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Splenectomy modifies hyperactive states of the dopaminergic system induced by morphine in C57BL/6J-bg^{J}/bg^{J} (beige-J) mice

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ABSTRACT

Genetic factors affect the locomotor activity induced by morphine, which mainly depends on the activation of dopaminergic systems, and morphine has distinct pharmacological activities in C57BL/6J-bg^{J}bg^{J} (beige-J) mice, which have genetic deficiencies in immunological function. We previously showed that beige-J mice exhibited greater locomotor activity and dopamine turnover, whereas splenectomy reduced this hyperlocomotion and dopamine turnover, which suggests that beige-J mice could be an experimental animal model for investigating hyperactivation of the dopaminergic system, and that the spleen may contribute to the susceptibility to activation of the dopaminergic system. Furthermore, morphine can induce hyperlocomotion mediated by activation of the dopaminergic system. Therefore, we examined the effects of splenectomy on the hyperlocomotion and regulation of the dopaminergic system induced by morphine in beige-J mice. Morphine induced hyperlocomotion, which was accompanied by activation of the dopaminergic system, in beige-J mice. Furthermore, splenectomy enhanced the hyperlocomotion and activation of the mesolimbic dopaminergic system induced by morphine in beige-J mice. Our findings indicate that substances originating from the spleen may regulate both spontaneous activation of the mesolimbic dopaminergic system and the μ-opioidergic system-mediated activation of the mesolimbic dopaminergic system by morphine through different modes of action. These results imply that beige-J mice could be a practical animal model for investigating the interactions between immune-modulation and the μ-opioidergic system and/or dopaminergic system.

1. Introduction

The C57BL/6J-bg^{J}bg^{J} (beige-J) strain of mouse originated as a spontaneous mutation from C57BL/6J lines and is of particular interest as a homolog of Chediak–Higashi syndrome in humans (Babior, 1985). The beige-J mouse has defects in lysosome-containing cells and other immunological functions, particularly natural killer (NK) cell and cytotoxic T-leukocyte functions (Roder and Duwe, 1979; Biron et al., 1987). Several behavioral studies have shown that beige-J mice, in addition to their immunologic defects, are also less sensitive to the antinociceptive effects of μ-opioid receptor agonists, such as morphine and [D-Ala^2, N-MePhe^4,Gly-ol^5]enkephalin (DAMGO) (Mathiasen et al., 1987). Studies on [^{3}H]-DAMGO binding have demonstrated that the density of opioid receptors in the brains of beige-J mice is comparable to that in the brains of their normal littermates and outbred albino mice (Raffa et al., 1988a; Pick et al., 1991). Based on these results, it is found that the antinociceptive defect in beige-J mice is not due to an insufficient number of μ-opioid receptors. This has led to the hypothesis that μ-opioid receptor-mediated signal transduction in the brain of beige-J mice might be altered (Raffa et al., 1988a,b).

The analysis of a drug's effects on locomotor activity in rodents is an important tool for investigating the psychotic states induced by psychoactive drugs. The administration of morphine can produce dose-related hyperlocomotion in certain strains of mice, and the mesolimbic dopaminergic system plays an important role in regulating exploratory and locomotor behaviors (Funada et al., 1993, 1994; Mori et al., 2012; Shibasaki et al., 2014). On the other hand, morphine alone does not induce hyperlocomotion in BALB/c mice (Ito et al., 2007). We recently demonstrated that beige-J mice showed greater locomotor activity and dopamine turnover than C-57BL/6J mice and ddY mice, whereas splenectomy reduced this spontaneous hyperlocomotion and high levels of dopamine

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turnover, which suggests that beige-J mice could be an experimental animal model for investigating hyperactivation of the dopaminergic system, and some circulating substances that originate in the spleen may modulate activation of the dopaminergic system (Mori et al., 2014). Splenectomy restores the morphine-induced antinociception in beige-J (Raffa et al., 1988b) and diabetic mice (Kamei et al., 1992) to almost normal levels. Furthermore, splenectomy has been shown to normalize the hyperactivity of diabetic mice (Kamei and Saitoh, 1997). Thus, it is possible that morphine does not induce any hyperlocomotion in beige-J mice, and splenectomy may affect the behavioral changes induced by morphine, particularly locomotor activity, in mice. Information on this subject may shed light on the complex interactions between immune-modulation and the μ-opioid receptor system or dopaminergic system.

The aim of the present study was to examine the effects of morphine on locomotor activity in beige-J mice, and the effects of splenectomy on locomotor activity induced by morphine in beige-J mice. We found that morphine-induced hyperlocomotion in beige-J mice was greater than that in ddY mice. Furthermore, morphine-induced hyperlocomotion in beige-J mice was enhanced by splenectomy. Therefore, the neurochemical properties of the activation of the mesolimbic system induced by morphine in beige-J mice were also documented.

2. Materials and methods

2.1. Animals

Male beige-J mice (National Institute on Genetics, Mishima, Japan), weighing 25–35 g at the beginning of the experiments, were used. Male ddY mice were obtained from Tokyo Animal Laboratories Inc. (Tokyo, Japan). The mice were housed at a room temperature of 22 ± 1 °C with a 12 h light-dark cycle (lights on 8:00 A.M. to 8:00 P.M.). Food and water were available ad libitum. Our studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals of Hoshi University School of Pharmacy and Pharmaceutical Science, which is accredited by the Ministry of Education, Culture, Sports and Technology of Japan.

2.2. Effects of morphine on locomotor activity

The locomotor activity of mice was measured by an ambulometer (ANB-M20, O'Hara Co. Ltd., Tokyo) as described previously (Funada et al., 1993). Briefly, a mouse was placed in a tilting-type round activity cage 20 cm in diameter and 19 cm high. Any slight tilt of the activity cage, which was caused by horizontal movement of the animal, was detected by microswitches. Mice were placed in the tilting cages for a habituation period of 30 min after the s.c. (1.0, 2.5, 5.0, 10 and 20 mg/kg) or i.c.v. (2.5 and 5.0 μg/mouse) administration of morphine, and total activity counts were automatically recorded for 180 min. I.c.v. drug administration was made under ether anesthesia in a volume of 5 μl according to the method of Haley and Mccormick (1957).

2.3. Splenectomized mice

Splenectomies were carried out under sterile and ether-anesthetized conditions. Sham-operated animals were subjected to the same handling, anesthesia, surgical exposure of the spleen, and wound closure as splenectomized animals, as described previously (Kamei et al., 1992). Seven days after surgery (sham or splenectomy), morphine-induced hyperlocomotion was measured.

2.4. Neurochemical analysis

The concentrations of dopamine (DA) and its metabolites 3,4-dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) were determined as described previously (Funada et al., 1993, 1994). Sham-operated and splenectomized mice were killed 60 min after s.c. injection of morphine (10 mg/kg, s.c.). The time of killing corresponded to the peak of morphine-induced hyperlocomotion. The brain was quickly removed and dissected into the limbic forebrain (containing the nucleus accumbens and olfactory tubercles) on an ice-cold glass plate according to the modified method of Ahtee et al. (1989). Briefly, the brain was turned to expose the dorsal surface and a vertical cut was made through the anterior commissure. The resulting frontal portion was turned to expose the ventral surface. A vertical cut was made through the rhinal fissure, and a small part that included the accessory olfactory bulb and olfactory nucleus was removed. The resulting block of tissue was turned to expose the section and the area bordered by the caudate putamen and the nucleus accumbens was cut vertically. The block of tissue that included the nucleus accumbens and olfactory tubercle was considered to be the main portion of the limbic forebrain.

The tissue samples were homogenized in 2 ml of 0.2 M perchloric acid containing 100 μM EDTA (2Na) and 100 ng isoproterenol, as an internal standard. For complete removal of the proteins, the homogenates were placed in cold water for 60 min. The homogenates were then centrifuged at $20{,}000 \times g$ for 20 min at 0 °C, and the supernatants were maintained at pH 3.0 using 1 M sodium acetate. Solution samples of 50 μl were injected by a refrigerated GILSON automatic sample injector (MODEL 231), and analyzed by high performance liquid chromatography (HPLC) with electrochemical detection (ECD). The HPLC system consisted of a delivery pump (880-PU, Jasco, Japan), an analytical column (Eicompak, MA-5ODS 4.6 × 150 mm, Eicom Co., Japan) and a guard column (Eicom Co.). The electrochemical detector (EC-100, Eicom Co.) included a graphite electrode (WE-3G Eicom Co.) and was used at a voltage setting of 0.7 V vs. an Ag/AgCl reference electrode. The mobile phase consisted of a 0.1 M sodium acetate/0.1 M citric acid buffer, pH 3.5, containing 13–15% methanol, sodium 1-octanesulfonate and EDTA (2Na). The flow rate was set to 1.0 ml/min with a column temperature of 25 °C.

2.5. Drugs

Morphine hydrochloride (Sankyo, Tokyo, Japan) was dissolved in sterile saline. Dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were purchased from Research Biochemicals, Inc. (Wayland, MA, U.S.A.), and dissolved in 0.02 N acetic acid for HPLC.

2.6. Data analysis

Behavioral data (total activity counts) were statistically evaluated with a one-way repeated measures analysis of variance (ANOVA) followed by a Newman–Keuls test for multiple comparisons. The time-course changes in behavioral data were analyzed using two-way repeated measures ANOVA. Neurochemical data were statistically evaluated with a one-way ANOVA followed by Dunnett's test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of morphine and/or splenectomy on locomotor activity

We previously demonstrated that beige-J mice exhibited greater spontaneous locomotor activity than ddY mice and C57/BL/6J mice,