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Direct and indirect 5-HT receptor agonists produce gender-specific effects on locomotor and vertical activities in C57 BL/6J mice

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ABSTRACT

It is well established that the dopamine (DA) and serotonin (5-HT) systems have extensive and complex interactions. However, the effects of specific 5-HT receptor agonists on traditionally DA-related behaviors remain unclear. Our goal in these studies was to characterize the effects of 5-HT receptor agonists on measures of locomotor activity and vertical rearing. The SSRIs fluoxetine and citalopram produced significant decreases in locomotor activity and vertical rearing at the highest doses used with females significantly more sensitive to citalopram. The 5-HT_{1A} agonist 8-OH-DPAT and the 5-HT_{2C} agonist MK 212 significantly decreased activity in both male and female mice, with females more sensitive to 8-OH-DPAT. In contrast, the 5-HT_{1B} agonist RU 24969 and the 5-HT_{2A} agonist DOI both increased activity, with DOI exhibiting differential effects with regard to sex. Finally, the 5-HT_3 agonist SR 57227 produced significant locomotor increases only in female mice at the lowest dose. The results of these experiments define locomotor profiles of several 5-HT agonists in male and female C57BL/6J mice, providing a foundation for further explorations of 5-HT receptor effects on activity.

1. Introduction

It is well established that dopamine (DA) and serotonin (5-HT) systems have extensive and complex interactions, especially within the basal ganglia (Alex and Pehek, 2007). The activity of the striatum, a sensory–motor–limbic integration region of the basal ganglia that controls locomotor and vertical activities in rodents and includes the caudate-putamen and nucleus accumbens, is modulated by many factors, in particular DA and 5-HT projections arising from the ventral midbrain and brainstem (Geyer, 1996). While the effects of direct and indirect DA receptor agonists on locomotor and vertical activities have been well-characterized (Berridge, 2006), the effects of 5-HT receptor agonists remain unclear. The ventral midbrain, where DA cell bodies are located, receives the heaviest 5-HT innervations in the brain (Herve et al., 1987), and 5-HT receptors regulate the activity of DA and GABA neurons in this region (Pessia et al., 1994; O'Dell and Parsons, 2004). There is also a strong 5-HT projection to the striatum which provides presynaptic 5-HT regulation of DA release (Alex and Pehek, 2007). In light of these extensive interactions, the effects of 5-HT receptor agonists on behaviors traditionally associated with activation of the dopaminergic system, such as locomotor and vertical activities (Smith, 1965; Christie and Crow, 1971; Costall et al., 1982), merit further study.

Fourteen 5-HT receptor subtypes have been identified to date, and at least five of these are known to have extensive interactions with the DA system, including the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3 receptors (Alex and Pehek, 2007). Many 5-HT receptor agonists are well-characterized with regard to anxiety-related, anti-depressant, hallucinogenic and other properties. However, the effects of these compounds on locomotor activity in mice remain relatively unknown. Although some locomotor studies of drugs such as the 5-HT_{1A} agonist, 8-OH-DPAT, have been performed in mice and rats, the doses used are limited, and studies center almost exclusively on examining the modulatory influences of specific 5-HT receptors on stimulant-induced

Abbreviations: DA, Dopamine; 5-HT, serotonin; SSRI, selective serotonin reuptake inhibitor; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tertraline; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2 amino propane; RU 24969, 5-methoxy-n1N-dimethyl-tryptamine and 5-methoxy-3(1,2,3,6,-tetrahydro-4-pyrindinyl)-1H-indole; MK 212, 6-Chloro-2-(1-piperazinyl)pyrazine hydrochloride; SR 57227, 1-(6-Chloro-2-pyridinyl)-4-piperidinamine hydrochloride; Fluoxetine, (±)-N-Methyl-γ-[4-(trifluoromethyl)phenoxy]benzenepropanamine hydrochloride; Citalopram, 1-[3-(Dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile hydrobromide.

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behaviors. Thus, a study of the effects of 5-HT receptor agonists on mouse locomotor activity could prove useful in further studies of drug-induced behaviors, as well as providing insight into the direct locomotor effects of the 5-HT agonists.

It is known that male and female mice differ in their responses to some psychoactive drugs that activate both the 5-HT and DA systems, such as amphetamine (Camp and Robinson, 1988; Robinson et al., 1982). Thus, it is possible that 5-HT receptor agonists with actions in the DA system might show sex differences. Additionally, the DA and 5-HT systems are known to have extensive interactions, particularly in the area of the striatum and nucleus accumbens, brain areas linked with the locomotor- and vertical activity-stimulating effects of drugs (Ikemoto, 2002). It is therefore likely that stimulation of specific 5-HT receptor subtypes in this area might have significant effects on locomotor and vertical activities, effects often mediated through the dopaminergic system (Kelly, 1975; Costall et al., 1982; Stanford et al., 2002), but which are not necessarily mediated exclusively by increases in DA (Murphy et al. 2001). Therefore, although locomotor and vertical activations or attenuation may be due to changes in DA via 5-HT receptor stimulation (Murphy et al., 2001), 5-HT receptor stimulation alone may also alter locomotor and vertical activities.

Although some 5-HT receptors are relatively well-characterized with regard to sex differences in brain distribution and the behavioral effects of stimulation, such as the 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors (Zhang et al., 1999; Blanchard et al., 1992; Bagdy, 1998), others remain poorly characterized, and no studies have examined sex differences in the effects of specific 5-HT receptor agonists on locomotor activity.

Despite what is known about the effects of 5-HT receptor agonists on mesolimbic DA firing rates and control of such behaviors such as drug self-administration (Rothman et al., 2007), the specific function of these receptors in DA-related behaviors, such as locomotor activity in mice, remains unclear. As locomotor and vertical activities are behaviors known to be regulated by DA signaling, our goal in these studies was to characterize the effects of several 5-HT receptor agonists on DA/5-HT interactions in mice. C57BL/6J is the most commonly used mouse strain in neuropharmacological research, and thus we chose to use this strain to characterize the locomotor effects of seven different serotonergic compounds; the selective serotonin reuptake inhibitors (SSRIs) fluoxetine and citalopram, the 5-HT_{1A} agonist 8-OH-DPAT, the 5-HT_{1B} agonist RU 24969, the 5-$HT_{2A/2C}$ agonist DOI, the selective 5-HT_{2C} agonist MK 212, and the 5-HT_3 agonist SR 57227. Naïve mice were tested with a variety of drug doses for locomotor distance traveled and measures of vertical rearing activity.

2. Methods

2.1. Animals

C57Bl/6J mice were obtained from Jackson laboratories (Bar Harbor, ME), and a breeding colony was established. Mice were housed in groups of three or four per cage with food and water *ad libitum* on a 12–h light–dark cycle with lights on at 7 am. All experiments used both male and female mice that were between 2 and 6 months old, and for each drug, the male and female mice being tested were age matched. Experimental protocols adhered to the National Institutes of Health Animal Care Guidelines and were approved by the Wake Forest University Institutional Animal Care and Use Committee.

2.2. Activity monitoring

Locomotor activity and vertical rearing were assessed using open field activity monitors (Med Associates, St. Albans, VT). The open field consisted of a square plexiglass container (27.0 cm × 27.0 cm × 20.3 cm) with three sixteen beam infrared arrays. Two arrays were placed on the periphery of the chamber at floor level for detection of locomotor activity (*X* and *Y* planes, measured approximately 0.25″ off the floor), while the third array was placed 2″ above the *X* and *Y* arrays to obtain measures of vertical activity (*Z* plane, measured 2.25″ off the floor). Data was collected using Med Associates Activity Monitoring Software (Med Associates, St. Albans, VT), and distance traveled was measured in cm over a given length of time. Vertical rearings were measured as number of beam breaks in the vertical plane over a given length of time. Behavioral analyses were conducted during the light phase between 9 am and 5 pm. The locomotor chambers contained no bedding, and were cleaned with 70% EtOH and dried thoroughly between testings.

2.3. Drug administration

Following a 2-h period where mice were allowed to habituate to the chambers, animals received either saline (0.1 mL injection volume) or drug dissolved in saline (unless specified otherwise), administered in a 0.1 mL volume i.p., by weight, at the doses described below. Separate injections were prepared for male and female mice based on average male and female weight for the cohort. Animals were divided into cohorts, each of which received all doses of a single drug type. Doses were randomized in a Latin-Square design. Data was collected using Med Associates proprietary software (Med Associates, St. Albans, VT) in 5-min bins for a period of 2 h following injection.

2.4. Statistics

Data was analyzed for distance traveled in cm and the number of vertical rears performed during the activity profile of the drug. Locomotor activity was grouped in bins for either the first 20 min after drug injection (fluoxetine, citalopram, MK 212), the first 30 min after drug injection (8-OH-DPAT), or the first 60 min after drug injection (RU 24969, DOI, SR 57227), depending on the active period of the drug. Active period of the drug was determined by AUC analysis of activity curves measured over 2 h following drug injection (for a series of representative locomotor activity traces over the full 2 h of recording following drug injection, see Supplemental data). The time course of each drug tested can be seen at representative doses for locomotor and vertical activities in Supplemental Figs. 1–9. Following summation of data over 20, 30 or 60 min, all groups were tested for outliers using the Grubb's Test for Outliers. Data was then grouped by sex and analyzed by a one-way ANOVA for the effect of drug in either male or female mice, with a corrected Bonferroni post-hoc analysis to determine specific effects of dose. For comparisons between sex, activity was compared as percent of saline values, to control for differences in saline response between sex. Data was analyzed for between sex effects and interactions between drug and sex by repeated-measures ANOVA with corrected Bonferroni post-hoc analysis. $p < 0.05$ was considered significant.

2.5. Drugs

The SSRIs (±)-N-methyl-γ-[4-(trifluoromethyl)phenoxy]benzenepropanamine hydrochloride (fluoxetine) and 1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile hydrobromide (citalopram), the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tertraline (8-OH-DPAT), and the 5-$HT_{2A/2C}$ receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2 amino propane ((±) DOI-hydrochloride) were purchased from Sigma (Sigma Aldrich, St. Louis, MO). The 5-HT_{1B} agonist 5-methoxy-n1N-dimethyltryptamine and 5-methoxy-3(1,2,3,6,-tetrahydro-4-pyrindinyl)-1H-indole (RU 24,969), the 5-HT_{2C} agonist 6-chloro-2-(1-piperazinyl)pyrazine hydrochloride (MK 212), and the 5-HT_3 agonist 1-(6-chloro-2-pyridinyl)-4-piperidinamine hydrochloride (SR 57227) were purchased from Tocris (Ellisville, MO). All drugs were given in a volume of 0.1 mL with concentrations determined by animal weight averages. Fluoxetine HCl was dissolved in ultra-pure water, while all other drugs were dissolved in 0.9% isotonic sterile saline.