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Effects of a 5-HT$_7$ receptor antagonist DR4004 on the exploratory behavior in a novel environment and on brain monoamine dynamics in mice

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Abstract

The present study examined whether serotonin (5-hydroxytryptamine; 5-HT)$_7$ receptors play a role in the modulation of emotionality in mice using the selective 5-HT$_7$ receptor antagonist 2*a*-[4-(4-phenyl-1,2,3,6-tetrahydropyridyl)butyl]-2*a*,3,4,5-tetrahydrobenzo (*c*,*d*)indol-2-(1*H*)-one (DR4004). The emotionality of mice was evaluated in terms of exploratory activity in the hole-board test. The mice treated with DR4004 (2.5–10 mg/kg, i.p.) displayed a dose-dependent decrease in locomotor activity by moving less distance in the hole-board, and statistically significant decreases were observed at 5 and 10 mg/kg. On the other hand, DR4004 (10 mg/kg, i.p.) did not affect spontaneous motor activity. In a neurochemical study, decreases in amygdaloid dopamine and 5-HT turnover were observed in mice in which locomotor activity in the hole-board test was attenuated following the administration of DR4004 (10 mg/kg, i.p.). Also, a simple linear regression analysis revealed that locomotor activity on the hole-board was significantly correlated with dopamine and 5-HT turnover in amygdala. Furthermore, co-injection of the selective dopamine reuptake inhibitor 1-(2-[bis(4-fluorophenyl)methoxy]ethyl)-4-(3-phenylpropyl)piperazine (GBR12909; 1.25–5 mg/kg, i.p.) or the selective 5-HT reuptake inhibitor fluvoxamine (20 mg/kg, i.p.) significantly reversed the DR4004 (10 mg/kg, i.p.)-induced decrease in locomotor activity in the hole-board test. These findings constitute the behavioral evidence that 5-HT$_7$ receptors may play a role in the modulation of emotionality. Furthermore, it is also suggested that amygdaloid dopamine and 5-HT neuronal systems may be involved in this modulation.

Keywords: 5-HT$_7$ (5-hydroxytryptamine7) receptors; Exploratory behavior; Hole-board test; Monoamines; Amygdala; (Mouse)

1. Introduction

The brain serotonin (5-hydroxytryptamine; 5-HT) nervous system has been implicated in various brain functions as well as in the pathophysiology and treatment of a wide variety of neuropsychiatric disorders (Bauer et al., 2002; Jones and Blackburn, 2002). A heterogeneous family of at least 14 distinct receptor subtypes has been shown to mediate the effect of 5-HT in the central nervous system (Hoyer and Martin, 1997). Among these, the 5-HT$_1$ and 5-HT$_2$ receptor subtypes have received particular attention as possibly being involved in mediating emotionality and as targets for the treatment of affective disorders such as anxiety and depression (Murphy et al., 1999; Jones and Blackburn, 2002). However, it is still unknown whether other 5-HT receptor subtypes play a role in the modulation of emotionality.

The 5-HT receptor subtype that was most recently identified by molecular cloning is the seven-transmembrane-spanning G-protein-coupled 5-HT$_7$ receptor (Ruat et al., 1993; Shen et al., 1993). Studies using autoradiography, in situ hybridization, radioligand binding and immunohistochemistry techniques have shown that 5-HT$_7$ messenger RNA (mRNA) and receptor protein have a similar abundant

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distribution in various brain regions, i.e., cerebral cortex, hippocampus, thalamus amygdala and hypothalamus (Ruat et al., 1993; Shen et al., 1993; Gustafson et al., 1996; Neumaier et al., 2001). However, it has been difficult to identify any physiological role for this receptor in the central nervous system, although there is some evidence which suggests that 5-HT_7 receptors may be involved in the regulation of circadian rhythms (Lovenberg et al., 1993; Ehlen et al., 2001).

It has been demonstrated that several receptor agonists and antagonists have affinity for 5-HT_7 receptors, but none of them is selective (Eglen et al., 1997). Thus, the development of selective ligands for 5-HT_7 receptors will be of utmost importance in pharmacologically determining the physiological role of this receptor subtype. Recently, Kikuchi et al. (1999) developed a 2*a*-[4-(4-phenyl-1,2,3,6-tetrahydropyridyl)butyl]-2*a*,3,4,5-tetrahydrobenzo(*c*,*d*)indol-2-(1*H*)-one (DR4004) as a selective 5-HT_7 receptor antagonist, which displaces the binding of [^{3}H]5-carboxamidotryptamine with high affinity and selectivity, and also inhibits the 5-HT-induced stimulation of cyclic AMP accumulation in a mammalian cell line (COS-7 cells) expressing 5-HT_7 receptors. This compound has recently been used as a tool for determining the actual functions of 5-HT_7 receptors (Ehlen et al., 2001; Matsumoto et al., 2002).

The expression and distribution of mRNA and proteins for 5-HT_7 receptors in the midline, thalamus and limbic structures (Ruat et al., 1993; Gustafson et al., 1996; Neumaier et al., 2001) suggest that they may play a role in the regulation of emotion. This hypothesis may be supported by evidence that some compounds that affect emotionality have an affinity for 5-HT_7 receptors; examples include antipsychotics, anxiolytics and antidepressants (Shen et al., 1993; Eglen et al., 1997). Furthermore, it has also been reported that chronic treatment with an antidepressant as well as exposure to stress stimuli affected the density or function of 5-HT_7 receptors (Sleight et al., 1995; Shimizu et al., 1996; Mullins et al., 1999; Yau et al., 2001). However, there is still no conclusive experimental evidence for the regulation of emotion by 5-HT_7 receptors. Therefore, the aim of the present study was to examine whether 5-HT_7 receptors play a role in the modulation of emotionality. Changes in the emotional behavior of mice produced by the selective 5-HT_7 receptor antagonist DR4004 were evaluated using our hole-board apparatus (Takeda et al., 1998, 2003; Tsuji et al., 2000, 2001). Furthermore, to elucidate the mechanisms involved in the expression of these behavioral changes, a neurochemical analysis on monoamine dynamics in mouse brain regions was also performed.

2. Materials and methods

The present studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals adopted by the Committee on Care and Use of Laboratory Animals of Tokyo Medical University and the Japanese Pharmacological Society.

2.1. Animals

Male ICR mice (Charles River, Japan) weighing 25–30 g were housed at a room temperature of 22 ±1 °C with a 12-h light–dark cycle (light on 6:00 a.m. to 6:00 p.m.). Food and water were available ad libitum.

2.2. Apparatus for hole-board test

Exploratory behaviors of mice in a novel environment were measured as previously described using an automatic hole-board apparatus (Muromachi Kikai, Japan) (Takeda et al., 1998, 2003; Tsuji et al., 2000, 2001). The apparatus consisted of a gray box (50 × 50 × 50 cm) with four equidistant holes 30 mm in diameter in the floor. An infrared beam sensor was installed on the wall (65 mm upper and 20 mm lower from floor) to detect the number and duration of rearing and head-dipping behaviors. The horizontal moving distance of mice in the hole-board (locomotor activity) was detected by an overhead color charge-coupled device (CCD) camera (the heads of mice were painted yellow to detect the locus in the hole-board by the color CCD camera and the distance that the painted head moved was used as an indicator of locomotor activity). Head-dipping behaviors were double-checked via an infrared beam sensor and the overhead color CCD camera. Thus, head-dipping behavior was counted only when both the head intercepted the infrared beam and the head was detected at the hole by the CCD camera. Data from the infrared beam sensor and the CCD camera were collected through a custom-designed interface (CAT-10, Muromachi Kikai, Japan) as a reflection signal. All data were analyzed and stored by a personal computer with analytical software (Comp ACT HBS; Muromachi Kikai, Japan).

2.3. Procedure for hole-board test

Groups of mice were injected with DR4004 (2.5–10 mg/kg), 1-(2-[bis(4-fluorophenyl)methoxy]ethyl)-4-(3-phenylpropyl)piperazine (GBR12909; 2.5-10 mg/kg), fluvoxamine (5–20 mg/kg), sulpiride (20 mg/kg) or vehicle (saline or 1% Tween 20 in saline; 10 ml/kg) intraperitoneally (i.p.). Thirty minutes later, each animal was placed in the center of the hole-board and allowed to freely explore the apparatus. Total moving distance, number and duration of rearing and head-dipping behaviors and latency to the first head-dip were automatically recorded for 5 min. In combination studies, GBR12909 (1.25–5 mg/kg, i.p.), fluvoxamine (5–20 mg/kg, i.p.), or sulpiride (20 mg/kg, i.p.) was co-injected with DR4004 (10 mg/kg, i.p.).

2.4. Apparatus and procedure for measurement of spontaneous motor activity

2.4.1. Measurement of the motor activity of mice that had been habituated to a novel environment

The motor activity of mice that had been habituated to a novel environment was measured using an activity-monitoring system (Supermex, Muromachi Kikai, Japan) as previously described (Takeda et al., 2002, 2003). Briefly, mice were placed