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Evidence for the Involvement of Dopamine in Ambulation Promoted by Menthol in Mice

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Abstract. The present study examines the mechanism that underlies the ability of menthol (ME), a major constituent of peppermint oil, to promote mouse ambulation. We initially confirmed that bupropion (BUP), a dopamine (DA) uptake inhibitor, promotes ambulation in ICR mice. Since the subcutaneous administration of ME produced similar effects in mice, we investigated the effects of ME on ambulation when combined with BUP. The results showed that BUP potentiated the effect of ME on mouse ambulation. We then examined effects of the DA antagonists chlorpromazine, haloperidol, fluphenazine, spiperone, and SCH12679 on the ability of BUP and ME to promote ambulation. All of these DA antagonists attenuated the effects of BUP and ME. Prior exposure to reserpine, which depletes monoamines, caused decreased sensitivity to the ability of BUP and of ME in promoting ambulation. The tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine, similarly decreased subsequent sensitivity to the effects of BUP and ME. These results suggest that DA is involved in the abilities of ME and BUP to promote ambulation in mice.

Keywords: menthol, ambulation, dopamine agonist, dopamine antagonist

Introduction

Various essential oils (EOs) derived from plants have traditionally been used to treat a variety of mental disorders. The medicinal use of EOs that originated in ancient Egypt has continued until the present. The aromatherapy movement that has spread worldwide shows promise as an alternative medicine (1, 2), despite the absence of a scientific basis for its effectiveness. On the other hand, the long history of EOs in therapy suggests that they do indeed have psychoactive effects. Our series of studies (3 – 5) revealed that some EOs affect mouse behavior.

Peppermint oil is believed to be useful in treating nervous disorders and mental fatigue (6). A previous study (5) has demonstrated that peppermint oil promotes ambulation in ICR mice, indicating that the effect is similar to that of psychostimulants. In addition, we also demonstrated that the effect arises from its active constituents such as menthol, menthone, isomenthone, 1,8-cineol, (*R*)-(+)-pulegone, menthyl acetate, and caryophyllene, all of which promote ambulation in mice. The intravenous administration of these constituents promotes ambulation at much lower doses than intraperitoneal administration. This observation suggests that the constituents become effective after absorption into the bloodstream. Although these compounds are thought to act on the central nervous system, the mechanism underlying their effects remains unclear.

Direct and indirect dopamine (DA) agonists administered to mice promote ambulation (7 – 10), which is abrogated by DA antagonists (11 – 16). DA might also be involved in the ability of non-dopaminergic drugs such as MK-801 and morphine to promote ambulation in mice (17, 18). These findings indicate that DA plays an important role in the control of mouse ambulation. We thus questioned whether DA is involved in the ability of constituents of peppermint oil to promote ambulation in mice.

We designed the present study to specifically determine whether DA is involved in the ability of menthol to promote ambulation in mice.

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Materials and Methods

Animals

Male ICR mice (Clea Japan, Tokyo) aged 7 – 10 weeks and weighing between 32 – 40 g were housed in Plexiglas cages (10 mice/cage) with a stainless-steel mesh top and excelsior bedding (Clea Japan). Commercial solid food (Clea Japan) and tap water were available ad libitum. The cages were placed in a room artificially illuminated by fluorescent lamps on a 12L:12D schedule (light period: 07:00 – 19:00), at a temperature of 25 ± 1°C.

All experiments proceeded in accordance with the guidelines of the Ethics Committee for Experimental Animals of the National Institute for Environmental Studies, Japan.

Drugs

We investigated the effects of menthol (ME) (Nacalai Tesque, Kyoto), the DA uptake inhibitor bupropion (BUP) (19, 20), as well as the DA antagonists, chlorpromazine (CPZ), haloperidol (HAL), fluphenazine (FLU), spiperone (SPI), and SCH12679 (SCH) (*R*(–)-1-phenyl-2,3,4,5-tetrahydro-1*H*-7,8-dimethoxy-3-benzazepine) (Research Biochemicals, Natick, MA, USA). We also examined effects of prior exposure to reserpine (RES) (Sigma-Aldrich, Tokyo), which depletes monoamines, and α-methyl-*p*-tyrosine (AMPT), an inhibitor of tyrosine hydroxylase (Wako Pure Chem., Osaka) on the ability of BUP and ME to promote ambulation. Menthol, RES, and AMPT were mixed with a small amount of Tween 80 (Nacalai Tesque) and then diluted in saline (0.9% NaCl). Haloperidol was dissolved in 0.1% acetic acid. Other drugs were dissolved in saline. All drugs except AMPT were administered subcutaneously in a volume of 1 ml/100 g body weight regardless of dosage. AMPT was administered intraperitoneally.

Measurement of ambulatory activity in ICR mice

We measured ambulatory activity, which is a type of spontaneous motor activity in mice, using a tilting-type ambulometer consisting of 10 bucket-like Plexiglas activity cages (20 cm in diameter) (SAM-10; O'Hara and Co., Tokyo) (21). Details of this apparatus have been reported elsewhere (22).

Experimental procedure

Experiment 1. Effect of subcutaneous administration of ME on ambulation in ICR mice: Mice were placed individually in activity cages; and after an adaptation period of 30 min, saline containing a small amount of Tween 80 or 100, 200, 400, or 800 mg/kg of ME was administered subcutaneously. Thereafter, ambulatory activity was measured continuously for 60 min.

Experiment 2. Effect of BUP on ambulatory activity in mice: Mice were adapted for 30 min, then saline or 2.5, 5, or 10 mg/kg of BUP was administered, and ambulatory activity was measured continuously for 60 min.

Experiment 3. Effect of ME combined with BUP on ambulatory activity in mice: Mice were adapted for 30 min, and then saline or 1.25, 2.5 or 5 mg/kg of BUP was administered. Ten minutes later, saline containing small a amount of Tween 80 or 100, 200, or 400 mg/kg of ME was administered to the same mice, and then ambulatory activity was continuously measured for 60 min.

Experiment 4. Effects of BUP or ME combined with DA antagonists on ambulatory activity in mice: We examined the effects of DA antagonists on the ability of BUP to promote mouse ambulation. The mice were adapted for 30 min, and then various doses of CPZ, HAL, FLU, SPI or SCH were administered. Ten minutes later, 10 mg/kg of BUP was administered to the same mice, and then ambulatory activity was continuously measured for 60 min. We examined the effects of DA antagonists on the ability of ME to promote ambulation. After an adaptation period of 30 min, we administered various doses of CPZ, HAL, FLU, SPI or SCH, followed 10 min later by 400 mg/kg of ME. We then continuously measured ambulatory activity of the mice for 60 min.

Experiment 5. Effects of RES or AMPT on the subsequent abilities of BUP and ME to promote ambulation in ICR mice: We examined the effect of RES on the abilities of BUP and ME to promote ambulation in mice. Saline or 1, 2, 4, 8 or 16 mg/kg of RES was administered to mice. One day later, we examined the abilities of BUP or ME to promote ambulation in the mice. After adaptation for 30 min, 10 mg/kg of BUP or 400 mg/kg of ME were administered and then ambulatory activity was continuously measured for 60 min.

We also examined the effect of AMPT, on the subsequent abilities of BUP and ME to promote ambulation. Saline or 25, 50 or 100 mg/kg of AMPT was administered to the mice. One day later, we examined the abilities of BUP and ME to promote ambulation. After adaptation for 30 min, 10 mg/kg of BUP or 400 mg/kg of ME were administered, and then ambulatory activity was continuously measured for 60 min.

Statistical analyses

We initially examined the time course of ambulatory activity after the administration of BUP or ME using repeated-measures analysis of variance (ANOVA). Differences in total ambulatory activity over 1 h were then examined using ANOVA, followed by Fisher's PLSD

test. When ME was combined with BUP, the data were analyzed using two-way ANOVA. $P<0.05$ was established as the level of significance.

Results

Experiment 1. Effect of subcutaneous administration of ME on ambulation in ICR mice

Figure 1a shows that the subcutaneous administration of ME apparently promoted ambulation in ICR mice. Repeated-measures ANOVA revealed that time ($F(5,475)=35.151$, $P<0.01$), dose ($F(4,95)=6.852$, $P<0.01$), and their interaction ($F(20,475)=2.964$, $P<0.01$) were statistically significant. Total ambulatory activity during 60 min after ME administration was also examined (Fig. 1b). Menthol apparently increased total ambulatory activity in a dose-dependent manner ($F(4,95)=7.104$, $P<0.01$) [Fisher's PLSD test: differences, saline – 100 mg/kg = −3.7 (critical value (c.v.) =167.20); saline – 200 mg/kg = −141.05 (c.v.=167.20); saline – 400 mg/kg = −277.6 (c.v.=221.38); saline – 800 mg/kg = −351.55 (c.v.=221.38)].

Experiment 2. Effect of BUP on ambulatory activity in mice

The subcutaneous administration of BUP also apparently promoted ambulation in ICR mice (Fig. 2: a and b). Repeated-measures ANOVA indicated that time ($F(5,330)=20.255$, $P<0.01$) and dose ($F(3,66)=13.141$, $P<0.01$) were statistically significant, whereas interaction between the two ($F(15,330)=1.27$, $P>0.05$) was not. Total ambulatory activity during 60 min after BUP injection (Fig. 2b) also increased in a dose-dependent manner ($F(3,66)=13.141$, $P<0.01$) [saline – 2.5 mg/kg = −33.75 (c.v.=196.95); saline – 5 mg/kg = −69.45 (c.v. =196.95); saline – 10 mg/kg = −358.05 (c.v.=241.81)].

Experiment 3. Effects of ME combined with BUP on ambulatory activity in mice

To examine the relationship between DA and the effect of ME, we examined the effects of a combination of ME and BUP on ambulation. Figure 3 shows ambulatory activity measured over 60 min when ME was administered after BUP.

Figure 4 shows the mean total ambulatory activity caused by ME with BUP. The data analyzed using two-way ANOVA revealed that the effects of ME and BUP

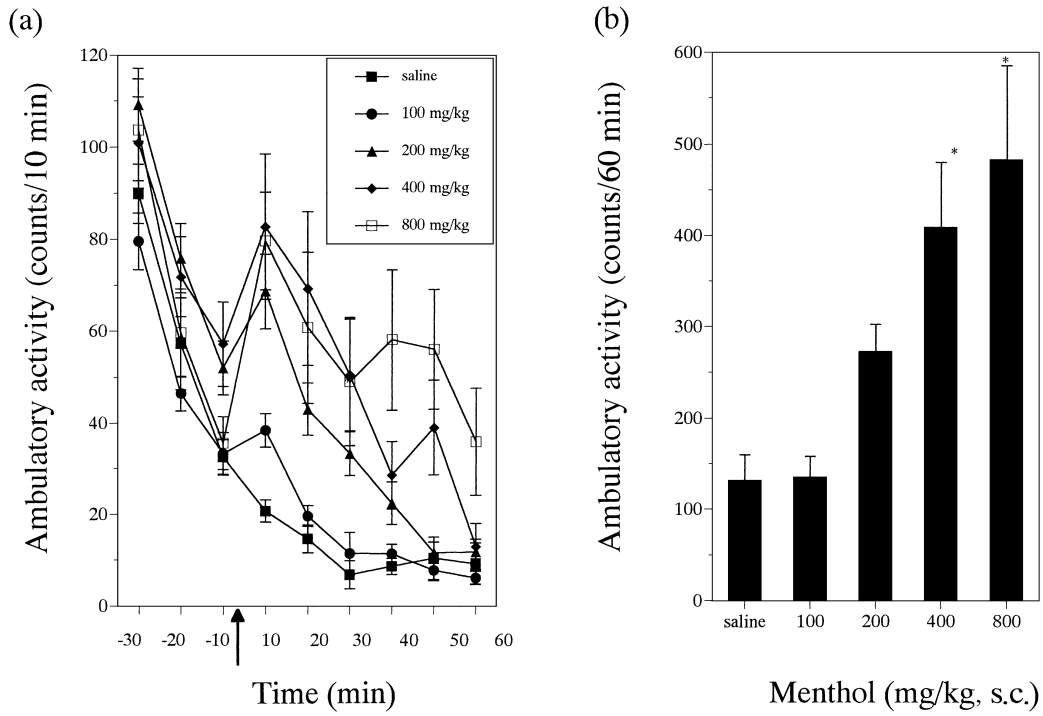

Fig. 1. Ambulatory activity in ICR mice after subcutaneous administration of menthol (ME). a: Changes in ambulation after subcutaneous administration of ME. Symbols represent mean values of ambulation for 10-min periods, and vertical lines indicate standard error of the mean (S.E.M.). Twenty animals received each dose (N = 20). Arrow indicates time of ME administration. b: Total ambulation over 60 min after administration of saline or various doses of ME. Filled columns indicate mean values of total ambulation for 60 min, and vertical lines indicate S.E.M. Data were analyzed by ANOVA, followed by Fisher's PLSD test (*$P<0.05$, **$P<0.01$ compared with control (vehicle administration) values).

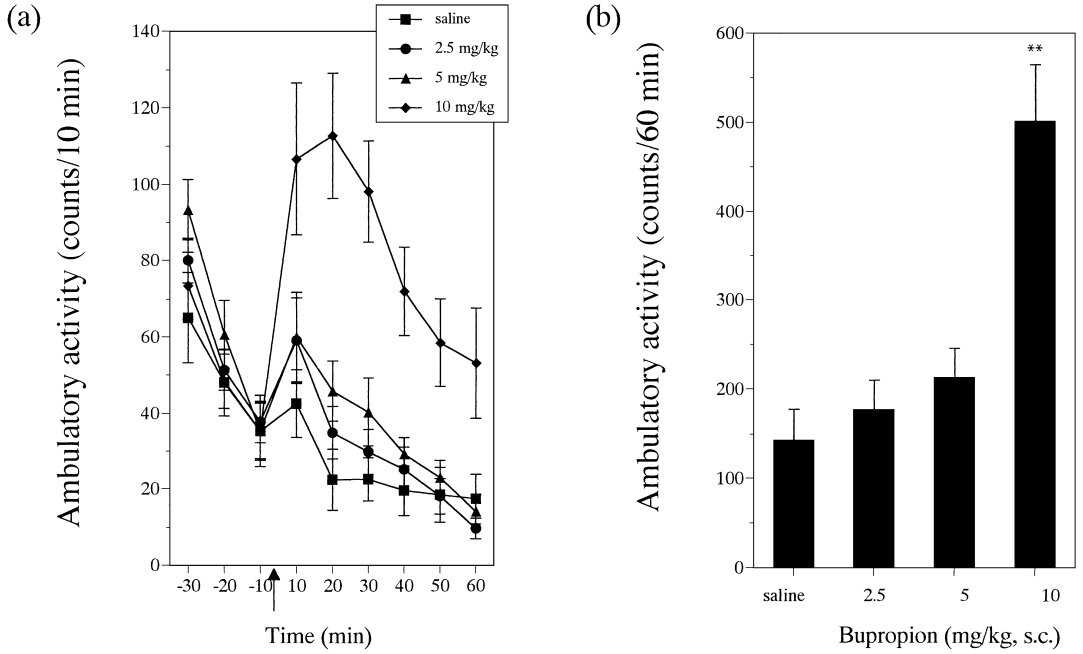

Fig. 2. Ambulatory activity in ICR mice after subcutaneous administration of bupropion (BUP), a dopamine uptake inhibitor. a: Changes in ambulation after subcutaneous administration of BUP (N = 10 – 20). b: Total ambulation for 60 min after administration of saline or various doses of BUP. See other details in the legend of Fig. 1.

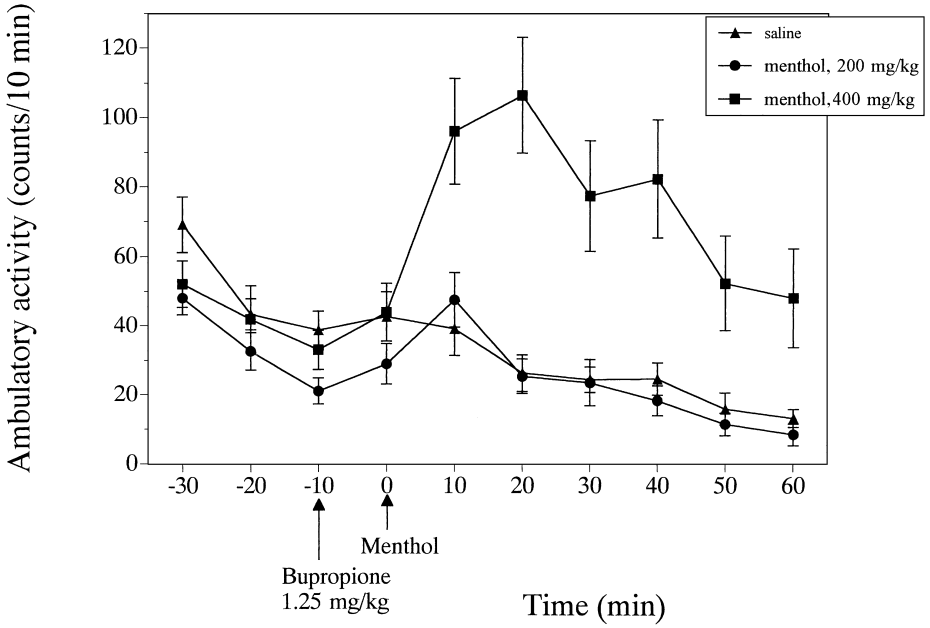

Fig. 3. Changes in ambulatory activity after administering BUP plus ME in ICR mice. After 30 min of adaptation, 1.25 mg/kg of BUP was administered, followed 10 min later by 200 or 400 mg/kg of ME. Ambulatory activity was measured for 60 min thereafter (N = 19 – 20). See other details in the legend of Fig. 1.

were statistically significant (ME, $F(3,299)=36.50$, $P<0.01$; BUP, $F(3,299)=21.494$, $P<0.01$), indicating that both factors promoted significant ambulation under our conditions. On the other hand, interaction between ME and BUP was not significant ($F(9,299)=1.771$, $P>0.05$). When the data of 400 mg/kg of ME were excluded and the remaining data were re-calculated by two-way ANOVA, the results were different. The effects

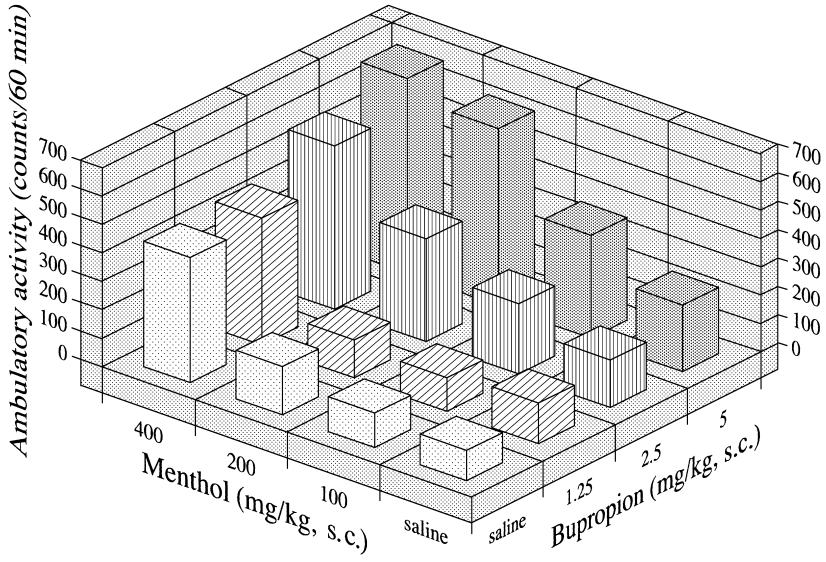

Fig. 4. Effect of various doses of BUP plus ME on ICR mouse ambulation. Columns indicate mean values of total ambulatory activity for 60 min after administration of BUP combined with ME to ICR mice (see Fig. 3) (N = 17 – 20).

of ME ($F(2,224)=16.886$, $P<0.01$) and BUP ($F(3,224)=32.447$, $P<0.01$) were significant and their interaction was also significant ($F(6,224)=4.908$, $P<0.01$), showing that BUP potentiated the effect of ME on mouse ambulation.

Experiment 4. Effects of BUP or ME combined with DA antagonists on ambulatory activity in mice

To examine the role of DA in the ambulation-promoting effect of ME, we examined the effects of ME combined with DA antagonists. We also examined the effects of BUP combined with DA antagonists as positive controls.

Effect of CPZ: Ambulation promoted by 10 mg/kg of BUP was attenuated by the combination of 0.25 – 1 mg/kg of CPZ (Fig. 5: a and b). The total ambulatory activity during 60 min examined by ANOVA revealed that CPZ significantly suppressed the ability of BUP to promote ambulation ($F(3,76)=21.762$, $P<0.01$) [saline – 0.25 mg/kg = 246.3 (c.v.=110.24); saline – 0.5 mg/kg = 226.25 (c.v.=110.24); saline – 1 mg/kg = 318.4 (c.v.=110.24)] and of 400 mg/kg of ME ($F(2,56)=26.563$, $P<0.01$) [saline – 0.5 mg/kg = 186.2 (c.v.=86.53); saline – 1 mg/kg = 221.61 (c.v.=87.66)] (Fig. 5: c and d).

Effect of HAL: Ambulation promoted by 10 mg/kg of BUP was significantly attenuated by 0.031 – 0.125 mg/kg of HAL ($F(4,95)=6.166$, $P<0.01$) [saline – 0.031 mg/kg = 93.85 (c.v.=91.48); saline – 0.062 mg/kg = 93.65 (c.v.=91.48); saline – 0.125 mg/kg = 212.35 (c.v.=121.12)] (Fig. 6: a and b). The effect of 400 mg/kg of ME was similarly attenuated by HAL ($F(3,71)=9.332$, $P<0.01$) [saline – 0.031 mg/kg = 68.08 (c.v.=56.05); saline – 0.062 mg/kg = 93.0 (c.v.=68.89); saline – 0.125 mg/kg = 134.15 (c.v.=68.89)] (Fig. 6: c and d).

Effect of FLU: Ambulation promoted by 10 mg/kg of BUP was apparently suppressed by 0.063 – 0.25 mg/kg of FLU ($F(3,76)=6.873$, $P<0.01$) [saline – 0.063 mg/kg = 63 (c.v.=101.93); saline – 0.125 mg/kg = 44.3 (c.v.=101.93); saline – 0.25 mg/kg = 218 (c.v.=135.21)] (Fig. 7: a and b). Ambulation promoted by 400 mg/kg of ME was also suppressed by FLU ($F(3,76)=7.631$, $P<0.01$) [saline – 0.063 mg/kg = 222.7 (c.v.=145.25); saline – 0.125 mg/kg = 204.35 (c.v.=145.25); saline – 0.25 mg/kg = 215.65 (c.v.=145.25)] (Fig. 7: c and d).

Effect of SPI: Ambulation promoted by 10 mg/kg of BUP was attenuated by 0.032 – 0.125 mg/kg of SPI ($F(3,75)=9.438$, $P<0.01$) [saline – 0.032 mg/kg = 114.15 (c.v.=119.21); saline – 0.063 mg/kg = 263.15 (c.v.=119.21); saline – 0.125 mg/kg = 274.07 (c.v.=120.76)] (Fig. 8: a and b). Ambulation promoted by 400 mg/kg of ME was also attenuated by SPI ($F(3,36)=7.14$, $P<0.01$) [saline – 0.032 mg/kg = 192.80 (c.v.=143.21); saline – 0.063 mg/kg = 307.90 (c.v.=143.21); saline – 0.125 mg/kg = 248.50 (c.v.=143.21)] (Fig. 8: c and d).

Effect of SCH: Ambulation promoted by 10 mg/kg of BUP was apparently attenuated by 2.5 – 10 mg/kg of SCH ($F(3,36)=13.858$, $P<0.01$) [saline – 2.5 mg/kg = 119.4 (c.v.=77.52); saline – 5 mg/kg = 166.0 (c.v.=77.52); saline – 10 mg/kg = 222.2 (c.v.=77.52)] (Fig. 9: a and b). Ambulation promoted by 400 mg/kg of ME was also attenuated by SCH. Although ANOVA did not show statistical significance ($F(3,76)=2.242$,

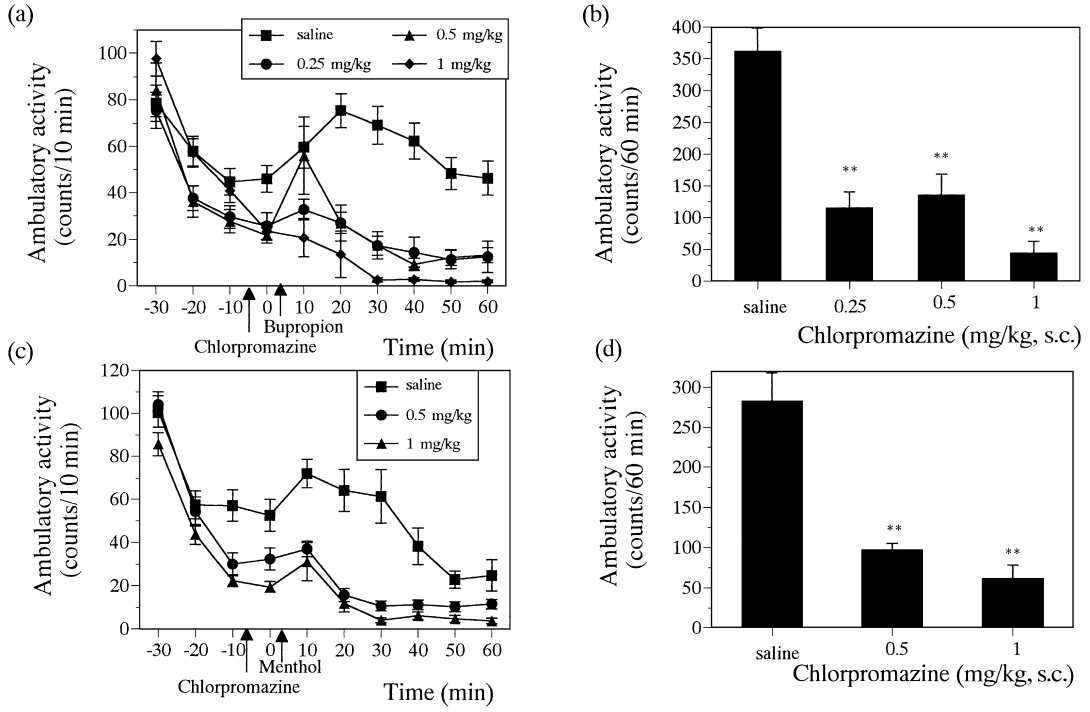

Fig. 5. Ambulatory activity after administration of chlorpromazine (CPZ) and 10 mg/kg of BUP or 400 mg/kg of ME. a: Changes in ambulation after administering various doses of CPZ plus BUP (N = 20). b: Total ambulation for 60 min after administering various doses of CPZ and BUP. c: Changes in ambulation after administering various doses of CPZ and ME (N = 19 – 20). d: Total ambulation for 60 min after administering various doses of CPZ and ME. See other details in the legend of Fig. 1.

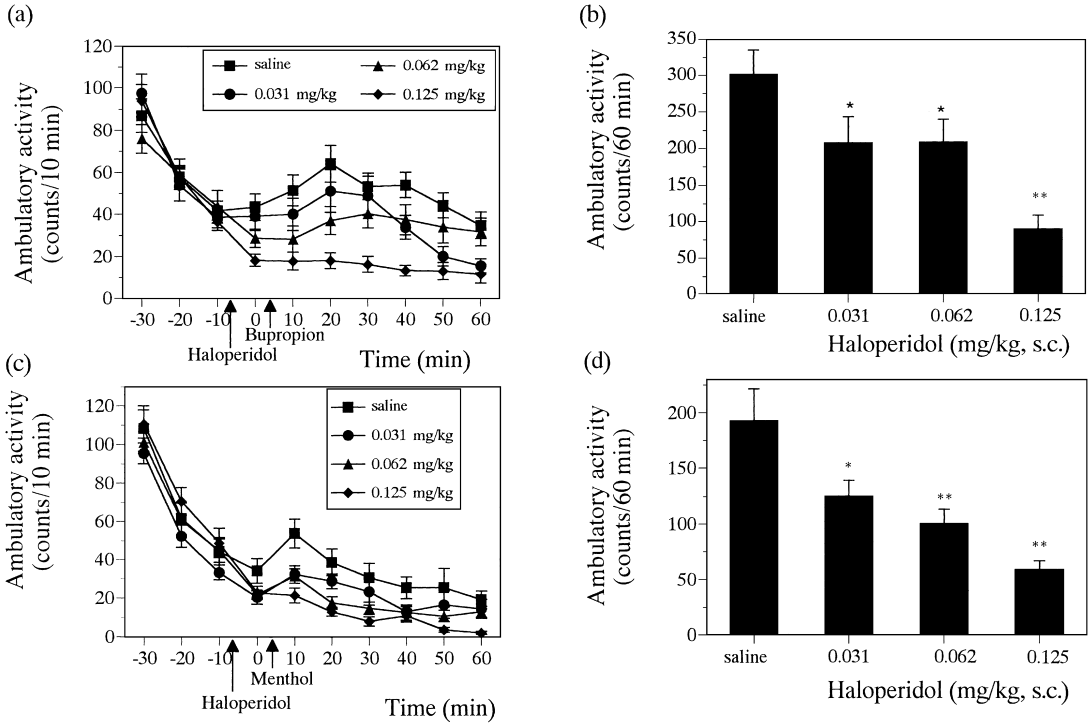

Fig. 6. Ambulatory activity after administering various doses of haloperidol (HAL) and 10 mg/kg of BUP or 400 mg/kg of ME to ICR mice. a: Changes in ambulation after various doses of HAL and 10 mg/kg of BUP (N = 20). b: Total ambulation for 60 min after various doses of HAL and 10 mg/kg of BUP. c: Changes in ambulation after various doses of HAL and 400 mg /kg of ME (N = 15 – 20). d: Total ambulation for 60 min after various doses of HAL and 400 mg/kg of ME. See other details in the legend of Fig. 1.

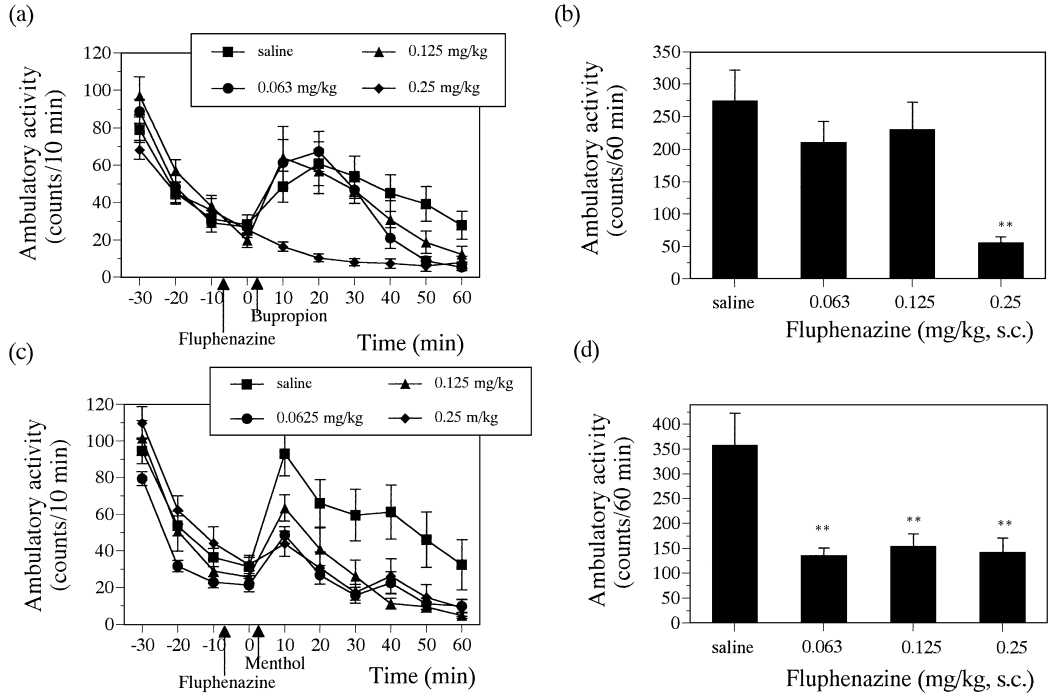

Fig. 7. Ambulatory activity after administering various doses of fluphenazine (FLU) with 10 mg/kg of BUP or 400 mg/kg of ME to ICR mice. a: Changes in ambulation after various doses of FLU and BUP (N = 20). b: Total ambulation for 60 min after various doses of FLU and BUP. c: Changes in ambulation after various doses of FLU and ME (N = 20). d: Total ambulation for 60 min after various doses of FLU and ME. See other details in the legend of Fig. 1.

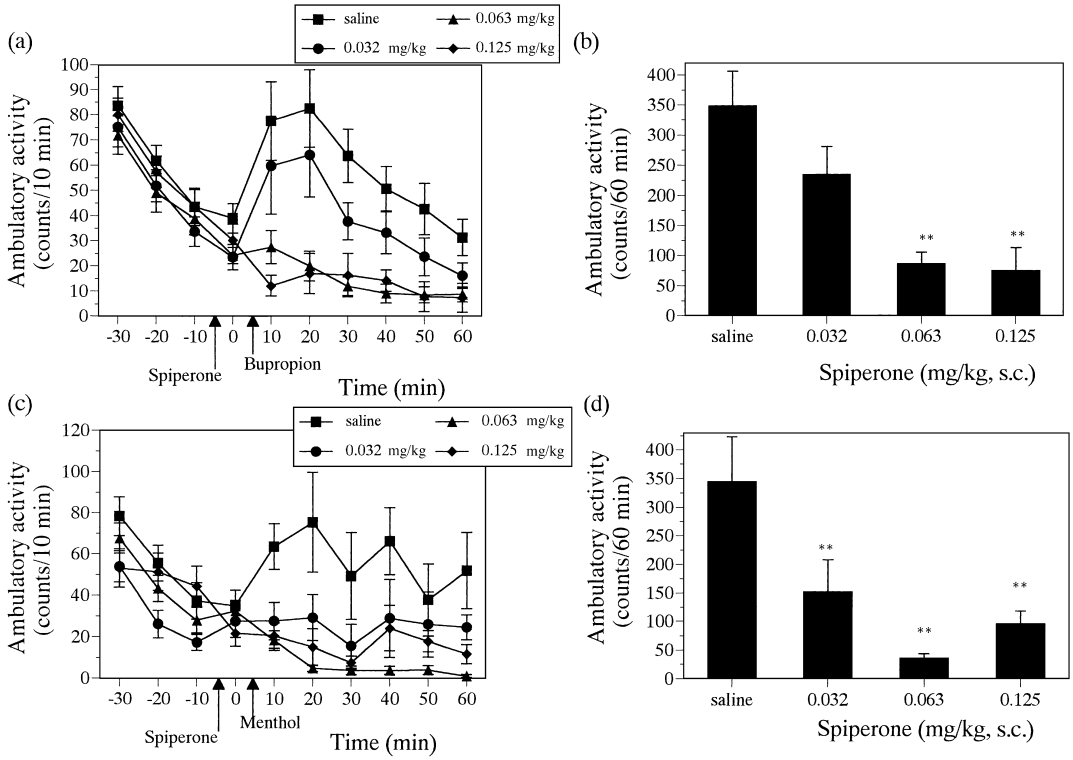

Fig. 8. Ambulatory activity after administering various doses of spiperone (SPI) with 10 mg/kg of BUP or 400 mg/kg of ME to ICR mice. a: Changes in ambulation after various doses of SPI and BUP (N = 19 – 20). b: Total ambulation for 60 min after various doses of SPI and BUP. c: Changes in ambulation after various doses of SPI and ME. (N = 10). d: Total ambulation for 60 min after various doses of SPI and ME. See other details in the legend of Fig. 1.

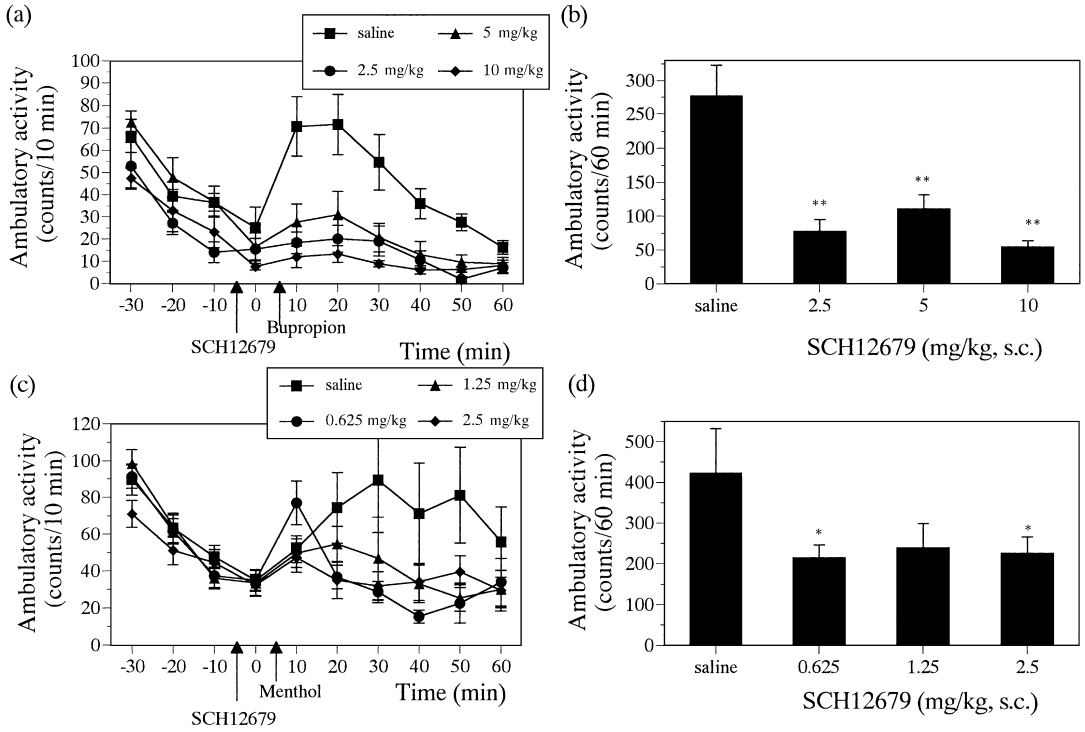

Fig. 9. Ambulatory activity after administering various doses of SCH12679 (SCH) with 10 mg/kg of BUP or 400 mg/kg of ME to ICR mice. a: Changes in ambulation after various doses of SCH and BUP (N = 10). b: Total ambulation for 60 min after various doses of SCH and BUP. c: Changes in ambulation after various doses of SCH and ME (N = 20). d: Total ambulation for 60 min after various doses of SCH and ME. See other details in the legend of Fig. 1.

P=0.092), Fisher's PLSD test demonstrated otherwise [saline – 0.625 mg/kg = 208.75 (c.v.=186.51); saline – 1.25 mg/kg = 185.15 (c.v.=186.51); saline – 2.5 mg /kg = 198.15 (c.v.=186.51)] (Fig. 9: c and d).

Experiment 5. Effects of prior RES or AMPT on the abilities of BUP and ME to promote ambulation

Figure 10, a and b, shows that prior exposure to RES decreased the sensitivity to BUP ($F(5,114)=11.376$, $P<0.01$) [saline – 1 mg/kg = –11.3 (c.v.=113.36); saline – 2 mg/kg = –42.2 (c.v.=113.36); saline – 4 mg /kg = 129.45 (c.v.=113.36); saline – 8 mg/kg = 195.90 (c.v.=113.36); saline – 16 mg/kg = 301.45 (c.v.= 113.36). Similarly, prior RES decreased sensitivity to the effect of ME ($F(2,57)=3.783$, $P<0.05$) [saline – 8 mg/kg = 124.0 (c.v.=156.35); saline – 16 mg/kg = 164.1 (c.v.=156.35)] (Fig. 10: c and d).

Prior AMPT produced decreased sensitivity to the effect of BUP ($F(3,73)=9.435$, $P<0.01$) [saline – 25 mg /kg = –60.9 (c.v. = 96.25); saline – 50 mg/kg = 104.25 (c.v.= 96.25); saline – 100 mg/kg = 184.06 (c.v.= 100.40)] (Fig. 11: a and b). Prior exposure to AMPT also decreased sensitivity to the effect of ME ($F(2,53) =4.818$, $P<0.05$) [saline – 25 mg/kg = 120.70 (c.v.= 135.53); saline – 50 mg/kg = 168.36 (c.v.=143.75)] (Fig. 11: c and d).

Discussion

Our previous study demonstrated that ME administered via i.p. injection promotes ambulation in ICR mice (5). The present study showed that this effect was also induced via a subcutaneous injection, showing that the effect is independent of the administration route. However, an i.p. injection of ME was effective at doses as low as 100 mg/kg, whereas the s.c. route required at least 400 mg/kg to exert a significant effect. In addition, the effect of ME disappeared quickly after i.p. injection, but remained for up to 60 min after s.c. injection. The absorption rate is generally higher for compounds injected i.p, than s.c., given that the density of blood vessels is higher in the abdomen than that under the skin. Thus, the pharmacokinetics associated with the injection routes could explain the different effects on behavior. This speculation is also supported by our previous findings in which an i.v. injection of ME immediately promoted ambulation at much lower doses (10 – 20 mg/kg) (5). Therefore, peripherally administered ME probably exerts behavioral effects after absorption into the bloodstream, and then by passing through the blood-

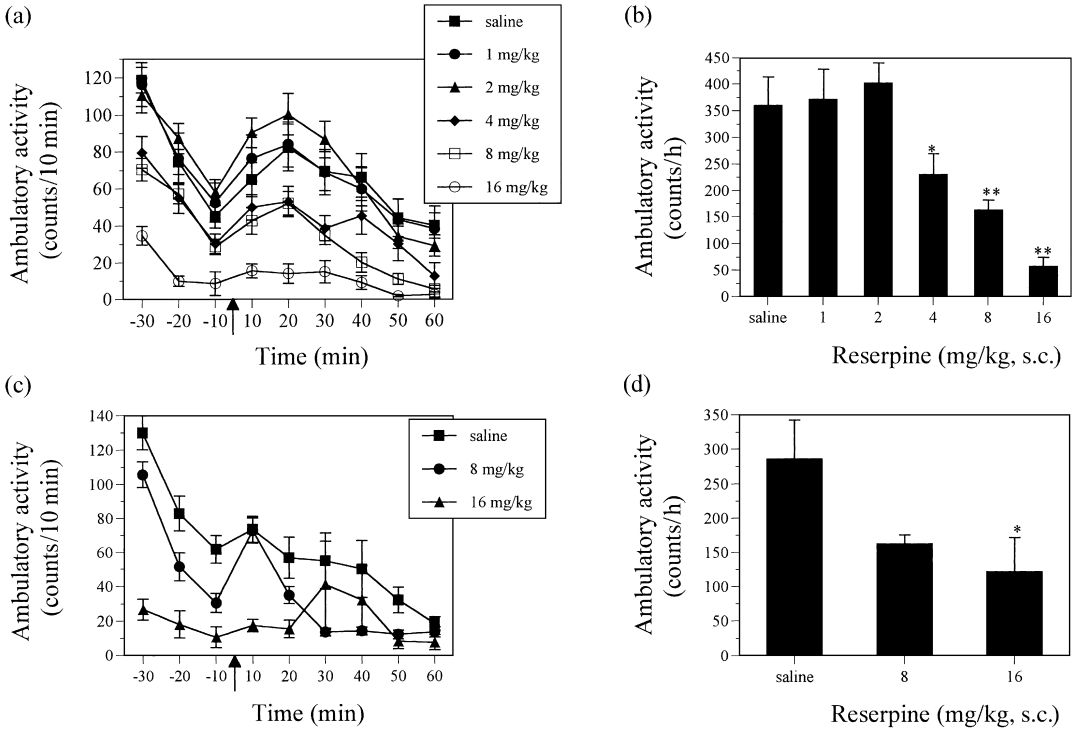

Fig. 10. Effect of prior exposure to various doses of reserpine (RES) on effect of 10 mg/kg of BUP or 400 mg/kg of ME in ICR mice. RES was administered one day before administration of BUP or ME. a: Changes in ambulation in mice administered with RES one day before BUP (N = 20). b: Total ambulation for 60 min after BUP. c: Changes in ambulation after ME in mice to which RES was administered one day before (N = 20). d: Total ambulation for 60 min after ME. See other details in the legend of Fig. 1.

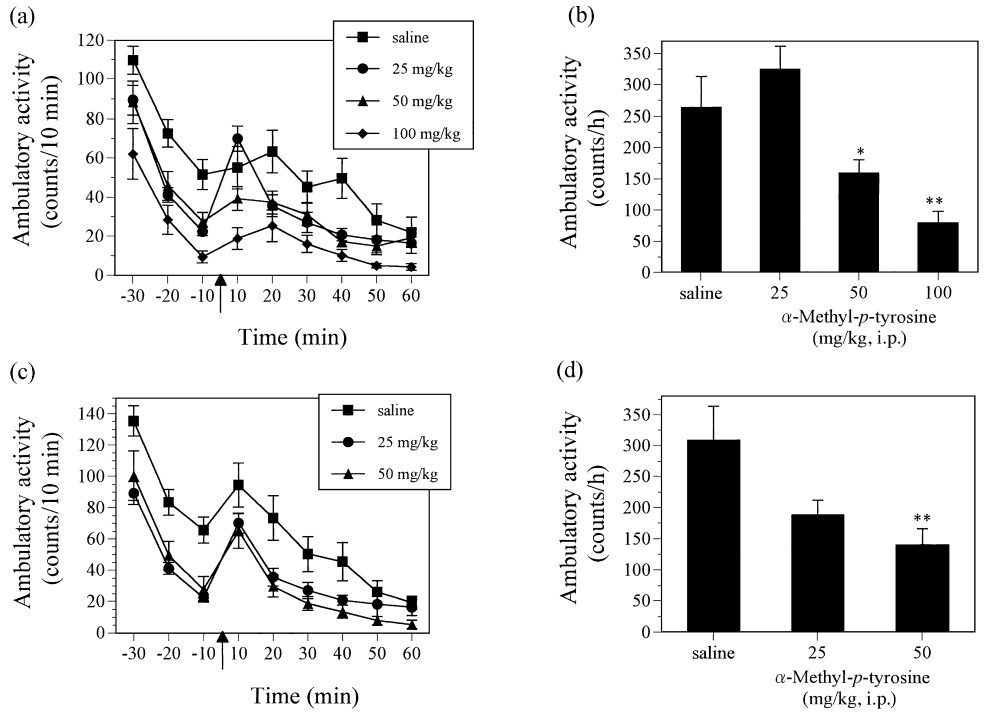

Fig. 11. Effect of prior exposure to various doses of α-methyl-*p*-tyrosine (AMPT) on the effect of 10 mg/kg of BUP or 400 mg /kg of ME in ICR mice. AMPT was administered one day before administration of BUP or ME. a: Changes in ambulation in mice administered with AMPT one day before BUP (N = 17 – 20). b: Total ambulation for 60 min after BUP. c: Changes in ambulation after ME in mice to which AMPT was administered one day before (N = 16 – 20). d: Total ambulation for 60 min after ME. See other details in the legend of Fig. 1.

brain barrier, where it acts upon neurons in the brain in the same manner as psychoactive drugs. However, the previous study did not provide evidence of the neuronal mechanism of how ME promotes ambulation.

The neurotransmitter DA plays an important role in the control of ambulation in ddY mice (7 – 18). Thus we examined the effect of BUP on ambulation of ICR mice. Since BUP inhibits synaptic DA uptake (19, 20), it may act as an indirect DA agonist on mouse ambulation. In fact, BUP promoted mouse ambulation, reconfirming that DA is also involved in this process in ICR mice. Therefore, we speculated that ME also acts on the mouse DA system since it also promotes ambulation. To test this hypothesis, we examined the effects of interactions between ME and drugs related to DA on ambulation.

We initially investigated the effects of a combination of ME and BUP on ambulation in ICR mice. The results showed that BUP potentiated the effect of 100 – 200 mg/kg of ME. This finding suggests that DA is involved in the ability of ME to promote ambulation since BUP inhibits DA uptake and produces the effect. High doses of ME produced ataxia (data not shown) but not stereotypy, suggesting that ME is not a direct DA agonist, since the direct DA agonist apomorphine produces stereotypy in rodents.

We then examined the effects of the DA antagonists CPZ, HAL, FLU, SPI and SCH on ambulation promoted by BUP and ME. Ambulation promoted by BUP was attenuated by these antagonists, indicating that DA is involved in the effects of BUP on mouse ambulation and that these compounds functioned as DA antagonists at the doses used in this experiment. The same doses of these antagonists also attenuated the effect of ME, suggesting that DA is involved in the mechanism underlying the effect of ME on mouse ambulation. On the other hand, these antagonists can act on other neurotransmitter receptors. Therefore, the effects of specific DA receptor antagonists on the ambulation-promoting effect of ME should be examined to confirm the role of DA in the effect of ME on mouse ambulation. Several DA receptor subtypes ($D_1 – D_4$) have been identified and subtype-specific DA receptor antagonists have been discovered. Thus, the DA receptor subtypes that might be involved in the mechanism underlie the ambulation-promoting effect of ME would be clarified if the effects of specific DA antagonists are examined.

RES depletes monoamines and its administration causes depletion of endogenous DA (28, 29). Thus, RES would abolish the subsequent ability of BUP and ME to promote ambulation if DA plays an important role in the effect of ME. We tested this notion and found that RES apparently decreased subsequent sensitivity to BUP and ME. This finding provided further evidence supporting the hypothesis that DA is involved in the ability of ME to promote mouse ambulation. In addition, we also examined the hypothesis using AMPT, which inhibits tyrosine hydroxylase and thus decreases levels of endogenous DA (29, 30). As with RES, the administration of AMPT subsequently decreased sensitivity to the effects of BUP and ME. This finding is in line with the results described above.

In summary, the present study demonstrated that the DA uptake inhibitor BUP potentiates the ability of ME to promote ambulation and that the effects of ME as well as of BUP were attenuated by various DA antagonists. In addition, prior exposure to the monoamine depletor RES and the tyrosine hydroxylase inhibitor AMPT decreased sensitivity to the effects of both ME and of BUP. These evidences suggest that DA is involved in the ability of ME to promote ambulation. However, the present study did not directly examine the DA system of the mouse brain. Thus the present study represents a first step towards understanding the mechanism underlying the effect of ME on mouse ambulation.

The target of ME remain unclear. TRPM8, a member of the TRP family, has recently been identified as a menthol receptor (31, 32). However, TRPM8 is located in the dorsal and trigeminal ganglia, and not in the mouse brain. Although TRPM8 can explain the mechanism of action of cold stimulus and ME, TRPM8 is probably not involved in the mechanism underlying the ability of ME to promote ambulation in mice. On the other hand, ME probably does not directly act on the DA receptor as a DA agonist, since a high dose of ME produced ataxia but not stereotyped behavior (data not shown), and prior RES and AMPT abolished the effect of ME. Menthol might inhibit DA uptake in the same manner as BUP, which was the positive control in the present study, but a large dose of BUP did not cause ataxia in mice (data not shown). Although the precise mechanism for the ME effect on mouse ambulation remains unclear, DA might mediate between an unknown target(s) for ME and promotion of mouse ambulation. Menthol may become a useful tool with which to investigate brain function.

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