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Effects of cabergoline and rotigotine on tacrine-induced tremulous jaw movements in rats

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ABSTRACT

Objectives: We examined the effects of two dopamine agonists, cabergoline and rotigotine, on tacrine-induced tremor and c-Fos expression in rats.
Methods: Rats received intraperitoneal injection of cabergoline (0.5, 1.0, or 5.0 mg/kg), rotigotine (1.0, 2.5, or 10.0 mg/kg), or vehicle 30 min before intraperitoneal injection of tacrine (5.0 mg/kg). The number of tremulous jaw movements (TJMs) after tacrine administration was counted for 5 min. Animals were sacrificed 2 h later under deep anesthesia, and the brain sections were immunostained in order to evaluate the c-Fos expression.
Results: Induction of TJMs by tacrine was dose-dependently reduced by pretreatment with cabergoline and rotigotine. The number of c-Fos-positive cells was significantly enhanced in the medial striatum, nucleus accumbens core, and nucleus accumbens shell after tacrine administration, and the enhanced expression of c-Fos in these three regions was significantly attenuated by cabergoline, while rotigotine suppressed c-Fos expression in two regions except the nucleus accumbens core.
Conclusions: These results suggest that tacrine-induced TJMs would be relieved by either cabergoline or rotigotine and that anticholinesterase-induced TJMs and the ameliorating effects of dopamine agonists would relate to neuronal activation in the striatum and nucleus accumbens.

1. Introduction

The symptoms of parkinsonism include several cardinal motor signs, such as bradykinesia, rigidity, postural instability and resting tremor. Tremors are clinically characterized as involuntary, rhythmic, and alternating movements in one or more body parts. Tremulous jaw movements (TJMs) are defined as rapid and repetitive deflections of the lower jaw in rodents that resemble chewing but are not directed at any particular stimulus (Salamone et al., 1998). TJMs are induced by intraperitoneal injection of dopamine antagonists (Glassman and Glassman, 1980), muscarinic agonist (Rupniak et al., 1983; Salamone et al., 1986), and acetylcholinesterase inhibitor, such as tacrine (Carriero et al., 1997; Mayorga et al., 1997). Thus, TJMs are induced by conditions that lead to parkinsonism in humans and have been validated as a valuable model of parkinsonian tremor (Salamone et al., 1998).

In comparison, muscarinic antagonists (Rupniak et al., 1983; Salamone et al., 1986), dopamine agonists (Cousins et al., 1997; Salamone et al., 2005), adenosine A_{2A} antagonist (Podurgiel et al., 2013; Salamone et al., 2008; Simola et al., 2004), and anti-epileptic drugs, such as zonisamide (Miwa et al., 2008), attenuate the induction of TJMs, indicating that the dopamine and acetylcholine systems are, at least in part, involved in the generation of TJMs. In the dopamine system, the stimulation of D_1 and/or D_2 receptors has been considered to be crucial to the suppression of TJMs (Cousins et al., 1997; Mayorga et al., 1999a; Salamone et al., 2005). In addition, the D_3 receptor has recently been focused on as a possible mediator of movement disorders (Joyce and Millan, 2007; Malik et al., 2004); however, the involvement of the D_3 receptor in the suppression of TJMs has been poorly examined. Cabergoline and rotigotine, dopamine agonists used clinically as antiparkinsonian drugs, have a higher affinity for the D_2 and D_3 receptors, respectively (Millan et al., 2002; Scheller et al., 2009). Comparing the effects of these dopamine agonists on TJMs would give a clue to revealing the role of dopamine receptor subtypes, including the D_3 receptor, in parkinsonian tremor. Thus, in this study, the effects of cabergoline and rotigotine on tacrine-induced TJMs were examined to estimate the underlying mechanisms of parkinsonian tremor.

Furthermore, to provide a cellular marker of neuronal activity following these pharmacological conditions, c-Fos immunoreactivity was examined in some brain areas that have been considered to be involved in the generation of TJMs. It has been believed that the striatum is a candidate associated with the generation of TJMs (Salamone et al., 1990,

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1998). Therefore, c-Fos expression in the striatum and other related areas was examined following intraperitoneal injection of tacrine, and the effects of cabergoline and rotigotine on tacrine-induced c-Fos expression were then evaluated.

2. Materials and methods

2.1. Subjects

Sixty-four adult male Sprague–Dawley rats (Charles River Laboratories, Japan), weighing 280–380 g, were housed in a temperature-controlled colony room on a 12:12 light:dark cycle with food and water freely available at the Experimental Animal Center of the University of Miyazaki. The experimental procedures used in the present study were approved by the Institutional Animal Care and Use Committee of the University of Miyazaki.

2.2. Drugs

Tacrine hydrochloride (Sigma Aldrich, Co., St. Louis, MO, USA) was dissolved in a vehicle of 0.9% saline solution. Cabergoline (Mitsubishi Tanabe Pharm. Co., Tokyo, Japan) and rotigotine (Sigma Aldrich, Co.) were suspended in a saline solution containing 0.25% methylcellulose (Sigma Aldrich, Co.). All drugs were injected intraperitoneally at a volume of 1.0 ml/kg.

2.3. Tacrine-induced TJMs after pretreatment with dopamine agonists, cabergoline, or rotigotine

Induction of TJMs was evaluated by counting the number of repetitive deflections of the lower jaw, and the number of TJMs was counted by an observer who used a mechanical hand counter and who was blind to the treatment. Just after injection of tacrine, rats were placed in a clear Plexiglas chamber (18 × 26 × 13 cm) with a wire mesh floor for 10 min as a habituation period, and TJMs were then observed for 5 min starting from 10 min after tacrine injection. Before the first test session, we conducted handlings for 5 min over 3 days and a habituation session for 15 min in an experimental chamber on the fourth day.

Different groups of rats were used to assess the effects of two dopamine agonists, cabergoline (n = 9) and rotigotine (n = 8), on the induction of TJMs in rats treated with tacrine. All rats performed one test session per week. During the test session, each rat received an intraperitoneal injection of cabergoline or rotigotine. All rats in the cabergoline group underwent the behavioral test with four doses of cabergoline (0, 0.5, 1.0 or 5.0 mg/kg). All rats in the rotigotine group underwent the behavioral test with four doses of rotigotine (0, 1.0, 2.5 or 10.0 mg/kg). One specific dose was used in one session, and the order of treated doses was pseudo-counterbalanced. Rats were returned to their home cage for 30 min after injection of each treatment. Then, to induce TJMs, rats received an intraperitoneal injection of 5.0 mg/kg tacrine.

2.4. c-Fos expression after administration of tacrine and/or dopamine agonists

The expression of c-Fos was evaluated in the medial striatum (MS), dorsal striatum (DS), ventrolateral striatum (VLS), nucleus accumbens core (AcbC), nucleus accumbens shell (AcbSh), globus pallidus (GP), entopeduncular nucleus (EP), subthalamic nucleus (STN), substantia nigra pars reticulata (SNr), ventrolateral nucleus of thalamus, and trigeminal motor nucleus. A total of 47 rats were randomly divided into the following six groups (n = 7–9/group): vehicle + saline, vehicle + tacrine (5.0 mg/kg), cabergoline (5.0 mg/kg) + saline, cabergoline (5.0 mg/kg) + tacrine (5.0 mg/kg), rotigotine (10.0 mg/kg) + saline, and rotigotine (10.0 mg/kg) + tacrine (5.0 mg/kg). After handlings for 5 min over 3 days, each of the dopamine agonists, cabergoline and rotigotine, or vehicle was intraperitoneally administered, and then, tacrine or saline was intraperitoneally injected 30 min later. Ninety minutes later, rats were deeply anesthetized with an overdose of sodium pentobarbital and perfused transcardially with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). The brains were removed and postfixed in the same fixative. After fixation, the samples were immersed in 10% sucrose in 0.1 M PB for 1 h and then in 30% sucrose in the same buffer for 2 days at 4 °C. Frozen coronal serial sections, 50 μm in thickness, were prepared on a freezing microtome. Although a pilot study showed that c-Fos immunoreactivity was inconspicuous in the GP, EP, STN, SNr, ventrolateral nucleus of thalamus, and trigeminal motor nucleus of all treatment groups, we made sections for those areas from four perfused brains per group. They kept in phosphate-buffered saline (PBS; pH 7.4), and processed for immunohistochemical staining for the c-Fos protein as free-floating sections. All sections were incubated in 0.1% hydrogen peroxide and 0.5% Triton X-100. After incubation in 10% normal goat serum (Histofine, SAB-PO(R) kit; Nichirei, Tokyo, Japan) for 20 min, the sections were reacted with a polyclonal rabbit anti-c-Fos antibody (sc-52; 1:5000; Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4 °C. The reaction products of biotinylated goat anti-rabbit antiserum and avidin-conjugated horseradish peroxidase (Histofine, SAB-PO(R) kit) were visualized by using 0.005% diaminobenzidine tetrahydrochloride (DAB; Sigma Aldrich, Co.) and 0.0003% hydrogen peroxide and were intensified by pretreatment with 0.25% cobalt chloride. The sections were mounted on gelatin-coated slide glasses, air-dried, dehydrated with alcohol and xylene, and coverslipped. Preabsorption of antibodies with the corresponding synthetic peptides or omission of the antibody from the protocol abolished staining.

The immunoreactivity of c-Fos was analyzed by light microscopy. Four sections from each structure of the brain were taken at the same level along the antero-posterior axis to avoid variance in the level of the structure in animals. The expression of c-Fos in each section was quantified by counting the number of c-Fos-immunoreactive cells in 0.25 × 0.25 mm squares by using a 20 × microscopic objective. The number of c-Fos-positive cells was counted bilaterally and averaged per structure. An observer who was blind to the treatment condition counted cells positive for c-Fos.

2.5. Statistical analysis

The behavioral and immunohistochemical data were analyzed by using a one-way analysis of variance (ANOVA) with repeated measures and a two-way ANOVA, respectively. An analysis of simple main effect and multiple comparisons were conducted if needed. Shaffer's modified sequentially rejective Bonferroni procedures and adjusted p-values were used for multiple comparisons. All p-values less than 0.05 were considered to indicate significance.

3. Results

3.1. Effects of dopamine agonists on tacrine-induced TJMs

Pretreatment with cabergoline produced a dose-dependent reduction of tacrine-induced TJMs. A one-way ANOVA with repeated measures carried out on the number of TJMs yielded a significant reduction [$F(3,24) = 5.19$, $p < 0.01$], and a post-hoc multiple comparison test revealed that the number of TJMs in a 5.0 mg/kg cabergoline treatment was significantly lower than that in a tacrine alone treatment ($p < 0.05$ for vehicle, Fig. 1, left).

Similarly, tacrine-induced TJMs were dose-dependently reduced by pretreatment with rotigotine. A one-way ANOVA with repeated measures carried out on the number of TJMs yielded a significant reduction [$F(3,21) = 5.59$, $p < 0.01$], and a post-hoc multiple comparison test