking after
AT, although such an effect was produced by repeated administration of comparatively higher doses.

For one of the mechanisms of the drinking facilitation, a change in the drinking type, namely a development of prandial drinking (7), is considered to be involved. In addition, other mechanisms, in particular a rate-dependency of drug effects, should be considered in the change in drinking behavior after administration of anticholinergic drugs. This is because many drugs tend to increase a low-rate behavior, while they decrease a high-rate behavior even after administration of the same dose (c.f. review, 10).

The higher doses of AT and SCP tested in this experiment failed to significantly increase the drinking during the 12:00–18:00 period. This negative effect is considered to be due to an over dosage and/or by a competition of the central and peripheral effects. The latter effect suppresses the drinking as described hereafter.

Furthermore, after administration of AT at 12:00, the drinking never decreased but rather increased during the dark period of 18:00–6:00 in which the rats’ drinking was much higher than in the light period of 6:00–18:00. This finding indicates again that in this experimental condition, the central cholinergic blockade is effective for increasing the drinking behavior and that role of rate-dependency of the drug effect is comparatively smaller in the AT- and SCP-induced drinking facilitation. In contrast, the same treatment with Mt-AT suppressed the drinking, indicating that the peripheral cholinergic blockade is only effective for suppressing the drinking behavior.

On the other hand, SCP produced no marked change in the drinking during the 18:00–12:00 period after the administration at 12:00. This result is considered to indicate the short-acting property of SCP.

AT, SCP and Mt-AT all suppressed the drinking during the 18:00–24:00 period when these drugs were administered at 18:00. These results are in agreement with the previous reports (1–6) that the cholinergic blockade is effective for suppressing high-rate drinking for several hours. The drinking-suppressing effect of AT was stronger than that of Mt-AT. This difference might reflect the presence or absence of the central effects.

Interestingly, AT at the doses of 5 and 10 mg/kg, administered at 18:00, increased the drinking during the 24:00–6:00 period, whereas the same treatment with Mt-AT still showed the drinking-suppressing effect. It is therefore highly probable that the drinking-facilitating effect of tertiary anticholinergic drugs appears at several hr after the administration in the case of a comparatively higher baseline drinking rate as reported by Soulairac (5) and Webb et al. (6).

SCP administration at 18:00 was ineffective for producing a marked change in the drinking during the 24:00–6:00 period and also the 6:00–18:00 period. This evidence supports again the short-acting property of this drug.

It can be concluded from the present results that in water non-deprived rats, the central muscarinic cholinergic blockade is sometimes effective for increasing drinking behavior, depending on the schedules of the drug administration and behavior observation as well as on the types of drinking behavior, although it has been generally considered that cholinergic blockade suppresses the drinking behavior. In contrast, the peripheral muscarinic cholinergic blockade is considered to monotonously suppress the drinking. Thus, in order to evaluate drug effects on the drinking behavior using water non-deprived rats, not only a time-related but also a dose-related investigation are required. In addition, a long-term behavior observation is also required after the drug administration to evaluate drug effect correctly.

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