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Research report

Stimulation of postsynapse adrenergic α_{2A} receptor improves attention/cognition performance in an animal model of attention deficit hyperactivity disorder

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HIGHLIGHTS

- Clonidine improved attention deficit in the 5-trial inhibitory avoidance model.
- Guanfacine improved attention deficit in the 5-trial inhibitory avoidance model.
- Adrenergic α_{2A} receptor stimulation mediated the effects of both compounds.
- A selective noradrenergic neurotoxin did not affect the effects of both compounds.

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ABSTRACT

A 5-trial inhibitory avoidance test using spontaneously hypertensive rat (SHR) pups has been used as an animal model of attention deficit hyperactivity disorder (ADHD). However, the roles of noradrenergic systems, which are involved in the pathophysiology of ADHD, have not been investigated in this model. In the present study, the effects of adrenergic α_2 receptor stimulation, which has been an effective treatment for ADHD, on attention/cognition performance were investigated in this model. Moreover, neuronal mechanisms mediated through adrenergic α_2 receptors were investigated. We evaluated the effects of both clonidine, a non-selective adrenergic α_2 receptor agonist, and guanfacine, a selective adrenergic α_{2A} receptor agonist, using a 5-trial inhibitory avoidance test with SHR pups. Juvenile SHR exhibited a shorter transfer latency, compared with juvenile Wistar Kyoto (WKY) rats. Both clonidine and guanfacine significantly prolonged the transfer latency of juvenile SHR. The effects of clonidine and guanfacine were significantly blocked by pretreatment with an adrenergic α_{2A} receptor antagonist. In contrast, the effect of clonidine was not attenuated by pretreatment with an adrenergic α_{2B} receptor antagonist, or an adrenergic α_{2C} receptor antagonist, while it was attenuated by a non-selective adrenergic α_2 receptor antagonist. Furthermore, the effects of neither clonidine nor guanfacine were blocked by pretreatment with a selective noradrenergic neurotoxin. These results suggest that the stimulation of the adrenergic α_{2A} receptor improves the attention/cognition performance of juvenile SHR in the 5-trial inhibitory avoidance test and that postsynaptic, rather than presynaptic, adrenergic α_{2A} receptor is involved in this effect.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by hyperactivity, inattention and impulsivity that affects 8–12% of children [1]. Although the etiology and pathophysiology of ADHD are not fully known, dysfunctional dopaminergic and noradrenergic systems are thought to play key roles [2]. Although stimulants, such as methylphenidate and amphetamine, have been regarded as effective pharmacological treatments, there are several drawbacks to the current medications that are available, including the existence of treatment-refractory patients and side effects, such as the potential for abuse [3]. Thus, there is a need for alternatives to the current medications, and a suitable and pharmacologically validated animal model is necessary to achieve this goal.

Fox et al. [4] reported the 5-trial inhibitory avoidance test using juvenile spontaneously hypertensive rats (SHR), which have been shown to exhibit abnormalities of catecholaminergic

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neural activity [5] and several major ADHD-like behaviors such as impulsivity, hyperactivity, and poor sustained attention [6,7], as an animal model for ADHD. In this test, juvenile SHR exhibited a significantly impaired performance, compared with normotensive juvenile Wistar Kyoto (WKY) rats, upon repeated exposure to mild aversive stimuli, such as a short duration (1 s) and a weak current (0.1 mA); methylphenidate, a prescribed and effective treatment for ADHD, improved the impaired performance of juvenile SHR when examined using this test [4,8]. Thus, the 5-trial inhibitory avoidance test using juvenile SHR may represent impaired attention/cognition and impulsivity, which are similar to the symptoms observed in patients with ADHD, and this model may be useful for evaluating the effects of compounds on attention/cognition and impulsivity. However, pharmacological validation of the 5-trial inhibitory avoidance test has not been fully conducted, and the possible involvement of noradrenergic pathways, which have been proposed to have an important role in attention and impulsivity, in this model remains unknown.

Recently, adrenergic α_2 receptor stimulation has been reported to be an effective means of treating ADHD. Indeed, the efficacies of both clonidine, a non-selective adrenergic α_2 receptor agonist, and guanfacine, a selective adrenergic α_{2A} receptor agonist, for the treatment of ADHD, including impaired attention and impulsivity, have been demonstrated in clinical settings [9–12]. However, the roles of adrenergic α_2 receptors in the 5-trial inhibitory avoidance test have not been investigated. In addition, although the involvement of the α_{2A} receptor in attention/cognition and impulsivity has been well acknowledged [9,13], the roles of other adrenergic α_2 receptor subtypes, particularly α_{2B} and α_{2C} receptors, in the actions of clonidine have not been addressed. Therefore, in the present study, we evaluated the effects of clonidine and guanfacine in the 5-trial inhibitory avoidance test using juvenile SHR. Moreover, because α_2 receptors are expressed both presynaptically and postsynaptically, some of the neuronal mechanisms mediated through the stimulation of adrenergic α_2 receptors were investigated.

2. Methods

2.1. Animals

Male WKY rats and SHR (4 weeks old at time of the experiment) were purchased from Charles River (Yokohama, Japan). All the rats were housed in plastic cages under a 12-h light:12-h dark cycle (lights on at 07:00 h). The rats were also maintained under controlled temperature ($23 \pm 3\,^{\circ}C$) and humidity ($50 \pm 20\%$). Food and water were available ad libitum. All the experiments were conducted in accordance with the criteria of the Taisho Pharmaceutical Co., Ltd. Animal Care Committee and met the Japanese Experimental Animal Research association standards, as defined in the Guidelines for Animal Experiments.

2.2. 5-Trial inhibitory avoidance test

The 5-trial inhibitory avoidance test was performed according to a previously described method [4,8], with certain modifications. Rats were examined in a step-through type cage (Muromachi Kikai Co., Ltd., Tokyo, Japan), which consisted of a bright compartment (width: 250 mm, depth: 210 mm, height: 170 mm; light level: about 110 lx), a dark compartment (width: 250 mm, depth: 210 mm, height: 170 mm) and a grid floor. The two compartments were separated by a plastic door (width: 50 mm, height: 50 mm). A rat was placed in the bright compartment. Then, the transfer latency until entering the dark compartment was measured using a stopwatch. Once the rat had transferred to the dark compartment, the door was closed and an electric shock (0.1 mA, 1 s) was applied through the grid using a shock generator (Muromachi Kikai Co., Ltd., Tokyo, Japan). After the application of the foot-shock, the rat was returned to the home cage. The process was then repeated for five trials, with an inter-trial interval of 1 min. The apparatus was cleaned with 70% ethanol between trials. The cut-off times were 60 s for trial 1 and 180 s for the other trials. The rats that entered into the dark compartment within the cut-off time of 60 s in trial 1 were selected for trials 2–5. When the transfer latency was beyond the cut-off time in trials 2–5, 180 s was registered as the result. The measurements were conducted in a blinded fashion.

2.3. Measurement of sensitivity to foot-shock

This experiment was conducted to investigate whether or not methylphenidate, clonidine, and guanfacine exerted non-specific effects, such as a perception alteration of the foot-shock. This test was performed according to a previously described method [4], with certain modifications. A rat was placed into the dark compartment under a condition that allowed the door between the two compartments to remain closed. The inescapable foot-shock current was gradually ramped up from 0.05 mA to 0.4 mA, and then gradually back to 0.05 mA over a period of 30 s. iMax and iMin were defined as the first current when the rats vocalized and when the rats ceased vocalization, respectively.

2.4. Measurement of noradrenaline concentration in the prefrontal cortex in SHR

To confirm that the noradrenaline level is depleted by N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4) in the prefrontal cortex, the noradrenaline level was measured using an enzyme-linked immunosorbent assay (ELISA) (Noradrenaline Research ELISA™; Labor Diagnostika Nord GmbH and Co. KG, Nordhorn, Germany). Rats were sacrificed by decapitation, and the prefrontal cortex (cingulate cortex and infralimbic area in Fig. 8 and Fig. 9 in a rat brain atlas [14]) was dissected bilaterally from a 4-mm-thick coronal section of the anterior pole of the brain. The tissues were homogenized in 0.01 M hydrochloric acid containing 4 mM sodium metabisulphite and 1 mM ethylenediamine tetraacetic acid. After centrifugation ($17{,}600 \times g$, 10 min, $4\,^{\circ}C$), 5 μL of the supernatant was applied to an ELISA to measure the noradrenaline concentration.

2.5. Drugs and treatments

Clonidine hydrochloride, guanfacine hydrochloride, yohimbine hydrochloride, 2-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isoindole maleate (BRL44408), imiloxan hydrochloride, and DSP-4 hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methylphenidate hydrochloride was synthesized at Taisho Medicinal Research Laboratories. Acridin-9-yl-[4-(4-methylpiperazin-1-yl)-phenyl]amine dihydrochloride (JP-1302) was purchased from Abcam (Cambridge, UK). All drugs except for yohimbine were dissolved in saline. Yohimbine was dissolved in distilled water. Clonidine, methylphenidate and guanfacine were injected subcutaneously 30 min before the 5-trial inhibitory avoidance test and the measurement of the foot-shock response. Yohimbine and BRL44408 were injected intraperitoneally 15 and 20 min before the injection of clonidine or guanfacine, respectively. Imiloxan and JP-1302 were injected subcutaneously 20 and 60 min before the injection of clonidine, respectively. DSP-4 was injected intraperitoneally 3 days before the 5-trial inhibitory avoidance test and the measurement of the prefrontal cortical noradrenaline contents. The drug administrations, except for yohimbine and BRL44408, were performed in