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Behavioural effects of monoamine reuptake inhibitors on symptomatic domains in an animal model of attention-deficit/hyperactivity disorder

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ABSTRACT

Attention-deficit/hyperactivity disorder (ADHD) is a heterogeneous neurobehavioural disorder. Several lines of evidence have implicated monoamine signalling systems, including transporters and receptors, in the pathogenesis of ADHD. We explored the heterogeneity of neural mechanisms that may possibly underlie symptomatic abnormalities in ADHD, by investigating the effects of monoamine reuptake inhibitors with differential spectrums for each monoamine transporter on ADHD-like behaviours in an animal model of ADHD, i.e. juvenile (6-week-old) male stroke-prone spontaneously hypertensive rats (SHRSP/Ezo). The impaired spontaneous alternation performance in a Y-maze task, demonstrated the inattentive features of SHRSP/Ezo, was improved by a selective DA reuptake inhibitor GBR-12909 (1 and 3 mg/kg, i.p.). Desipramine (1, 3 and 10 mg/kg, i.p.) and milnacipran (30 mg/kg, i.p.), which possess a noradrenaline (NA) reuptake inhibitory activity, also ameliorated inattentive behaviour. Increased locomotor activity in open-field apparatus and total arm entries in a Y-maze task, which demonstrate the hyperactive features of SHRSP/Ezo, were improved by desipramine and milnacipran, but impaired by a high dose of GBR-12909 (10 mg/kg, i.p.). A selective serotonin (5-HT) reuptake inhibitor fluvoxamine (10 and 30 mg/kg, i.p.), did not affect inattention but significantly suppressed hyperactivity at a high dose (30 mg/kg, i.p.). Moreover, a low dose of fluvoxamine (3 mg/kg, i.p.) ameliorated the increased open arm spent time in an elevated plus-maze without affecting total arm entries, indicating an effect on impulsive features based on the anxiolytic characteristics of SHRSP/Ezo. These behavioural effects of monoamine reuptake inhibitors support the heterogeneity of monoaminergic systems, which are responsible for ADHD-like behaviours in SHRSP/Ezo. These findings may provide pharmacological evidence for the development of ADHD treatments that target more appropriate monoamine transporters.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is the most common, childhood-onset psychiatric disorder, affecting approximately 5% of school-aged children and frequently persisting into adulthood (Barkley, 1998; Szatmari et al., 1989). ADHD is a heterogeneous neurobehavioural disorder that is occasionally accompanied by learning deficits, and is prevalent among boys (Lahey et al., 1994; Taylor et al., 2004). Diagnosis is based on the observation of certain behavioural symptoms. The criteria for ADHD given by DSM-IV (American Psychiatric Association, 2000) include three behavioural subtypes, which are defined as the predominantly inattentive subtype, the predominantly hyperactive and impulsive subtype, and the combined subtype.

The pathogenesis of ADHD is incompletely understood, because it is complicated by comorbidity with other psychiatric disorders (i.e. conduct disorder, dis-social personal disorder, autism symptoms, and anxiety and mood disorders) (Sharp et al., 2009). ADHD is highly heritable, as estimated from twin and family studies (Biederman et al., 1995; Durston, 2008), and is exacerbated by environmental factors such as psychological events (Lahey et al., 1994; Solanto et al., 2001). Moreover, there is growing evidence of complex interactions between genetic and environmental factors (Swanson et al., 2007).

A majority of specific genes implicated in ADHD encode components of monoamine signalling systems, including the dopamine (DA) transporter (DAT), DA receptors (DRD_4 and DRD_5), noradrenaline (NA) transporter (NAT), NA receptors ($ADRA_{1A}$ and $ADRA_{2A}$)

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; DA, dopamine; DAT, dopamine transporter; NA, noradrenaline; NAT, noradrenaline transporter; 5-HT, serotonin (5-hydroxytriptamine); SERT, serotonin transporter; SHRSP, stroke-prone spontaneously hypertensive rat.

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and DA β-hydroxylase (Dougherty et al., 1999; Krause et al., 2000; Thapar et al., 2005; Faraone and Khan, 2006); and the serotonin (5-HT) transporter (SERT), 5-HT receptors (HTR_{1A}, HTR_{1B}, HTR_{2A} and HTR_4) and tryptophan hydroxylase (Hawi et al., 2002; Quist et al., 2003; Smoller et al., 2006; Sharp et al., 2009; Archer et al., 2011). Numerous studies of these components provide a basis for the dopamine theory of ADHD. There is a significant association between ADHD and variants of the DAT and DA receptor genes; the most common drug used for the treatment of ADHD, methylphenidate, acts on DAergic synapses as an indirect agonist. Imaging studies of ADHD patients also show changes in brain regions activated by DA. Thus, DAT is a major candidate that is implicated in the pathophysiology of ADHD and anti-ADHD drug mechanisms (Barr et al., 2001).

The monoamine transporters, DAT, NAT and SERT, are located on the plasma membrane of pre-synaptic nerve terminals for DA, NA and 5-HT, which modulate the synaptic dynamics of these neurotransmitters. The activity of monoamine transporters has significant consequences for neuronal activity and neurological/psychiatric disorders including ADHD. Indeed, methylphenidate acts on DAT (Barr et al., 2001), and is thought to correct an underlying DAergic deficit in ADHD patients. However, the precise mechanism whereby methylphenidate exerts its therapeutic effects is incompletely understood, though some argue in favour of the involvement of NA (Arnsten, 2006) or 5-HT (Gainetdinov et al., 1999). In addition, monoamine transporters are not overly selective of their targets, and are region-specific in their expression. For example, it is reported that in the prefrontal cortex (PFC), where DAT density of dopaminergic neurons is low (Sesack et al., 1998), NAT discharges DA reuptake (Carboni et al., 1990; Tanda et al., 1997; Yamamoto and Novotney, 1998; Morón et al., 2002). These characteristics of monoamine transporters, the key molecules for understanding the pathophysiology of ADHD, may partly explain the enigmatic action of methylphenidate as an ADHD drug. Thus, from a clinical perspective, the elucidation of the therapeutic effects of monoamine reuptake inhibitors with a variety of pharmacological spectrums on monoamine transporters is of great importance for rational understanding of ADHD neurobiology.

ADHD is a behavioural disorder, the symptomatic and neurological correlates of which are better understood with animal models. A variety of animal models has been reported to exhibit ADHD-like behavioural features (Davids et al., 2003), but few are likely to satisfy the complete set of validation criteria as ADHD animal models. We previously reported that an inbred substrain of stroke-prone spontaneously hypertensive rats (SHRSP/Ezo), a substrain of spontaneously hypertensive rats (SHR) (Okamoto et al., 1974), exhibits ADHD-like behaviours (inattention in a Y-maze task, hyperactivity in an open field test and impulsiveness due to hypo-anxiety in an elevated plus-maze test) (Ueno et al., 2003). Recently, SHRSP/Ezo have been deposited to National BioResource Project-Rat in Japan (NBRP-Rat, http://www.anim.med.kyoto-u.ac.jp/nbr/homejp.htm), in which SHR and SHRSP substrains were characterized by the comprehensive phenotypes and the genotypes determined by global analysis of single nucleotide polymorphism. It was notable that hyperactivity was a common behavioral phenotype among SHRSP substrains examined, however, only SHRSP/Ezo fulfilled the behavioural validation criteria as ADHD animal models (Yamaguchi et al., 2012a). In addition, attention deficit assessed by spontaneous alternation behaviour in the Y-maze was male biassed (Ueno et al., 2002a,b,c, 2003). Of note is that juvenile male SHRSP/Ezo exerted dopaminergic dysfunction in the frontal cortex as reported in ADHD patients (Kimura et al., in press). Thus, male SHRSP/Ezo demonstrated a combined subtype-like behavioural disturbance, which was ameliorated by methylphenidate (Ueno et al., 2003). However, our previous studies showed that its therapeutic efficacy is different among behavioural domains, i.e. low doses of methylphenidate are effective for attention deficits estimated based on alternation behaviour in the Y-maze, whereas low doses are effective but high doses are detrimental to hyperactivity estimated based on locomotor activity in the open field. None of the test dosage had unequivocal effects on impulsive behaviour, as tested by an elevated plus-maze (Ueno et al., 2002b). These results led us to hypothesize that heterogeneous monoaminergic mechanisms may underlie the symptomatic abnormalities observed in this ADHD animal model.

The present study aimed to explore the heterogeneity of monoaminergic mechanisms that may underlie symptomatic abnormalities in ADHD. Thus, we elucidated the effects of monoamine reuptake inhibitors with differential pharmacological spectrums for monoamine transporters, on ADHD-like behavioural domains in an ADHD animal model, SHRSP/Ezo. Special attention was paid to the possible involvement of 5-HTergic mechanisms in the impulsive behaviour of this model. Clarifying the heterogeneity that underlies the neurobiology of ADHD is of great importance for a rational understanding of this disorder and improving its treatment. Behavioural and pharmacological studies using monoamine reuptake inhibitors in an ADHD animal model could potentially be useful in this regard.

2. Materials and methods

2.1. Subjects

Male SHRSP/Ezo (6-week-old) was bred in our laboratory (Health Sciences University of Hokkaido, Hokkaido, Japan). Rats were housed in a room with a 12-h light/dark cycle at 22 ± 2 °C with free access to food and water. All experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Health Sciences University of Hokkaido.

2.2. Behavioural analysis

2.2.1. Spontaneous alternation behaviour in a Y-maze

Spontaneous alternation behaviour in a Y-maze, which requires attention (Katz and Schmaltz, 1980) and working memory (Sarter et al., 1988) was evaluated as a measure of inattentive behaviour. Each arm of the Y-maze was 45 cm long, 10 cm wide and 35 cm high, and the three arms were positioned at equal angles. Rats were placed in the centre of the arms and allowed to move freely during an 8-min test session. The illumination of the arms was maintained at 200 lx during the test. An arm entry was defined as the entry of all four paws into an arm. The maximum alternation was defined as the total number of arms entered minus 2, and the percentage of alternation behaviour was calculated as (actual alternations/maximum alternations) × 100.

2.2.2. Locomotor activity in an open-field apparatus

Locomotor activity was evaluated as a measure of hyperactive behaviour using an open-field apparatus. Rats were placed in the centre of the apparatus (90 × 90 × 40 cm) and allowed to move freely during a 60-min test session. The illumination of the apparatus was maintained at 200 lx throughout the test. The distance travelled and total crossings were recorded and analyzed automatically using the Lime-Light2 software package (Actimetrics, Inc., Wilmette, IL, USA). Total crossings were defined as the total number of crossings of lines (all four paws) divided into squares (10 × 10 cm) in the open-field.

2.2.3. Anxiety-related behaviour in an elevated plus-maze test

Anxiety-related behaviour in an elevated-plus maze was used as a measure of impulsive behaviour (Pellow et al., 1985; Pellow and File, 1986; Ueno et al., 2002b). A plus-maze, with two opposite open arms (50 × 10 cm) and two enclosed-arms (50 × 10 × 40 cm), was elevated 50 cm above the floor. The arms extended from a central platform (10 × 10 cm). Rats were placed individually in the central platform of the maze and allowed to enter freely into the arms throughout a 10-min test session. The illumination of the arms was kept at 200 lx throughout the test. The number of open- and enclosed-arm entries,