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Conditioned place preference studies with atomoxetine in an animal model of ADHD: Effects of previous atomoxetine treatment

Ike C. dela Peña [a], Hyung Seok Ahn [a], Jong Hoon Ryu [b], Chan Young Shin [c], Il Ho Park [a], Jae Hoon Cheong [a,*]

[a] Uimyung Research Institute for Neuroscience, Sahmyook University, 26-21 Kongkreung-dong, Nowon-gu, Seoul 139-742, South Korea
[b] Department of Oriental Pharmaceutical Science, Kyunghee University, Dongdaemun-gu, Hoegidong, Seoul 130-701, South Korea
[c] Center for Geriatric Neuroscience Research, Institute of Biomedical Science and Technology, Konkuk University, Seoul 143-701, South Korea

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ABSTRACT

To investigate the putative rewarding effects of atomoxetine, a non-stimulant medication for Attention-deficit/hyperactivity disorder (ADHD), we conducted conditioned place preference (CPP) tests in an animal model of ADHD, the spontaneously hypertensive rat (SHR). The effects of drug pre-exposure were also evaluated, thus, parallel experiments were done in rats which have undergone 14 days of atomoxetine treatment. The responses of SHR were compared with the rat strain representing the "normal" heterogeneous population, the Wistar rats. Neither rat strain showed significant CPP to atomoxetine. However, previous atomoxetine treatment produced place preference responses in rats, more profoundly in Wistar rats conditioned with the low and moderate atomoxetine doses. In conclusion, acute exposure to atomoxetine does not have any rewarding effect, however, drug pretreatment produces responses characteristic of reward or psychological dependence, more specifically in the "normal" *vs*. the ADHD animal model. The present findings call for more studies with atomoxetine, especially those that investigate the effects of long-term or chronic drug treatment.

1. Introduction

Pharmacotherapy has been considered as the primary clinical approach for treating Attention-deficit/hyperactivity disorder (ADHD) (Heil et al., 2009), a neurodevelopmental disorder that affects 3–7% of school-aged children (Jasinski et al., 2008) and about 4% of adults (Faraone and Biederman, 2005). ADHD treatment has been dominated by the use of catecholaminergic stimulants (amphetamine and methylphenidate), drugs having significant abuse liability (Heil et al., 2009). Therefore, despite the reported clinical benefits of stimulant ADHD medications, many question the safety of these interventions, especially as ADHD has a high comorbidity with substance use disorder (Molina and Pelham, 2003; Wilens, 2000; Wilson and Levin, 2005). A review of literature stated that stimulant medications are misused or diverted not only by "healthy" individuals, but also by ADHD patients themselves (Wilens et al., 2008a). Evidence from preclinical and clinical studies also reported increased risk for substance use later in adulthood in subjects pre-exposed to stimulant medications (for reviews see Volkow, 2003; Volkow and Swanson, 2008).

Atomoxetine [(−)-*N*-methyl-gamma-(2-methylphenoxy)-1phenylpropylamine; LY139603; Stratera®] represents a class of ADHD drugs without any stimulant-like properties. Clinical trials have shown its efficacy in alleviating ADHD symptoms in children and adults (Simpson and Perry, 2003; Spencer et al., 1998). *In vivo* and *in vitro* studies have shown the potency and selectivity of atomoxetine as a norepinephrine reuptake inhibitor (Bolden-Watson and Richelson, 1993; Bymaster et al., 2002). Norepinephrine may also contribute to the pathophysiology of ADHD. Thus, drugs that could alter norepinephrine levels in the brain, especially in the prefrontal cortex, have important therapeutic implications (Arnsten, 2006).

Pharmacovigilance and a majority of preclinical studies indicate that atomoxetine is devoid of abuse liability (Gasior et al., 2005; Heil et al., 2002; Jasinski et al., 2008; Lile et al., 2006). There are some animal studies, however, which reported discordant findings (Sasaki et al., 1995; Spealman, 1995). Regardless, there are but a few studies that investigate the effects of long-term atomoxetine treatment. These kinds of investigations are needed as they may provide additional insights into the controversy. In the present study, we conducted conditioned place preference (CPP) tests, to delve into the potential rewarding effect of atomoxetine. We wanted to make our findings comparable to the clinical situation thus, experiments were conducted in an "appropriate" ADHD animal model, adolescent Spontaneously Hypertensive rats (SHR) (Sagvolden et al., 2009). The responses of the SHR were compared

* Corresponding author. Tel.: +82 23391605; fax: +82 23391617.
E-mail address: cheongjh@syu.ac.kr (J.H. Cheong).

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with the rat strain representing the "normal" heterogeneous population, the Wistar rats, as demonstrated in previous studies (dela Peña et al., 2010, 2011a, 2011b). Atomoxetine is clinically administered on a daily basis for treatment of ADHD. Therefore, we investigated the effects of repeated atomoxetine treatment in rats, to find out if repeated atomoxetine dosing could result to greater liking or "abuse" of the drug, as suggested by others (Jasinski et al., 2008).

2. Materials and methods

2.1. Subjects

Adolescent (postnatal day [PND] 28–45) (Spear and Brake, 1983) Wistar and SHR rats were obtained from Charles River Japan, via Orient Bio. Korea. They were housed in groups in a temperature— (22 ± 2 °C) and humidity— (55 ± 5%) controlled animal room on a 12 h/12 h light/dark (6 AM–6 PM) schedule. Food and water were available *ad libitum* except during behavioral testing. Animal treatment and maintenance were carried out in accordance with the Principles of Laboratory Animal Care (NIH publication no. 85–23 revised 1985) and the Animal Care and Use Guidelines of Sahmyook University, Korea.

2.2. Drugs

Atomoxetine hydrochloride (20 mg/kg; Eli Lilly and Co., Indianapolis, IN, USA) was suspended in physiologic saline (0.9% w/v of NaCl). Subsequent dilutions (5 and 1.25 mg/kg doses) were also made in physiologic saline. The atomoxetine doses used in the present study were similar with those in our previous work (dela Peña et al., 2011b) to facilitate comparisons on the effects of ADHD drugs in the "normal" *vs.* the ADHD animal model. Drugs were prepared a day before experiments, and given in a volume of 1 mg/kg body weight via oral administration (p.o.).

2.3. Apparatus

Two-compartment place preference apparatus made of polyvinylchloride were used in this study. Each of the compartments measures 47 × 47 × 47*H* cm and had distinctive visual and tactile cues. One compartment was black with smooth floor and the other had white-painted walls with rough, black floor. A guillotine door divided the apparatus and it served as a partition between compartments during the conditioning phase of the CPP test. Animal movement and behavior were video-recorded and analyzed using Ethovision (Noldus, Netherlands) system.

2.4. Procedure

Two kinds of experiments were performed in line with our objectives. Some experiments were conducted in drug-naïve rats that underwent CPP when they were about 4 weeks old. In other experiments, rats were treated with atomoxetine (1.25, 5 and 20 mg/kg) or saline (p.o.) in their home cages (for 14 days) before CPP tests. Drug administration started at the time when the rats were 3 weeks old so that CPP tests would be conducted while they are still in their peri-adolescence (PND 28-45) (Spear and Brake, 1983).

CPP tests were performed as outlined in our previous study (dela Peña et al., 2010), with some modifications. Each test consisted of three phases: habituation and preconditioning, conditioning and post-conditioning. During the first two days of the first phase (preconditioning), rats were allowed to explore both compartments of the CPP apparatus for 15 min. On the third day, the same method was followed except that the time spent in both sides of the box was measured using automated systems (Ethovision Noldus, Netherlands). After determining the rats' initially-preferred compartment, approximately half of the rats per group was assigned to the black compartment as the drug-paired side, while the other half to the other (dela Peña et al., 2010, 2011b; Meririnne et al., 2001). If their staying time was less than 200 s, they were excluded from further testing. This was done to eliminate different levels of bias across groups which might unknowingly confound our results. During the conditioning phase, animals were paired with atomoxetine (1.25, 5 or 20 mg/kg) or saline (control group) in their non-preferred compartment. On alternate days, they were given saline and confined to their preferred compartment for 30 min. The control group received saline every day. After 6 days of conditioning, rats were tested for changes in place preference (post-conditioning phase). As in the preconditioning phase, the staying time of each rat in the compartments of the CPP apparatus was recorded.

2.5. Data analysis

All results are presented as means and standard error of means (± S.E.M.). Place preference data were expressed as the difference in time spent in the atomoxetine- or saline- (for control group) paired compartment during the post- and preconditioning phases. Two-way ANOVA was used to identify strains or treatment effects, or interaction between the two factors. If significant effects were found in anyone of the factors, unpaired *t*-test was employed for further analysis. The accepted level of significance was set at $P<0.05$. All statistical analyses were conducted using GraphPad Prism Version 5 software (California, USA).

3. Results

Place preference was not expressed in rats conditioned with atomoxetine at the three dosages (Fig. 1). Specifically, two-way ANOVA showed similarity in responses between strains [$F(1, 52) = 0.14$, $P>0.05$] and the lack of rewarding effect of atomoxetine in all dosages [$F(3,52) = 0.07$, $P>0.05$]. In contrast, positive place preference responses were observed in some rat groups pre-treated and conditioned with atomoxetine (Fig. 2). Two-way ANOVA showed main strain [$F(1,48) = 4.64$, $P<0.05$] and treatment effects [$F(3,48) = 3.36$, $P<0.05$], with Wistar rats demonstrating more remarkable CPP to atomoxetine. Treatment and conditioning with atomoxetine at the dosages of 1.25 [$t(14) = 2.77$, $P<0.01$] and 5 mg/kg [$t(14) = 1.80$, $P<0.05$] produced significant place preference in Wistar rats. Treatment and conditioning with the lowest atomoxetine dose (1.25 mg/kg) [$t(10) = 2.07$, $P<0.05$] produced CPP in SHR.

4. Discussion

The abuse liability of atomoxetine, a non-stimulant medication for ADHD, was tested by examining if the drug produces rewarding

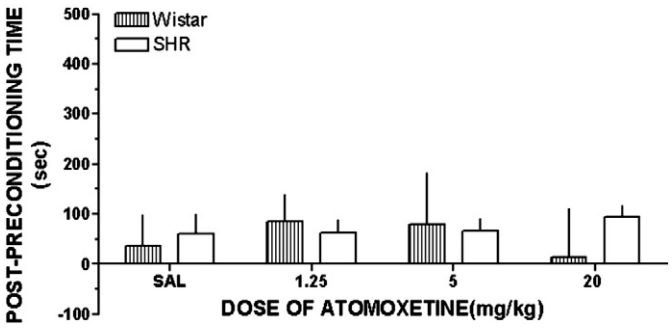

Fig. 1. Atomoxetine does not induce place preference response in Wistar and SHR. Each bar represents the means ± S.E.M. of the difference in the time spent in the atomoxetine- or saline- (for control group) paired side during the post- and preconditioning phases, grouped according to strain and treatment. ($n = 7$–8 animals per group).