Pharmacology, Biochemistry and Behavior 91 (2009) 315–320

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Evidence for dopamine involvement in ambulation promoted by menthone in mice

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ARTICLE INFO

Article history:
Received 3 October 2007
Received in revised form 17 July 2008
Accepted 25 July 2008
Available online 31 July 2008

Keywords:
Menthone
Peppermint
Dopamine
Ambulation
Mouse

ABSTRACT

The present study examines the mechanism that underlies the ability of menthone (MTN), a constituent of peppermint oil, to promote mouse ambulation. Since bupropion (BUP), a dopamine (DA) uptake inhibitor, promotes mouse ambulation, the effect of MTN combined with BUP on ambulation was investigated. The results showed that BUP with MTN produced an additive interaction on mouse ambulation. The effects of DA antagonists chlorpromazine, fluphenazine, haloperidol, SCH12679 and spiperone on the ability of MTN to promote ambulation were then examined. All of these antagonists attenuated the effects of MTN. Prior exposure to the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine, which inhibits catecholamines synthesis, decreased subsequent sensitivity to the effect of MTN. These results suggest that DA is involved in the ability of MTN to promote ambulation in mice.

1. Introduction

Mental disorders have traditionally been treated with various essential oils (EOs) derived from plants. 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 (Balchin, 1997; Buckle, 1999; Perry and Perry, 2006), 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. My series of studies (Umezu, 1999, 2000; Umezu et al., 2001, 2002, 2006) revealed that some EOs affect mouse behavior.

Peppermint oil is believed to be useful in treating nervous disorders and mental fatigue (Tisserand, 1993). One study (Umezu et al., 2001) has demonstrated that peppermint oil promotes ambulation in ICR mice, indicating that the effect is similar to that of psychostimulants. In addition, 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 (Umezu et al., 2001). These constituents promote ambulation at much lower doses when administered intravenously than intraperitoneally. 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 underlying mechanism of their effects remains unclear.

Direct and indirect dopamine (DA) agonists administered to mice promote ambulation (Kuribara and Tadokoro, 1984; Hirate and Kuribara, 1991; Asami et al., 1986; Kuribara and Uchihashi, 1993a), which is abrogated by DA antagonists (Kuribara and Uchihashi, 1993b, 1994; Kuribara, 1994a,b, 1995a, 1996). Dopamine might also be involved in the ability of non-dopaminergic drugs such as MK-801 and morphine to promote ambulation in mice (Kuribara et al., 1992; Kuribara, 1995b). These findings indicate that DA plays an important role in the control of mouse ambulation promoted by psychoactive drugs. Whether or not DA is involved in the ability of peppermint oil constituents to promote ambulation in mice remains unknown.

Our previous study (Umezu and Morita, 2003) revealed that the DA uptake inhibitor bupropion (BUP) potentiates the effect of menthol (ME) on mouse ambulation when they are simultaneously administered. In addition, various DA antagonists decreased the ability of ME to promote ambulation in mice as well as that of BUP. Reserpine, which depletes DA, or α-methyl-*p*-tyrosine, a tyrosine hydroxylase inhibitor, reduces the subsequent ability of both ME to promote mouse ambulation and BUP. These results suggest that DA is involved in the ability of ME to promote ambulation and in the effect of BUP. The remaining question is whether DA is involved in the abilities of other peppermint constituents to promote ambulation.

The present study examined MTN, another major constituent of peppermint oil. MTN is known to have an action as a skin penetration enhancer (Zhao et al., 2001; Pillai and Panchagnula, 2003; Narishetty and Panchagnula, 2004) as well as menthol (Krishnaiah and Bhaskar, 2004; Ammar et al., 2006; Abu Hena Mostofa Kamal et al., 2006; Liu et al., 2006). To my knowledge, its potency, absorption and distribution are not clarified yet. It is reported that MTN is metabolized

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doi:10.1016/j.pbb.2008.07.017

to neomenthol and 7-hydroxy menthone and then excreted as glucuronides (Parke, 1968; Miyazawa and Nakanishi, 2006). Pharmacological action(s) of MTN is not known at all except the ambulation-promoting effect at the present time. Thus the present study uses pharmacological methods to examine the role of DA in the ability of MTN to promote ambulation in mice.

2. Materials and methods

2.1. Animals

Male ICR mice (Clea Japan, Tokyo) aged 7–10 weeks and weighing between 35 and 42 g were housed in aluminum cages (3 mice/cage) with a stainless-steel mesh top and paper bedding. Commercial solid food (Clea Japan) and tap water were provided *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 room temperature of 25±1 °C. Each animal was used only once for all experiments in this study.

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

2.2. Drugs

The effects of menthone (MTN; 5-Methyl-2-(1-methylethyl) cyclohexanone), the DA uptake inhibitor bupropion (BUP) (Gazzara and Andersen, 1997; Munzar and Goldberg, 2000), and the DA antagonists, chlorpromazine (CPZ), fluphenazine (FLU), haloperidol (HAL), SCH12679 (SCH) and spiperone (SPI) (Sigma-Aldrich, Tokyo) were examined. The effects of prior exposure to α-methyl-*p*-tyrosine (AMPT), an inhibitor of tyrosine hydroxylase (Wako Pure Chem., Osaka) (Wacan et al., 2006) on the ability of MTN to promote ambulation were also investigated. Menthone was diluted in olive oil and AMPT was mixed with a small amount of Tween 80 (Nacalai Tesque, Kyoto) and then diluted in saline (0.9% NaCl). Haloperidol was dissolved in 0.1% acetic acid. Other drugs were dissolved in saline. Both MTN and AMPT were administered intraperitoneally, and the other drugs were applied subcutaneously. The administered volume was 1 ml/100 g body weight regardless of dosage.

2.3. Measurement of ambulatory activity in ICR mice

Ambulatory activity, which is a type of spontaneous motor activity in mice, was measured using a tilt-type ambulometer consisting of 10 bucket-like Plexiglas activity cages (20 cm in diameter) (SAM-10; O'Hara and Co., Tokyo) (Umezu et al., 1998, 2001; Umezu and Morita, 2003). Details of this apparatus have been reported elsewhere (Hirabayashi et al., 1978).

2.4. Experimental procedure

2.4.1. Experiment 1. Effect of intraperitoneal administration of MTN on ambulation in ICR mice

Mice were placed individually in activity cages, and after 30 min of adaptation, olive oil, or 100, 200, 400 or 800 mg/kg of MTN was administered intraperitoneally. Thereafter, ambulatory activity was continuously measured for 60 min.

2.4.2. Experiment 2. Effects of MTN 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, olive oil or 50, 100 or 200 mg/kg of MTN was administered to the same mice, and then ambulatory activity was continuously measured for 60 min.

2.4.3. Experiment 3. Effect of MTN combined with DA antagonists on ambulatory activity in mice

The mice were adapted for 30 min, and then 0.25–1 mg/kg of CPZ, 0.063–0.25 mg/kg of FLU, 0.032–0.125 mg/kg of HAL, 2.5–10 mg/kg of SCH, or 0.032–0.125 mg/kg of SPI was administered. Ten minutes later, 400 mg/kg of MTN was administered to the same mice, and then ambulatory activity was measured for 60 min. As a control experiment, effects of combined administration of the same doses of CPZ, FLU, HAL, SCH or SPI with olive oil were also examined in a same manner.

2.4.4. Experiment 4. Effects of AMPT on the subsequent ability of MTN to promote ambulation in ICR mice

Saline, 50 or 100 mg/kg of AMPT was administered to mice. One day later, the ability of MTN to promote ambulation in the mice was examined. After adaptation for 30 min, olive oil or 400 mg/kg of MTN was administered and then ambulatory activity was continuously measured for 60 min.

2.5. Statistical analyses

The time course of ambulatory activity after the administration of MTN was initially examined using the repeated-measures analysis of variance (ANOVA). Differences in total ambulatory activity over 1 h were then analyzed using ANOVA, followed by Fisher's PLSD test. When MTN was combined with BUP, the data were analyzed using two-way ANOVA. Effects of DA antagonists and pre-administration of AMPT were analyzed using ANOVA. $P<0.05$ was established as the level of significance.

3. Results

3.1. Experiment 1. Effect of intraperitoneal administration of MTN on ambulation in ICR mice

Fig. 1a shows that the intraperitoneal administration of MTN apparently promoted ambulation in ICR mice. Repeated-measures ANOVA revealed that dose ($F(4, 95)=7.38$, $P<0.05$) and time course ($F(5, 475)=9.56$, $P<0.05$) were statistically significant, whereas their

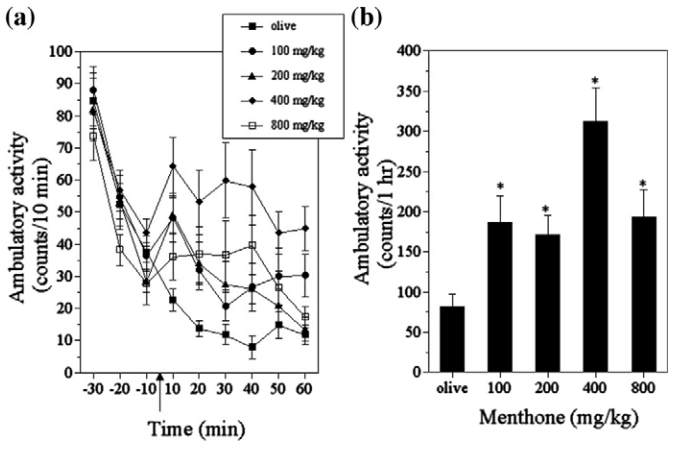

Fig. 1. Ambulatory activity in ICR mice after intraperitoneal administration of menthone (MTN). (a) Changes in ambulation after intraperitoneal administration of MTN. Symbols represent mean values of ambulation for 10-min periods, and vertical lines indicate standard error of the mean (S.E.M.). The number of animals for each dose group was twenty ($N=20$). Arrow indicates time of MTN administration. (b) Total ambulation over 60 min after administration of olive oil or various doses of MTN. 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 (*$P<0.05$ compared with control values obtained after olive oil administration).

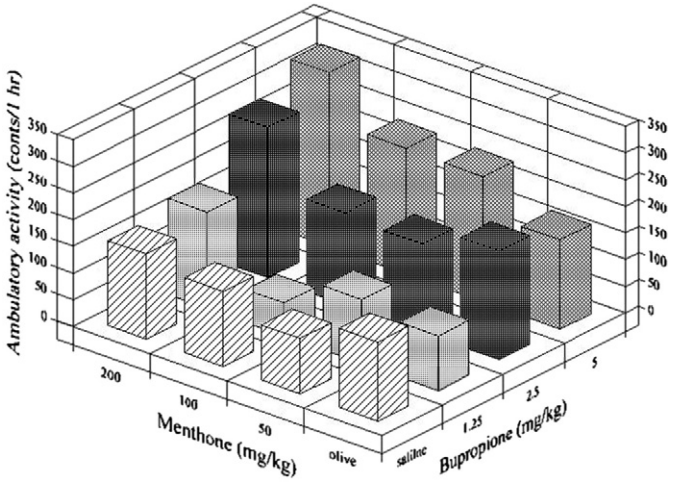

Fig. 2. Effect of various doses of BUP plus MTN on ICR mouse ambulation. Columns indicate mean values of total ambulatory activity for 60 min after administration of BUP combined with MTN to ICR mice (N=19–20).

interaction (F(20, 475)=1.53, P>0.05) was not. Total ambulatory activity during 60 min after MTN administration was also examined (Fig. 1b). Menthone apparently increased total ambulatory activity (F(4, 95)=6.96, P<0.05) [Fisher's PLSD test: differences, olive−100 mg/kg=−103.9 (critical value (c.v.)=87.445), P=0.0204; olive−200 mg/kg=−88.4 (c.v.=87.445), P=0.0476; olive−400 mg/kg=−230.3 (c.v.=87.445), P<0.0001; olive−800 mg/kg=−110.25 (c.v.=87.445), P=0.014].

3.2. Experiment 2. Effects of MTN combined with BUP on ambulatory activity in mice

To examine the relationship between DA and the effect of MTN, the effects of a combination of MTN and BUP on ambulation were investigated. Fig. 2 shows the mean total ambulatory activity caused by MTN with BUP. The data analyzed using two-way ANOVA revealed that the effects of MTN and BUP were statistically significant (MTN, F(3, 301)=7.154, P<0.05; BUP, F(3, 301)=14.408, P<0.05), indicating that both factors promoted significant ambulation under the tested experimental conditions. On the other hand, interaction between MTN and BUP was not significant (F(9, 301)=1.195, P>0.05), showing that their interaction was additive.

3.3. Experiment 3. Effect of MTN combined with DA antagonists on ambulatory activity in mice

To examine the role of DA in the ambulation-promoting effect of MTN, the effects of MTN combined with various DA antagonists were examined. Fig. 3 shows the results.

Ambulation promoted by 400 mg/kg of MTN was attenuated by the combination of 0.25–1 mg/kg of CPZ (Fig. 3a). Total ambulatory activity during 60 min examined by ANOVA revealed that CPZ significantly suppressed the ability of MTN to promote ambulation (F(3, 76)=2.876,

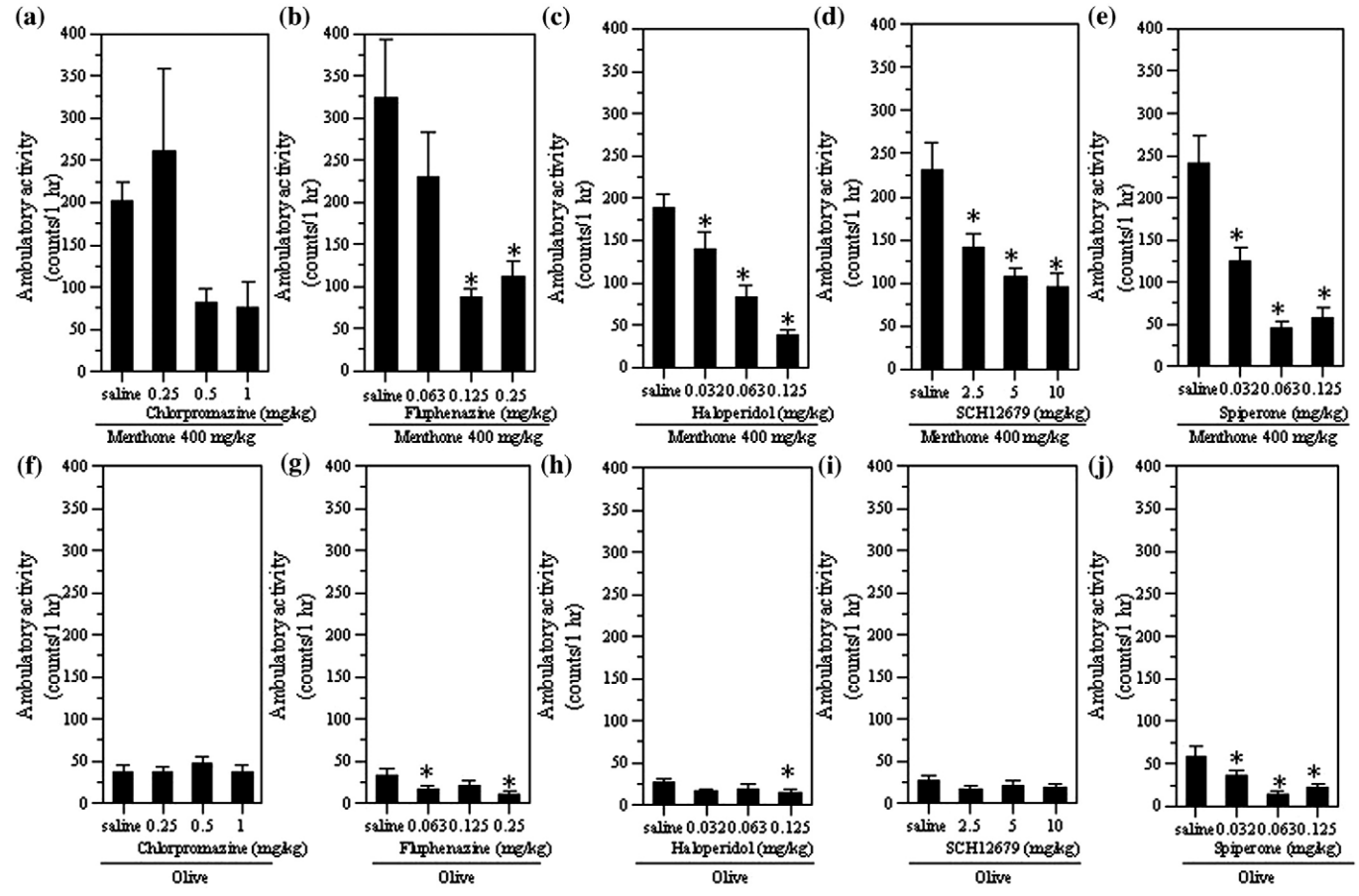

Fig. 3. Total ambulatory activity for 60 min after administration of 400 mg/kg of MTN with (a) 0.25–1 mg/kg of chlorpromazine (CPZ) (N=20), (b) 0.063–0.25 mg/kg of fluphenazine (FUL) (N=20), (c) 0.032–0.125 mg/kg of haloperidol (HAL) (N=20), (d) 2.5–10 mg/kg of SCH12679 (SCH) (N=19–20) or (e) 0.032–0.125 mg/kg of spiperone (SPI) (N=19–20), and total ambulatory activity for 60 min after administration of olive oil with (f) 0.25–1 mg/kg of CPZ (N=18), (g) 0.063–0.25 mg/kg of FUL (N=16–18), (h) 0.032–0.125 mg/kg of HAL (N=18), (i) 2.5–10 mg/kg of SCH (N=7–16) or (j) 0.032–0.125 mg/kg of SPI (N=18) in ICR mice. See details of legend in Fig. 1b.

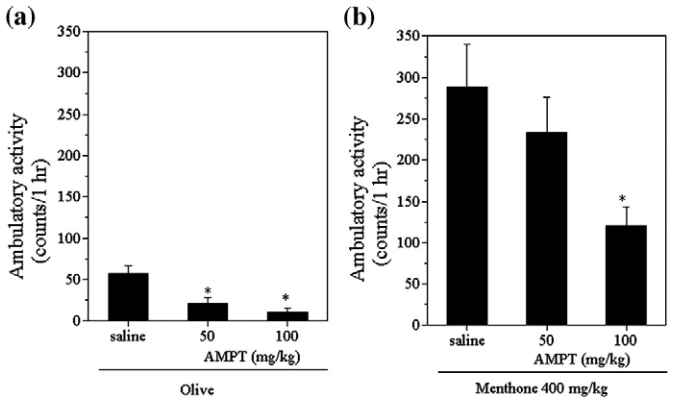

Fig. 4. Effect of prior exposure to various doses of α-methyl-*p*-tyrosine (AMPT) on effect of 400 mg/kg of MTN in ICR mice ($N=20$). AMPT was administered one day before MTN. See details of legend in Fig. 1b.

$P<0.05$), although Fisher's PLSD test failed to indicate statistical significance between the saline control and each dosed group [saline−0.25 mg/kg=−58.45 (c.v.=151.128), $P=0.4435$; saline−0.5 mg/kg=120.5 (c.v.=151.128), $P=0.1164$; saline−1 mg/kg=125.15 (c.v.=151.128), $P=0.1032$]. Ambulation promoted by 400 mg/kg of MTN was also significantly attenuated by 0.063–0.25 mg/kg FUL (Fig. 3b) ($F(3, 76)=6.07$, $P<0.05$) [saline−0.063 mg/kg=93.15 (c.v.=126.121), $P=0.1454$; saline−0.125 mg/kg=237.55 (c.v.=126.121), $P=0.0003$; saline−0.25 mg/kg=212.6 (c.v.=126.121), $P=0.0012$], 0.032–0.125 mg/kg of HAL (Fig. 3c) ($F(3, 76)=20.144$, $P<0.05$) [saline−0.032 mg/kg=48.65 (c.v.=41.567), $P=0.0224$; saline−0.063 mg/kg=106.65 (c.v.=41.567), $P<0.0001$; saline−0.125 mg/kg=151.5 (c.v.=41.567), $P<0.0001$], 2.5–10 mg/kg of SCH (Fig. 3d) ($F(3, 75)=9.004$, $P<0.05$) [saline−2.5 mg/kg=88.955 (c.v.=58.318), $P=0.0033$; saline−5 mg/kg=123.05 (c.v.=57.565), $P<0.0001$; saline−10 mg/kg=136.05 (c.v.=57.565), $P<0.0001$], 0.032–0.125 mg/kg of SPI (Fig. 3e) ($F(3, 75)=20.355$, $P<0.05$) [saline−0.032 mg/kg=114.729 (c.v.=56.376), $P=0.0001$; saline−0.063 mg/kg=194.35 (c.v.=55.648), $P<0.0001$; saline−0.125 mg/kg=182.5 (c.v.=55.648), $P<0.0001$]. On the other hand, CPZ (Fig. 3f) ($F(3, 68)=0.394$, $P>0.05$) [saline−0.25 mg/kg=1.444 (c.v.=21.454), $P=0.8935$; saline−0.5 mg/kg=−9.278 (c.v.=21.454), $P=0.3912$; saline−1 mg/kg=−3.5 (c.v.=21.454), $P=0.7458$], HAL (Fig. 3h) ($F(3, 68)=1.591$, $P>0.05$) [saline−0.032 mg/kg=10.333 (c.v.=11.780), $P=0.0845$; saline−0.063 mg/kg=6.944 (c.v.=11.78), $P=0.2435$; saline−0.125 mg/kg=11.833 (c.v.=11.780), $P=0.049$], SCH (Fig. 3i) ($F(3, 37)=1.741$, $P>0.05$) [saline−2.5 mg/kg=16.021 (c.v.=17.446), $P=0.707$; saline−5 mg/kg=13.688 (c.v.=18.974), $P=0.1523$; saline−10 mg/kg=15.132 (c.v.=17.446), $P=0.0871$], did not produce significant effect on ambulation when combined with olive oil. FLU (Fig. 3g) ($F(3, 64)=4.271$, $P<0.05$) [saline−0.063 mg/kg=16.243 (c.v.=12.771), $P=0.0135$; saline−0.125 mg/kg=11.812 (c.v.=13.141), $P=0.0773$; saline−0.25 mg/kg=22.188 (c.v.=12.771), $P=0.0009$] and SPI (Fig. 3j) ($F(3, 68)=7.402$, $P<0.05$) [saline−0.032 mg/kg=21.222 (c.v.=19.905), $P=0.037$; saline−0.063 mg/kg=43.5 (c.v.=19.905), $P<0.0001$; saline−0.125 mg/kg=36.111 (c.v.=19.905), $P=0.0006$] significantly decreased ambulatory activity when combined with olive oil, however, the differences between saline-treated control values and FLU or SPI administered values were much smaller than those when combined with MTN. Thus, all examined DA antagonists attenuated the ability of MTN to promote ambulation.

3.4. Experiment 4. Effects of AMPT on the subsequent ability of MTN to promote ambulation in ICR mice

To examine the role of DA in the ambulation-promoting effect of MTN furthermore, the effect of DA depletor α-methyl-*p*-tyrosine (AMPT) on the MTN effect was examined. Pretreatment with AMPT significantly attenuated the ambulation-promoting effect of MTN (Fig. 4). ($F(2, 57)=4.377$, $P<0.05$). Differences between saline-treated value and AMPT-treated values were much smaller in animals received combination with olive oil [saline−50 mg/kg=36.118 (c.v.=23.346), $P=0.0032$; saline−100 mg/kg=46.431 (c.v.=23.346), $P=0.0002$] than in animals received combination with MTN [saline−50 mg/kg=55.2 (c.v.=115.945), $P=0.6371$; saline−100 mg/kg=168.05 (c.v.=115.945), $P=0.0197$], although pretreatment with AMPT also significantly attenuated ambulation when olive oil was administered ($F(2.47=8.975$, $P=0.005$).

4. Discussion

My previous study demonstrated that MTN injected i.p. promotes ambulation in ICR mice (Umezu et al., 2001) and the present study confirmed this finding. The previous study also demonstrated that a much lower dose of MTN promotes ambulation when injected i.v. Therefore, peripherally administered MTN probably exerts behavioral effects after absorption into the bloodstream, and then by passing through the blood brain barrier, where it acts upon neurons in the brain in the same manner as psychoactive drugs. However, because the neuronal mechanism of the effect of MTN remained unclear, the present study used pharmacological methods to investigate this process.

Many psychoactive drugs promote mouse ambulation (Kuribara and Tadokoro, 1983, 1984; Asami et al., 1986; Iijima et al., 1986; Hirate and Kuribara, 1991; Kuribara and Uchihashi, 1993a; Kuribara et al., 1990, 1991; Kuribara 1994c,d, 1995c), and numerous studies (Kuribara and Uchihashi, 1993b, 1994; Kuribara, 1994a,b, 1995a,d, 1996) suggest that the neurotransmitter DA commonly plays an important role in such promotion in ddY mice. Therefore, the present study focused on the involvement of DA in the ability of MTN to promote ambulation. In order to test this notion, pharmacological interactions between MTN and various DA relating drugs were investigated.

BUP inhibits DA uptake (Gazzara and Andersen, 1997; Munzar and Goldberg, 2000), therefore, BUP produces actions as DA indirect agonist. BUP promotes ambulation in mice (Umezu and Morita, 2003) as well as other DA indirect agonists such as methamphetamine and cocaine (Kuribara and Uchihashi, 1993a,b, 1994; Kuribara, 1994a,b,c,d, 1995a,d, 1996). We previously confirmed the ambulation-promoting effect of BUP was attenuated by combined administrations with various DA antagonists (Umezu and Morita, 2003), showing that the effect of BUP on mouse ambulation could be attributed to its effect as DA indirect agonist. The present result showed that BUP and MTN significantly promote and interact additively to further promote mouse ambulation. These findings suggest that DA is involved in the ability of MTN to promote ambulation, therefore, MTN additively interacts with BUP.

The effects of the DA antagonists CPZ, FLU, HAL, SCH and SPI on ambulation promoted by MTN were examined using doses that reduce the ability of BUP to promote ambulation (Umezu and Morita, 2003). Therefore, the doses were sufficient to antagonize the effect of BUP as a DA indirect agonist on mouse ambulation. All DA antagonists consistently decreased the ambulation-promoting effect of MTN. Since the same doses of CPZ, SCH and HAL did not reduce ambulation significantly when combined with olive oil, the effects of these DA antagonists on MTN effect are not attributable to reduction of behavioral baseline. Although FLU and SPI significantly decreased ambulatory activity when combined with olive oil, however, the differences between saline-treated control values and FLU or SPI administered values were much smaller in animals received olive oil administration than in animals received MTN administration. Therefore, changes of behavioral baseline by FLU and SPI cannot sufficiently account for the decrease of ambulation promoted by MTN. Thus, all DA antagonists used in the present study reduced the ability of MTN to promote mouse ambulation. These results of DA antagonists provide further evidences supporting the hypothesis that DA is involved in the ambulation-promoting effect of MTN in ICR mice.

MTN might have act directly on DA receptors as an agonist. This notion was examined using AMPT, which inhibits catecholamine synthesis and results in DA depletion and reduction of DA efflux (Fulford and Marsden, 2007; Watanabe et al., 2005). It has been known that pretreatment with AMPT reduces effects of DA indirect agonists such as methamphetamine (Thomas et al., 2008) but not effects of DA receptors direct agonists (Wacan et al., 2006). Therefore, either the effect of MTN on ambulation would have been decreased by the prior administration of AMPT if DA mediates the MTN effect or MTN would have promoted mouse ambulation if MTN directly acts on DA receptors. Prior administration of the described doses of AMPT reduces the ambulation-promoting effect of BUP (Umezu and Morita, 2003). Although pretreatment with AMPT caused decrease of baseline of ambulation, differences between saline-treated value and AMPT-treated values were much smaller in animals received olive oil administration than in animals received MTN administration, therefore, changes of baseline of ambulation caused by pretreatment with AMPT do not sufficiently account for reduction of ambulation-promoting ability of MTN, showing that AMPT decreased subsequent sensitivity to MTN. This result suggests that MTN does not directly act on DA receptors as an agonist, but rather promotes ambulation through changing the extracellular DA level like BUP.

The results for MTN obtained in this study are the same as those for BUP that were observed in the previous study (Umezu and Morita, 2003), suggesting that mechanism underlying the ambulation-promoting effect of MTN could overlap with that of BUP, that is, DA might be involved in the ambulation-promoting effect of MTN. BUP increase extracellular DA level through inhibiting DA uptake. Similarly, other DA indirect agonists such as methamphetamine and cocaine also increase extracellular DA level through inhibition of DA uptake and/or facilitating DA release, and promote mouse ambulation. These indirect DA agonist cause stereotyped behaviors in animals at high doses. However, high doses of MTN produce ataxia but not stereotyped behaviors in mice, thus, the effect of MTN is distinct from those DA indirect agonists on this point. Therefore, although MTN produces the effect on mouse ambulation through DA mechanism, the detailed effect of MTN on DA system could be different from those DA indirect agonists.

MTN might inhibit DA uptake and/or facilitate DA release as well as DA indirect agonists described above, and at the same time, MTN might act on another neurotransmitter system that produces ataxia. However, it is unclear whether MTN acts on another neurotransmitter system or not at the present time. Another possibility is that DA system might be downstream of MTN target(s), that is, MTN indirectly increase extracellular DA level after acting MTN target(s). It is known that MK-801 produces ambulation-promoting effect and ataxia in mice (Iijima et al., 1986; Kuribara et al., 1992). MK-801 directly acts on NMDA receptors as an antagonist but not on DA receptors, however, DA system is downstream of glutamate system, therefore, NMDA receptor antagonism results in activation of DA system, which produces the ambulation-promoting effect. However, an effect of MTN on NMDA receptors has not been known at all. In addition, the present study did not directly examine the DA system of the mouse brain. Thus, although the present results suggest the involvement of DA in the ambulation-promoting effect of MTN, the present study represents a fist step towards understanding the mechanism underlying the effect of MTN on mouse ambulation.

It is notable that results observed in this study are identical with those of ME (Umezu and Morita, 2003), of which chemical structure is similar to that of MTN. The mechanism underlying their ambulation-promoting effects might be identical, and could synergistically interact through the same mechanism to promote mouse ambulation, which would explain how peppermint oil promotes mouse ambulation. The contents of MTN and ME in the peppermint oil used in the previous study were 20.01% and 36.98%, respectively (Umezu et al., 2001), indicating that these are major constituents of peppermint oil. Thus, the effect of peppermint oil is largely attributed to the effects of MTN and ME. Peppermint oil has traditionally been used to treat mental fatigue (Tisserand, 1993). This efficacy of peppermint oil is probably true since it promotes ambulation and DA might be involved in the effects of MTN and ME like psychostimulants, such as amphetamine and methamphetamine that produce mental excitation and thus reduce mental fatigue. Therefore, peppermint oil could help to change mood by reducing mental fatigue. However, natural oils are generally expensive since the cultivation of plants is laborious, time-consuming and only a small amount of essential oils can be extracted. Because MTN and ME can be synthesized, they can be produced in large scale at reasonable cost. Therefore, MTN and ME could present an alternative to peppermint oil.

Acknowledgement

This study was supported by the Smoking Research Foundation, Tokyo.

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