Pharmacology, Biochemistry and Behavior 94 (2010) 497–502

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Evidence for dopamine involvement in ambulation promoted by pulegone in mice

Toyoshi Umezu *

Environmental Chemistry Division, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

ARTICLE INFO

Article history:
Received 16 September 2008
Received in revised form 29 January 2009
Accepted 6 November 2009
Available online 14 November 2009

Keywords:
Pulegone
Peppermint
Dopamine
Ambulation
Mouse

ABSTRACT

I investigated whether dopamine (DA) is involved in the ambulation promoted by pulegone (PUL), a constituent of peppermint oil, in ICR mouse. Co-administration of PUL and bupropion (BUP) had an additive effect on their ambulation-promoting activities. When administered with PUL, the DA antagonists chlorpromazine, fluphenazine, haloperidol, SCH12679, and spiperone all attenuated the effect of PUL on ambulation. In addition, pretreatment with the DA depletor reserpine produced no subsequent sensitivity to the effect of PUL. Taken together, DA may be involved in the ability of PUL to promote ambulation in ICR mice but PUL may not be a direct DA agonist. The chemical structure of PUL is similar to menthol and menthone, and thus they may all be acting through a common mechanism.

1. Introduction

Interest in aromatherapy, which originated in ancient Egypt, has been increasing world-wide, and it is being revalued as an alternative medicine. Aromatherapy includes various treatment methods such as body and/or foot bathing, massage, and finger-pressure therapy; however, the most remarkable feature is the use of various kinds of plant-derived essential oils (EOs), hence the term aromatherapy. It has traditionally been believed that aromatherapy is able to treat various mental disorders (Tisserand, 1993) and that EOs are essential for producing such therapeutic effects. However, the absence of a scientific basis for their effectiveness has been noted (Balchin, 1997; Buckle, 1999). One possible explanation for the apparent efficacy of EOs on mental disorders is that EOs may possess psychoactive effects. Accumulating evidence (Perry and Perry, 2006), including that from our laboratory (Umezu, 1999, 2000, 2009; Umezu et al., 2001, 2002, 2006), is indicating that some EOs indeed produce pharmacological effects on animal behaviors similar to those of psychoactive drugs.

Peppermint oil has been believed to be useful in treating mental fatigue (Tisserand, 1993), which is not unreasonable as the oil promotes ambulation in ICR mice in a manner similar to psychostimulants (Umezu et al., 2001). This effect of peppermint oil is attributable to its constituent elements such as menthol (ME), menthone (MTN), isomenthone, 1,8-cineol, pulegone, menthyl acetate and caryophellene, as these constituents also promote mouse ambulation (Umezu et al., 2001). Peppermint oil and its constituents may have psychostimulant-like actions; however, whether their ambulation-promoting effects are a result of these pharmacological action(s) on CNS function is unclear, to say nothing of the mechanism(s) underlying the effects.

Dopamine (DA), a neurotransmitter, is thought to play an important role in controlling mouse ambulation. This notion is supported by the following findings: (1) direct and indirect DA agonists promote mouse ambulation (Kuribara and Tadokoro, 1984; Hirate and Kuribara, 1991; Asami et al., 1986; Kuribara and Uchihashi, 1993a); (2) such effects of DA agonists can be attenuated by combined administration with various DA antagonists (Kuribara and Uchihashi, 1993b, 1994; Kuribara, 1994a,b, 1995a, 1996); and (3) DA antagonists can also diminish the ambulation-promoting effects of non-DA agonists such as MK-801 and morphine (Kuribara et al., 1992; Kuribara, 1995b). Therefore, DA might also be involved in the mouse ambulation promoted by peppermint oil and its constituents.

Pharmacological methods that employ various agonists and antagonists that specifically act against some neurotransmitter systems are very useful for examining neurochemical mechanism(s) that might underlie behavioral effect(s) of the chemical in question in the absence of other successful methods. We previously used this technique to examine whether DA is involved in the ambulation-promoting effect of ME and MTN, major constituents of peppermint oil, in ICR mice (Umezu and Morita, 2003; Umezu, 2009). In those studies, we used a DA indirect agonist, bupropion (BUP), and various DA receptor antagonists. In addition, DA depletors such as reserpine and alpha-methyl-*p*-tyrosine were also used to determine whether peppermint constituents act directly on DA receptors. These examinations revealed that ME and MTN possess similar pharmacological properties as the DA indirect agonist BUP: (1) BUP, ME and MTN promote ambulation in ICR mice; (2) BUP and ME or MTN produces synergistic interactions on their ambulation-promoting effects when

* Tel.: +81 29 850 2874; fax: +81 29 850 2880.
E-mail address: umechan2@nies.go.jp.

0091-3057/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2009.11.001

BUP and ME or MTN are simultaneously administered; (3) various DA antagonists reduce the abilities of BUP, ME and MTN to promote ambulation; and (4) DA depletors diminish the abilities of BUP, ME and MTN to promote ambulation (Umezu and Morita, 2003; Umezu, 2009). Thus, DA may be involved in the abilities of ME and MTN to promote ambulation in ICR mice but they may not be DA receptors direct agonists as well as BUP, a DA indirect agonist. Since ME and MTN are the major constituents of peppermint oil, DA may also be involved in the ambulation-promoting effect of the oil in ICR mice. However, the roles of other constituent elements in the ambulation-promoting effect of peppermint oil remain unclear. One important issue is whether DA is commonly involved in the ambulation-promoting effects of unexamined constituents of peppermint oil such as pulegone (PUL).

PUL is another major constituent of peppermint oil, constituting 3.2% of the peppermint oil used in our previous study (Umezu et al., 2001). PUL also promotes mouse ambulation. PUL has been known to be a skin penetration enhancer (Pillai and Panchagnula, 2003); antibacterial (Bekhechi et al., 2007; Sutour et al., 2008; Salehi et al., 2005); antifungal (Goncalves et al., 2007); insecticidal (Coats et al., 1991); antinociceptive (de Sausa et al., 2007); and to have antihistamine activity on guinea-pigs (Ortiz de Urbina et al., 1990). However, these previous findings do not provide any insight into the mechanism underlying its ambulation-promoting effect. The data on ME and MTN (Umezu and Morita, 2003; Umezu, 2009) suggest that DA is also involved in the ability of PUL to promote mouse ambulation. Thus, the current study examines this notion using the same pharmacological methods as those used for ME and MTN.

2. Materials and methods

The present study applied the same pharmacological protocol as those used for ME and MTN (Umezu and Morita, 2003; Umezu, 2009).

2.1. Animals

Male ICR mice (Clea Japan, Tokyo, Japan; 7–10 weeks old and body weight 35–42 g) were used. Commercial solid food (Clea Japan) and tap water were available *ad libitum*. The animals were kept under a 12 light:12 dark schedule (light period: 07:00–19:00) and at a constant room 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.

2.2. Drugs

The present study used PUL (5-Methyl-2-(1-methylethylidene) cyclohexanone), the DA indirect agonist BUP, the DA antagonists chlorpromazine (CPZ), fluphenazine (FLU), haloperidol (HAL), SCH12679 (SCH), and spiperone (SPI) and the DA depletor reserpine. These chemicals were purchased from Sigma-Aldrich (Tokyo). Olive oil, Tween 80 and NaCl were purchased from Nacalai Tesque (Kyoto), and acetic acid was from Wako Pure Chemicals (Osaka). These chemicals were used in preparing injection vehicles for DA-related agents.

2.3. Measurement of ambulatory activity in ICR mice

The present study measured the ambulatory activity of ICR mice using a tilt-type ambulometer (SAM-10; O'Hara and Co., Tokyo, Japan) to assess how DA relating agents modulate the ambulation-promoting effect of PUL. Details of the apparatus have already been reported (Hirabayashi et al., 1978; Umezu et al., 1998, 2001; Umezu and Morita, 2003; Umezu, 2009).

2.4. Experimental protocol

The ambulatory activity of individual ICR mouse was continuously measured in the following experiments, and the activity during the 60 min after the final administration of the agents was also recorded. Prior to each experiment, mice underwent a 30-min adaptation period in the activity cage. Olive oil and PUL were administered by intraperitoneal injection, and DA-related agents and their vehicles were administered subcutaneously.

2.4.1. Effect of intraperitoneal administration of PUL on ambulation

After the 30-min adaptation period, olive oil or 100, 200, 400 or 800 mg/kg of PUL was administered to the mice followed by a 60-min measurement of ambulatory activity.

2.4.2. Interaction between PUL and BUP on ambulatory activity

After the 30-min adaptation period, saline or 1.25, 2.5 or 5 mg/kg of BUP was administered, followed 10 min later by administration of olive oil or 100 or 200 mg/kg of MTN.

2.4.3. Effects of DA antagonists on the ambulation-promoting effect of PUL

After the 30-min adaptation period, 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, followed 10 min later by administration of 200 mg/kg of PUL. As control experiments, the effects of the combined administration of the same doses of CPZ, FLU, HAL, SCH or SPI with olive oil were also examined under the same protocol.

2.4.4. Effects of pretreatment with RES on the ambulation-promoting effect of PUL

Saline or 8 or 16 mg/kg of RES was administered and 1 day later, after the 30-min adaptation period 400 mg/kg of PUL was administered.

2.5. Statistical analysis

The time course of ambulatory activity after administration of PUL was initially examined using 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 PUL was combined with BUP, data were analyzed using two-way ANOVA. The effects of the DA antagonists and pre-administration of RES were analyzed using ANOVA. $P<0.05$ was established as the level of significance.

3. Results

3.1. Effect of intraperitoneal administration of PUL on ambulation

Intraperitoneal administration of PUL significantly promoted ambulation in the ICR mice. Fig. 1a shows the time course of the ambulatory activity after administration of olive oil or various doses of PUL. The effects of dose, time course and their interaction were statistically significant (repeated-measures ANOVA; dose ($F(4, 252) = 2.727$, $P<0.05$); time course ($F(5, 1260) = 90.452$, $P<0.05$; their interaction ($F(20, 1260) = 6.754$, $P<0.05$). Fig. 1b shows the total ambulatory activity during the 60 min after PUL administration. PUL increased total ambulatory activity in a dose-dependent but bell-shaped manner ($F(4, 253) = 2.728$, $P<0.05$; Fisher's PLSD test: differences from control, 100 mg/kg = −55.56, $P>0.05$; 200 mg/kg = −125.85, $P=0.043$; 400 mg/kg = −181.9, $P<0.0001$; 800 mg/kg = −114.15, $P>0.05$).