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Effects of serotonin–norepinephrine reuptake inhibitors on locomotion and prefrontal monoamine release in spontaneously hypertensive rats

Masato Umehara [a,1], Yukio Ago [a,1], Kazumi Fujita [a], Naoki Hiramatsu [a], Kazuhiro Takuma [a], Toshio Matsuda [a,b,*]

[a] Laboratory of Medicinal Pharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan
[b] United Graduate School of Child Development, Osaka University, Kanazawa University, Hamamatsu University School of Medicine, Chiba University and University of Fukui, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan

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ABSTRACT

Catecholamine neurotransmission in the prefrontal cortex plays a key role in the therapeutic actions of drugs for attention-deficit/hyperactivity disorder (ADHD). Recent clinical studies show that several serotonin–norepinephrine reuptake inhibitors have potential for treating ADHD. In this study, we examined the effects of acute treatment with serotonin–norepinephrine reuptake inhibitors on locomotion and the extracellular levels of monoamines in the prefrontal cortex in spontaneously hypertensive rats (SHR), an animal model of ADHD. Adolescent male SHR exhibited greater horizontal locomotion in an open-field test than male WKY control rats. Psychostimulant methylphenidate (0.3 and 1 mg/kg), the selective norepinephrine reuptake inhibitor atomoxetine (1 and 3 mg/kg), and serotonin–norepinephrine reuptake inhibitors duloxetine (10 mg/kg), venlafaxine (10 and 30 mg/kg) and milnacipran (30 mg/kg) reduced the horizontal activity in SHR, but did not affect in WKY rats. The selective norepinephrine reuptake inhibitor reboxetine (10 mg/kg) and the tricyclic antidepressant desipramine (10 and 30 mg/kg) also reduced the horizontal activity in SHR, whereas the selective serotonin reuptake inhibitor citalopram (30 mg/kg) did not. Microdialysis studies showed that atomoxetine, methylphenidate, duloxetine, venlafaxine, milnacipran, and reboxetine increased the extracellular levels of norepinephrine and dopamine in the prefrontal cortex in SHR. Citalopram did not affect norepinephrine and dopamine levels in the prefrontal cortex, although it increased the serotonin levels. Neither duloxetine nor venlafaxine increased the dopamine levels in the striatum. These findings suggest that serotonin–norepinephrine reuptake inhibitors, similar to methylphenidate and atomoxetine, have potential for ameliorating motor abnormality in the SHR model.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common developmental disorders in children, with a prevalence of 5–10% (Polanczyk et al., 2007; Scahill and Schwab-Stone, 2000). Moreover, 2.9–4.4% of the adult population have continuing ADHD (Faraone and Biederman, 2005; Kessler et al., 2006). Although the etiology of ADHD is not known, it is likely that dysfunction of catecholaminergic signaling plays a key role, particularly in prefrontal cortical regions (Arnsten, 2009). The pharmacotherapy of ADHD is based on evidence that norepinephrine and dopamine are highly involved in motor control and several domains of cognition, including working memory, attention, and executive function. Atomoxetine blocks selectively the norepinephrine transporter, whereas methylphenidate blocks the dopamine and norepinephrine transporters (Bymaster et al., 2002; Easton et al., 2007; Gehlert et al., 1995; Tatsumi et al., 1997). In vivo microdialysis studies have shown that these drugs increase extracellular levels of norepinephrine and dopamine in brain cortical regions (Berridge et al., 2006; Bymaster et al., 2002; Koda et al., 2010; Weikop et al., 2007). It should be noted that methylphenidate has opposite effects, depending on the dose. Low doses (0.25–1 mg/kg, i.p.) of methylphenidate improve cognitive function without locomotor-activating effects (Berridge et al., 2006), whereas high doses (10 mg/kg) induce hyperlocomotion (Koda et al., 2010). Methylphenidate at doses that induce hyperlocomotion increase extracellular dopamine levels in the striatum and nucleus accumbens, where has been associated with reinforcing and locomotor-activating effects (Bymaster et al., 2002; Koda et al., 2010). In addition, recent study using

* Corresponding author at: Laboratory of Medicinal Pharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6879 8161; fax: +81 6 6879 8159.
E-mail address: matsuda@phs.osaka-u.ac.jp (T. Matsuda).
[1] These authors equally contributed to this work.

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high-speed chronoamperometric recording techniques shows the sub-regional differences in dopamine release and uptake in the striatum and nucleus accumbens of rodent ADHD models (Miller et al., 2012). Serotonin–norepinephrine reuptake inhibitors are widely used for the treatment of major depressive disorder. Microdialysis studies show that duloxetine, venlafaxine, and milnacipran increase not only the extracellular levels of serotonin and norepinephrine but also those of dopamine in rat prefrontal cortex (Kihara and Ikeda, 1995; Kitaichi et al., 2005; Koch et al., 2003; Millan et al., 2001), but not affect dopamine levels in the striatum (Millan et al., 2001), when compared at similar doses. Several clinical trials have examined the effectiveness of venlafaxine (Findling et al., 2007; Zarinara et al., 2010) and duloxetine (Bilodeau et al., in press; Mahmoudi-Gharaei et al., 2011) for treating ADHD symptoms including inattention, oppositionality, and hyperactivity. These studies suggest that serotonin–norepinephrine reuptake inhibitors have potential in the treatment of ADHD. However, no study has examined the effects of serotonin–norepinephrine reuptake inhibitors on behavior in an animal model.

In this study, we examined the effects of duloxetine, venlafaxine, and milnacipran in comparison to atomoxetine and methylphenidate on locomotion and prefrontal monoamine systems in spontaneously hypertensive rats (SHR), an animal model of ADHD (Arime et al., 2011; Sagvolden et al., 2005). To elucidate the roles of both monoamine systems in the effects of serotonin–norepinephrine reuptake inhibitors, we also examined the effects of the selective serotonin reuptake inhibitor citalopram, the selective norepinephrine reuptake inhibitor reboxetine, and the tricyclic antidepressant desipramine which mainly inhibits the reuptake of norepinephrine and also inhibits the uptake to a lesser extent of serotonin.

2. Materials and methods

2.1. Animals and drug treatments

All animal studies were approved by the Animal Care and Use Committee of the Graduate School of Pharmaceutical Sciences, Osaka University. All studies followed the Guiding Principles for the Care of Laboratory Animals as described in the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize animal suffering, and to reduce the number of animals used. Three-week-old male SHR/NCrlCrlj and WKY/NCrlCrlj rats were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan) and housed in cages (26 cm × 38 cm × 20 cm) in each group of four animals under controlled environmental conditions (23 ± 3 °C; 12:12-h light–dark cycle, lights on at 0800 hours, food and water ad libitum) for at least 1 week before use in the experiments. Four–five-week-old male rats were used in the behavioral and microdialysis experiments. We used 285 male SHR and 206 male WKY rats in total and in single use for each purpose. The doses of atomoxetine and methylphenidate were selected according to the previous studies (Tamburella et al., 2012; Ueno et al., 2002). The doses of serotonin–norepinephrine reuptake inhibitors, norepinephrine reuptake inhibitors, and serotonin reuptake inhibitors used in the behavioral experiments were determined referring to the previous studies that the doses of these drugs increase the norepinephrine and/or serotonin release in the prefrontal cortex (Koch et al., 2003; Millan et al., 2001). We first performed the behavioral experiments and then performed the microdialysis experiments. Therefore, the doses used in the microdialysis studies were based upon the findings in the behavioral tests. The animal numbers used in this study are shown in Table 1. The following drugs were used: atomoxetine hydrochloride, citalopram hydrobromide, milnacipran hydrochloride, reboxetine mesylate, venlafaxine hydrochloride (Tocris Bioscience, Bristol, UK), desipramine hydrochloride, methylphenidate hydrochloride (Sigma, St Louis, MO, USA), and duloxetine hydrochloride (AvaChem Scientific LLC, San Antonio, TX, USA). All drugs were dissolved in distilled water containing 5% glucose and were administered in a volume of 1 ml/kg intraperitoneally (i.p.).

Table 1
The drug treatment and numbers of animals used in the behavioral and microdialysis experiments.

Treatment		Horizontal activity		Microdialysis (SHR)	
		SHR *n*	WKY *n*	Prefrontal cortex *n*	Striatum *n*
Atomoxetine	Vehicle	9	7	4	4
	0.3 mg/kg	8	7	–	–
	1 mg/kg	8	7	–	–
	3 mg/kg	8	7	4	5
Methylphenidate	Vehicle	7	6	–	–
	0.1 mg/kg	7	6	–	–
	0.3 mg/kg	7	6	–	–
	1 mg/kg	7	6	3	5
Duloxetine	Vehicle	8	8	–	–
	1 mg/kg	8	7	–	–
	3 mg/kg	8	8	–	–
	10 mg/kg	8	8	4	4
Venlafaxine	Vehicle	7	7	–	–
	3 mg/kg	7	7	–	–
	10 mg/kg	7	7	–	–
	30 mg/kg	7	7	4	4
Milnacipran	Vehicle	6	6	–	–
	3 mg/kg	6	6	–	–
	10 mg/kg	6	6	–	–
	30 mg/kg	6	6	4	–
Reboxetine	Vehicle	7	6	–	–
	1 mg/kg	7	6	–	–
	3 mg/kg	7	6	–	–
	10 mg/kg	7	6	3	–
Desipramine	Vehicle	7	6	–	–
	3 mg/kg	7	6	–	–
	10 mg/kg	7	6	–	–
	30 mg/kg	7	5	–	–
Citalopram	Vehicle	8	6	–	–
	3 mg/kg	8	6	–	–
	10 mg/kg	8	6	–	–
	30 mg/kg	8	6	4	–

n: animal number used.

2.2. Measurement of Horizontal activity in an open-field

Previous studies show that adolescent SHR exhibit greater horizontal locomotion and total distance traveled than WKY rats in both a novel and familiar (habituated) open-field (Li and Huang, 2006; Tamburella et al., 2012; van den Bergh et al., 2006). In this study, all rats were exposed to the open-field apparatus (acrylic cylinder; 40 cm height × 40 cm diameter) for two consecutive days before the test to eliminate any effects because of handling and injection stress. On day 1, each naïve rat was removed from the home cage by hand, individually placed in the center of the apparatus under ambient lighting of 150–170 lx, and then allowed to explore the open-field for 2 h. On day 2, the rat was removed from the home cage by hand, injected with the vehicle, and then placed in the same open-field apparatus for 2 h immediately after the vehicle injection. In the day 3 test session, the rat was removed from the home cage by hand, injected with the drug or vehicle, and then placed in the same open-field apparatus immediately after the drug or vehicle injection. The horizontal activity for 2 h was measured as the total counts