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The novel adenosine A_{2A} antagonist Lu AA47070 reverses the motor and motivational effects produced by dopamine D2 receptor blockade

Lyndsey E. Collins [a,1], Thomas N. Sager [b], Anette G. Sams [c], Adam Pennarola [a], Russell G. Port [a], Mona Shahriari [a], John D. Salamone [a,*]

[a] Dept. of Psychology, University of Connecticut, Storrs, CT 06269-1020, USA
[b] Neuroscience Drug Discovery, Divison of Neurodegeneration, H. Lundbeck A/S, Ottiliavej 9, Valby 2500, Denmarkharmacology Target Research, H. Lundbeck A/S, 9 Ottiliavej, Valby 2500, Denmark
[c] Neuroscience Drug Discovery, Discovery Chemistry & DMPK, H. Lundbeck A/S, Ottiliavej 9, Valby 2500, Denmark

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ABSTRACT

Dopamine D2 and adenosine A_{2A} receptors interact to regulate aspects of motor and motivational function, and it has been suggested that adenosine A_{2A} antagonists could be useful for the treatment of parkinsonism and depression. The present experiments were performed to characterize the effects of Lu AA47070, which is a phosphonooxymethylene prodrug of a potent and selective adenosine A_{2A} receptor antagonist, for its ability to reverse the motor and motivational effects of D2 antagonism. In the first group of studies, Lu AA47070 (3.75–30 mg/kg IP) was assessed for its ability to reverse the effects of the D2 receptor antagonist pimozide (1.0 mg/kg IP) using several measures of motor impairment, including catalepsy, locomotion, and tremulous jaw movements, which is a rodent model of parkinsonian tremor. Lu AA47070 produced a significant reversal of the effects of pimozide on all three measures of parkinsonian motor impairment. In addition, Lu AA47070 was able to reverse the effects of a low dose of the D2 antagonist haloperidol on a concurrent lever pressing/chow feeding task that is used as a measure of effort-related choice behavior. The ability of Lu AA47070 to reverse the effects of D2 receptor blockade suggests that this compound could have potential utility as a treatment for parkinsonism, and for some of the motivational symptoms of depression.

1. Introduction

Within the last few years, increasing evidence has demonstrated an important role for adenosine in modulating the functional circuitry of the basal ganglia (Mally and Stone, 1996, 1998; Ferré et al., 1997, 2001, 2004; Svenningsson et al., 1999; Stromberg et al., 2000; Hauber et al., 2001; Morelli and Pinna, 2002; Bara-Jimenez et al., 2003; Simola et al., 2006; Salamone et al., 2008b). Several subtypes of adenosine receptors are involved in striatal function, and anatomical studies have shown that the adenosine A_{2A} receptor subtype has a very high degree of expression within both the neostriatum and the nucleus accumbens (Svenningsson et al., 1999; Wang et al., 2000; Chen et al., 2001). In striatal areas, adenosine A_{2A} receptors are present in very high densities on both ventral and dorsal striatopallidal neurons, which also tend to co-express DA D2 receptors and enkephalin (Schiffman et al., 1991; Fink et al., 1992; Rosin et al., 1998; Svenningsson et al., 1999; Hillion et al., 2002; Fuxe et al., 2007; Mingote et al., 2008; Vontell et al., 2010). Adenosine A_{2A} and DA D2 receptors are thought to form heterodimers, and it has been suggested that they also converge onto the same cAMP-related signal transduction pathways (Ferré et al., 1997; Svenningsson et al., 1999; Ferré et al., 2008). This combination of anatomical and neurochemical findings has led to the suggestion that adenosine A_{2A} receptor antagonists could be useful as antiparkinsonian drugs (Ferré et al., 1997; Svenningsson et al., 1999; Morelli and Pinna, 2002; Salamone, 2010a).

Several studies with animal models have demonstrated that antagonism of adenosine A_{2A} receptors can produce motor effects that are consistent with antiparkinsonian actions (Aoyama et al., 2000; Ferré et al., 2001; Morelli and Pinna, 2002; Schwarzschild et al., 2002; Correa et al., 2004; Simola et al., 2004; Pinna et al., 2005; Ishiwari et al., 2007; Tronci et al., 2007; LeWitt et al., 2008; Salamone et al., 2008a,b; Betz et al., 2009; Collins et al., 2010a,b, 2011). Adenosine A_{2A} antagonists have been shown to reverse the locomotor suppression, catalepsy, and muscle rigidity that are induced by interference with striatal DA transmission (Shiozaki et al., 1999; Hauber et al., 2001; Wardas et al., 2001; Correa et al., 2004; Ishiwari et al., 2007; Salamone et al., 2008a,b; Trevitt et al., 2009a). The adenosine A_{2A} antagonists MSX-3 and KF 17837 significantly reversed the locomotor suppression induced by DA D1 and D2 receptor antagonism (Correa

* Corresponding author at: Division of Behavioral Neuroscience, Dept. of Psychology, University of Connecticut, Storrs, CT, 06269-1020, USA.
E-mail address: john.salamone@uconn.edu (J.D. Salamone).
[1] Present Address: Cognitive Neuroscience Division, Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University College of Physicians and Surgeons, New York, NY.

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et al., 2004; Ishiwari et al., 2007; Collins et al., 2010a), and adenosine A_{2A} receptor knockout mice showed reduced levels of haloperidol-induced catalepsy compared to wild type mice (Chen et al., 2001). Furthermore, adenosine A_{2A} antagonists have been studied for their antitremor effects in rodent models. Tremulous jaw movements induced by DA antagonism, DA depletion and cholinomimetic drugs are a well characterized animal model of drug-induced tremor (Salamone et al., 1990, 1998, 2001, 2005a, 2008a,b; Salamone and Baskin, 1996; Cousins et al., 1998; Rodriguez Diaz et al., 2001; Cenci et al., 2002; Simola et al., 2004, 2006; Ishiwari et al., 2005; Miwa, 2007; Miwa et al., 2008, 2009; Vanover et al., 2008; Betz et al., 2009; Collins et al., 2010b, 2011). These oral movements in rats have many of the characteristics of parkinsonian tremor (Cousins et al., 1997; Salamone et al., 1998, 2005a,b, 2008a,b; Collins et al., 2010b, 2011), and several studies have shown that adenosine A_{2A} antagonists can substantially attenuate drug-induced tremulous jaw movements (Correa et al., 2004; Simola et al., 2004, 2006; Tronci et al., 2007; Salamone et al., 2008a; Collins et al., 2010b, 2011). Data from human clinical studies also have supported the hypothesis that adenosine A_{2A} antagonists could be useful as antiparkinsonian agents (Bara-Jimenez et al., 2003; Hauser et al., 2003, 2008; Jenner, 2005; LeWitt et al., 2008; Stacy et al., 2008; Gillespie et al., 2009; Pinna, 2009; Factor et al., 2010; Fernandez et al., 2010; Knebel et al., 2010; Mizuno et al., 2010; Salamone, 2010b).

In addition to studies related to parkinsonian motor dysfunctions, researchers have begun to characterize the effects of A_{2A} receptor agonists and antagonists on aspects of cognition (Takahashi et al., 2008) and motivation (O'Neill and Brown, 2006; Font et al., 2008; Mingote et al., 2008). Behavioral activation and expenditure of effort are fundamental aspects of motivation (Salamone, 1988, 1992, 2010a; Salamone and Correa, 2002) and considerable evidence indicates that nucleus accumbens DA and adenosine interact in regulating these functions (Salamone and Correa, 2009; Salamone et al., 2005b, 2009a,b, 2010). Studies of effort-related choice behavior typically allow animals to select between options that vary in terms of the work requirement of the instrumental actions and the value of the reinforcers that can be obtained. Research on effort-related choice has involved the use of a T-maze barrier task (Salamone et al., 1994; Cousins et al., 1996; Walton et al., 2002, 2003; Denk et al., 2005; Floresco and Ghods-Sharifi, 2007; Bardgett et al., 2009; Correa et al., 2009), or effort discounting procedures (Floresco et al., 2008; Bardgett et al., 2009). Additional experiments have employed a concurrent fixed ratio 5 (FR5)/chow feeding procedure (Salamone et al., 1991, 2002, 2003, 2007). With this task, rats can select either lever pressing on an FR5 schedule for a highly preferred food (i.e., high carbohydrate precision pellets) or approaching and consuming a freely available but less preferred food (rodent chow). Trained rats eat little of the freely available lab chow, and instead spend most of their time lever pressing for the preferred food. Low doses of D1 or D2 family antagonists, or intra-accumbens injections of D1 or D2 antagonists, suppress food-reinforced lever pressing, but substantially elevate chow intake (Salamone et al., 1991, 1996, 2002; Cousins et al., 1994; Koch et al., 2000; Nowend et al., 2001; Sink et al., 2008; Farrar et al., 2010). Recent evidence indicates that drugs acting on adenosine A_{2A} receptors also affect behavioral activation and effort-related processes. Microinjections of the adenosine A_{2A} agonist CGS 21680 into the nucleus accumbens produced effects on instrumental behavior and effort-related choice that resembled those produced by accumbens DA depletions or antagonism (Font et al., 2008; Mingote et al., 2008). Several studies have shown that adenosine A_{2A} antagonists such as MSX-3 and istradefylline (KW-6002) are capable of reversing the effort-related effects of the DA D2 antagonists haloperidol and eticlopride in rats tested on the concurrent FR5/feeding choice procedure (Farrar et al., 2007, 2010; Salamone et al., 2009a,b; Worden et al., 2009; Nunes et al., 2010), and on the T-maze barrier choice task (Mott et al., 2009; Pardo et al., 2010). It has been suggested that the DA/adenosine interaction that is evident in animal studies of behavioral activation and effort-based choice may also be related to human clinical symptoms such as anergia, psychomotor retardation, and fatigue in depression and other disorders (Salamone et al., 2006, 2007, 2009a,b, 2010; Treadway and Zald, 2011). This hypothesis is consistent with reports indicating that adenosine A_{2A} antagonists are effective in animal models of depression (El Yacoubi et al., 2003; Hodgson et al., 2009; Hanff et al., 2010).

In view of the preclinical and clinical data gathered thus far, the development and testing of novel adenosine A_{2A} antagonists is becoming an important research priority. The present studies represent an assessment of a novel adenosine A_{2A} antagonist prodrug, Lu AA47070, which is a phosphonooxymethylene prodrug of a potent and selective adenosine A_{2A} receptor antagonist (4-(3,3-dimethyl-butyrylamino)-3,5-difluoro-*N*-thiazol-2-yl-benzamide; Sams et al., 2011). The parent compound is a competitive antagonist of adenosine A_{2A} receptors, with a K_i of 5.9 nM, and a relatively high binding selectivity for A_{2A} receptors relative to A_1 receptors (69-fold), A_{2B} receptors (45-fold), and A_3 receptors (>1000-fold; Sams et al., 2011). The first three experiments studied the ability of Lu AA47070 to reverse the effects of the DA D2 antagonist pimozide on tremulous jaw movements, locomotor suppression, and catalepsy, under the same conditions used previously for the assessment of the adenosine A_{2A} antagonists MSX-3 and istradefylline (Salamone et al., 2008a). These conditions (i.e., repeated administration of 1.0 mg/kg pimozide) are optimized for induction of tremulous jaw movements, but also allow for assessment of locomotion and catalepsy. The fourth experiment assessed the effects of Lu AA47070 on the alterations in effort-related choice behavior induced by a low dose of the DA antagonist haloperidol in rats responding on the concurrent FR5 lever pressing/chow feeding task. It was hypothesized that Lu AA47070 would reverse the behavioral effects of D2 receptor antagonism across these different procedures.

2. Materials and methods

2.1. Animals

A total of 106 adult male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) with no prior drug experience were used in the present experiments. The rats weighed 350–450 g during the course of the experiment and had ad libitum access to lab chow and water. They were group-housed in a colony that was maintained at approximately 23 °C and had a 12-hour light/dark cycle (lights on at 0700 h). These studies were conducted according to University of Connecticut and NIH guidelines for animal care and use.

2.2. Drug treatment procedures and dose selection

Pimozide and haloperidol were purchased from Sigma Aldrich Chemical (St. Louis, MO), and were in a 0.3% tartaric acid solution (final pH = 4.0), and the tartaric acid solution also was used as the vehicle control for the haloperidol injections. Lu AA47070 (phosphoric acid mono-{2-[(*E*/*Z*)-4-(3,3-dimethyl-butyrylamino)-3,5-difluoro-benzoylimino]thiazol-3-ylmethyl} ester) is a water soluble prodrug of 4-(3,3-dimethyl-butyrylamino)-3,5-difluoro-*N*-thiazol-2-yl-benzamide. Lu AA47070 was obtained from H. Lundbeck A/S (Copenhagen, Denmark) and was dissolved in a 4.0% NaOH solution, which was also used as the vehicle control. The pH of the Lu AA47070 solution was adjusted by adding 1.0 N NaOH until the drug was completely in solution (pH = 7.4). For the studies of tremulous jaw movements, locomotion and catalepsy, the subchronic 1.0 mg/kg (IP) pimozide treatment procedure that was shown to induce tremulous jaw movements in the present studies was based upon previously published experiments showing induction of jaw movements at this dose (Ishiwari et al., 2005; Betz et al., 2007, 2009; Salamone et al., 2008a; Collins et al., 2010b). These methods are optimized for the production

of jaw movement activity, but also allow for the parallel assessment of locomotion and catalepsy. The procedure of screening animals by assessing them for tremulous jaw movements the day before the drug challenge day was the same as that used in previous studies (Ishiwari et al., 2005; Salamone et al., 2008a; Collins et al., 2010b). This was done in order to ensure a robust jaw movement response on the drug challenge day. Only a small percentage of animals (i.e., <5%) failed to show a substantial jaw movement response to pimozide (i.e., <15 tremulous jaw movements) on day 7. The doses of Lu AA47070 chosen were based upon extensive pilot work and were comparable to doses of adenosine A_{2A} antagonists utilized in other tremulous jaw experiments (Simola et al., 2004; Tronci et al., 2007; Salamone et al., 2008a,b; Betz et al., 2009; Collins et al., 2010b). For the operant conditioning experiments, the dose of haloperidol (0.1 mg/kg IP) was selected based upon previous studies (Salamone et al., 1991, 1996, 2009a,b; Farrar et al., 2007). Although higher doses of haloperidol can suppress food intake, this 0.1 mg/kg dose did not suppress intake of chow or operant pellets, and did not alter preference between them (Salamone et al., 1991).

2.3. Behavioral procedures

2.3.1. Tremulous jaw movements

Observations of rats took place in a 30×30×30 cm clear Plexiglas chamber with a wire mesh floor, which was elevated 42 cm from the table top. This allowed for the viewing of the animal from several angles, including underneath. Tremulous jaw movements were defined as rapid vertical deflections of the lower jaw that resembled chewing but were not directed at any particular stimulus (Salamone et al., 1998). Each individual deflection of the jaw was recorded using a mechanical hand counter by a trained observer, who was blind to the experimental condition of the rat being observed. Separate studies with two observers demonstrated an inter-rater reliability of $r = 0.97$ ($p < 0.01$) using these methods.

2.3.2. Catalepsy

Catalepsy was tested by placing both forelimbs of the rat onto a stationary horizontal metal bar, raised 12.5 cm above a wooden platform. The rat was then allowed to stabilize itself with its hindpaws resting on the platform. Latency for the animal to cease having both forelimbs on the metal bar was timed. Three trials were conducted, and the latencies for each trial were averaged. A maximum of 2 min on the catalepsy bar was allowed for each trial. In a previous paper using these exact methods, 1.0 mg/kg pimozide on day 8 produced a mean of 22.7 ± 2.4 s of catalepsy, while vehicle injection produced a mean of 1.7 ± 1.2 s (Salamone et al., 2008a).

2.3.3. Locomotor activity

Locomotor activity was assessed by placing the rat into an automated activity chamber (28 cm×28 cm×28 cm) enclosed in a sound-attenuating shell. The floor of the chamber was elevated 6 cm above the chamber bottom and was composed of two moveable wire-mesh panels, (25 cm×12 cm), which were further divided into four quadrants by means of a central metal rod between the two panels. As the rat entered each quadrant, a slight vertical movement of the mesh panels closed a microswitch located outside of the locomotion chamber. This depression was detected and recorded by a computer program, written in MedPC, as a single activity count (Med Associates, Inc., Georgia, VT). The locomotor activity session was 10-min in length. These methods of measuring locomotion have been used previously to assess the effects of DA and adenosine antagonists on locomotion (Collins et al., 2010a; Salamone et al., 2008a).

2.3.4. Operant concurrent FR5/chow feeding task

Operant behavior sessions were conducted in lever pressing chambers (28×23×23 cm; Med Associates). Rats were initially trained to lever press on a continuous reinforcement schedule (30-min sessions; 45-mg pellets, Bioserve, Frenchtown, NJ, were used for all operant behavior tests) and then were trained on the FR5 schedule (30-min sessions, 5 days/week) for several additional weeks. Rats were then tested on the concurrent FR5/chow-feeding procedure. With this task, weighed amounts of lab chow (Lab Diet, 5P00 Prolab RMH 3000, Purina Mills, St. Louis, MO; typically 15–20 g, three large pieces) were concurrently available on the floor of the chamber during the FR5 sessions. At the end of the session, rats were immediately removed from the chamber, and food intake was determined by weighing the remaining food (including spillage). Rats were trained until they attained stable baseline response rates (i.e., consistent responding over 1200 lever presses per 30 min), after which drug testing began. For most baseline days, rats did not receive supplemental feeding; however, over weekends and after drug tests, rats typically received supplemental chow in the home cage. On baseline and drug treatment days, rats normally consumed all the operant pellets that were delivered during each session.

2.4. Experiments

2.4.1. Experiment 1: ability of Lu AA47070 to reverse the tremulous jaw movements induced by subchronic administration of the DA D2 antagonist pimozide

A group of 81 rats (n = 11–20/group) was used to assess the effects of subchronic systemic injections of the DA D2 antagonist pimozide on tremulous jaw movements. All rats received an injection of 1.0 mg/kg pimozide IP for 8 consecutive days. On day 7 of the subchronic injections, rats were assessed for the induction of tremulous jaw movements in a 5 min period. Any rat that showed less than 15 tremulous jaw movements on day 7 was excluded from further testing. On day 8, 3 h and 30 min after the daily pimozide injection, each animal randomly received an injection of either Lu AA47070 or vehicle control in one of the following doses (vehicle control; 3.75 mg/kg; 7.5 mg/kg; 15 mg/kg; 30 mg/kg Lu AA47070). Twenty minutes later, animals were placed in the Plexiglas observation chamber and allowed to habituate for 10 min. Immediately following habituation, the number of jaw movements in a 5-min observation period was assessed as described above.

2.4.2. Experiment 2: ability of Lu AA47070 to reverse the catalepsy induced by subchronic administration of the D2 antagonist pimozide

Immediately following the tremulous jaw movement assessment carried out in experiment 1, rats were tested for the induction of catalepsy, as described above.

2.4.3. Experiment 3: ability of Lu AA47070 to reverse the locomotor suppression induced by subchronic administration of the D2 antagonist pimozide

After completion of the catalepsy testing in experiment 2, locomotor activity was assessed in a 10-min session, using the procedure outlined above.

2.4.4. Experiment 4: effect of Lu AA47070 on haloperidol-induced changes in effort-related choice behavior

Following the initial training with the concurrent FR5/chow feeding procedure described above, rats (n = 14) were tested after receiving combined drug treatments. For this experiment, the following treatments were used: tartaric acid vehicle (50 min before testing) plus saline vehicle IP (30 min before testing), 0.1 mg/kg haloperidol IP (50 min before testing) plus saline vehicle IP (30 min before testing), and 0.1 mg/kg haloperidol IP (50 min before testing) plus various doses of Lu AA47070 injected IP (0.5, 1.5, 5.0 and 15.0 mg/kg; 30 min before testing). Behavioral measures included number of lever presses and amount of chow consumed. Treatments were given once per week, in a randomly varied order. Although 1.0 mg/kg pimozide was used to induce tremulous jaw movements, 0.1 mg/kg haloperidol was used for the operant studies because this is a standard drug condition

in our laboratory that has been worked out in detail in previous operant studies with MSX-3 and istradefylline (Farrar et al., 2007; Salamone et al., 2009a,b).

2.5. Data analyses

Behavioral data for experiments 1–3 were analyzed using a between groups analysis of variance (ANOVA). For the operant behavior experiment, data were analyzed by repeated measures ANOVA. A computerized statistical program (SPSS 10.1 for Windows) was used to perform all analyses. When there was a significant ANOVA, planned comparisons using the overall error term were used to assess the differences between each dose and the control condition; the total number of comparisons was restricted to the number of treatments minus one (Keppel, 1991).

3. Results

3.1. Experiments 1–3: ability of Lu AA47070 to reverse the tremulous jaw movements, catalepsy, and locomotor suppression induced by subchronic administration of the D2 antagonist pimozide

Fig. 1A shows the effects of systemic injections of Lu AA47070 on tremulous jaw movements. Co-administration of Lu AA47070 significantly reversed the tremulous jaw movements induced by the DA D2 antagonist pimozide ($F(4,76) = 3.259$; $p < 0.05$). Planned comparisons revealed that the 30.0 mg/kg dose of Lu AA47070 differed significantly from vehicle control ($p = 0.001$). The effects of Lu AA47070 on catalepsy are shown in Fig. 1B. The novel adenosine antagonist prodrug Lu AA47070 was able to significantly reverse pimozide-induced catalepsy ($F(4,76) = 4.933$; $p = 0.001$). Planned comparisons demonstrated that all four of the doses of Lu AA47070 (3.75 mg/kg, 7.5 mg/kg, 15.0 mg/kg, and 30.0 mg/kg) were able to significantly reverse catalepsy when compared to vehicle control. Fig. 1C depicts the effects of Lu AA47070 administration on DA D2 antagonist-induced locomotor suppression. Lu AA47070 was able to significantly increase locomotion in pimozide-treated rats ($F(4,76) = 10.470$; $p < 0.001$). Planned comparisons showed that the 7.5 mg/kg, 15.0 mg/kg, and 30.0 mg/kg doses of Lu AA47070 all increased locomotion compared to the pimozide plus vehicle control.

3.2. Experiment 4: reversal of the effects of DA antagonism on effort-related choice behavior with co-administration of Lu AA47070

The results of the fourth experiment are shown in Fig. 2. There was an overall significant effect of drug treatment on lever pressing (Fig. 2A top; $F(5,65) = 54.8$, $p < 0.001$). Planned comparisons showed that haloperidol produced a significant reduction in lever pressing compared to vehicle control ($p < 0.05$). In addition, co-administration of Lu AA47070 with haloperidol produced a significant increase in lever pressing compared to haloperidol plus vehicle, with the 1.5, 5.0 and 15.0 mg/kg doses of Lu AA47070 producing significant differences relative to haloperidol plus vehicle (planned comparisons; $p < 0.05$). There also was an overall significant effect of drug treatment on chow intake (Fig. 2B bottom; $F(5,65) = 32.3$, $p < 0.001$). Planned comparisons indicated that haloperidol produced a significant increase in chow intake compared to vehicle control ($p < 0.05$). Planned comparisons revealed that co-administration of Lu AA47070 with haloperidol produced a significant decrease in chow intake relative to haloperidol plus vehicle, with the 5.0 and 15.0 mg/kg doses of Lu AA47070 being significantly different from haloperidol plus vehicle ($p < 0.05$). For both lever pressing and chow intake, post-hoc analysis with the Tukey test ($\alpha = 0.05$) indicated that the haloperidol plus 15.0 mg/kg Lu AA47070 condition did not significantly differ from the vehicle plus vehicle condition.

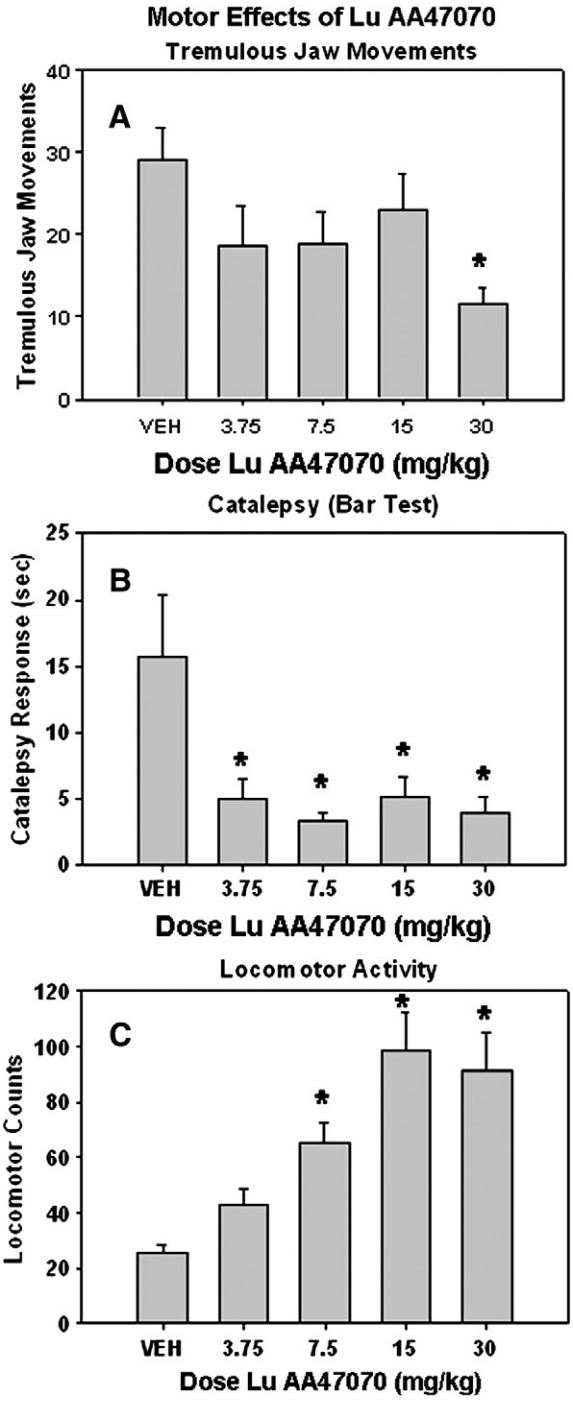

Fig. 1. Effect of Lu AA47070 on pimozide-induced tremulous jaw movements, catalepsy, and suppression of locomotion. A. Mean (±SEM) number of individual jaw movements (per 5 min observation period) after injection of tartaric acid vehicle plus1.0 mg/kg pimozide or pimozide plus various doses of Lu AA47070. B. Mean (±SEM) catalepsy response (in seconds) after injection of tartaric acid vehicle plus1.0 mg/kg pimozide or pimozide plus various doses of Lu AA47070. C. Mean (±SEM) number of locomotor counts after injection of tartaric acid vehicle plus 1.0 mg/kg pimozide or pimozide plus various doses of Lu AA47070. *$p < 0.05$, different from vehicle plus pimozide.

4. Discussion

The present experiments were conducted to determine if the novel adenosine A_{2A} antagonist prodrug Lu AA47070 could show a behavioral profile similar to other adenosine A_{2A} antagonists on tests related to motor function and effort-based choice behavior. In the first group of experiments, rats were assessed using a

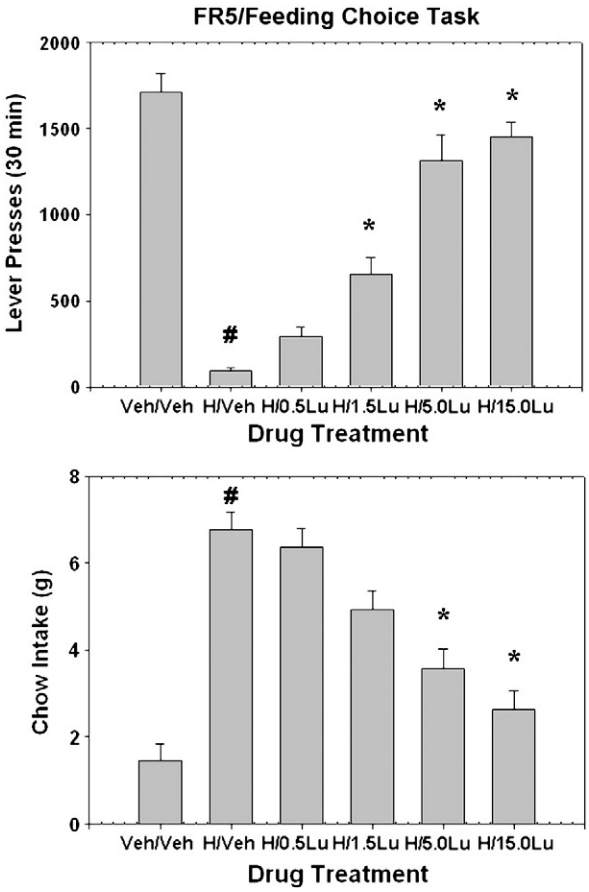

Fig. 2. The effects of Lu AA47070 on haloperidol-induced changes in performance on the concurrent lever pressing/chow feeding choice procedure. Rats (n = 14) were treated with tartaric acid vehicle plus saline vehicle (Veh/Veh), 0.1 mg/kg haloperidol plus vehicle (H/Veh), and 0.1 mg/kg haloperidol plus various doses of haloperidol plus Lu AA47070 (H/Lu). Top. Mean (±SEM) number of lever presses (FR5 schedule) during the 30 min session. Bottom. Mean (±SEM) gram quantity of chow intake. #$p<0.05$, significantly different from the Veh/Veh control; *$p<0.05$ significantly different from haloperidol plus vehicle.

neurological battery consisting of three measures of motor function that are thought to be related to parkinsonism: tremulous jaw movements, catalepsy, and locomotion (Salamone et al., 2008a). The same behavioral procedures were used in the Salamone et al. (2008a) paper, and in that study, it was shown that subchronic administration of 1.0 mg/kg of the DA D2 antagonist pimozide induced tremulous jaw movements and catalepsy, and reduced locomotion, relative to vehicle-treated rats. In the present study, co-administration of Lu AA47070 reversed the motor impairments induced by subchronic administration of 1.0 mg/kg pimozide. All four doses of Lu AA47070 tested (3.75 mg/kg, 7.5 mg/kg, 15.0 mg/kg, and 30.0 mg/kg) were capable of reducing pimozide-induced catalepsy. Three of the four doses of Lu AA47070 injected (7.5 mg/kg, 15 mg/kg, and 30 mg/kg) stimulated locomotion in pimozide-treated rats. Furthermore, the highest dose of Lu AA47070 (30.0 mg/kg) also attenuated the tremulous jaw movements induced by subchronic pimozide administration. The ability of Lu AA47070 to reverse parkinsonian motor impairments induced by DA antagonism is consistent with the growing body of literature suggesting that adenosine A_{2A} antagonists are capable of attenuating some of the motor dysfunctions induced by interference with DA transmission, such as reduced locomotion (Shiozaki et al., 1999; Aoyama et al., 2000; Correa et al., 2004; Ishiwari et al., 2007; Collins et al., 2010a), rigidity (Wardas et al., 2001), catalepsy (Hauber, 1998; Hauber et al., 2001; Kanda et al., 1994; Salamone et al., 2008a), and tremulous jaw movements (Correa et al., 2004; Tronci et al., 2007; Salamone et al., 2008a,b; Trevitt et al., 2009b; Collins et al., 2010b, 2011). Moreover, the present results with Lu AA47070 are consistent with a previous report (Salamone et al., 2008a) demonstrating that the A_{2A} antagonists MSX-3 and istradefylline attenuated the motor impairments induced by pimozide in rats assessed using the same neurological battery utilized in the current experiments.

Adenosine A_{2A} receptors and D2 receptors are co-localized on the same enkephalin-positive medium spiny neurons of the striatum (Fink et al., 1992; Svenningsson et al., 1999; Ferré et al., 2001; Hillion et al., 2002; Fuxe et al., 2003, 2007). This co-localization may allow these receptors to interact either through the formation of heteromeric complexes, or through convergence onto the same cAMP-related signal transduction pathways (Ferré et al., 1997; Svenningsson et al., 1999; Ferré et al., 2001, 2008). The interaction between A_{2A} receptors and D2 receptors on striatal medium spiny neurons provides a plausible mechanism for explaining the drug interactions observed in the present studies. The ventrolateral neostriatum is the brain area most closely associated with the production of tremulous jaw movements (Jicha and Salamone, 1991; Finn et al., 1997; Cousins et al., 1998; Salamone et al., 1998, 2008a), and evidence indicates that local injection of the adenosine A_{2A} antagonist MSX-3 directly into the ventrolateral neostriatum attenuated pimozide-induced tremulous jaw movements (Salamone et al., 2008a). In addition, systemic co-administration of the adenosine A_{2A} antagonist istradefylline significantly attenuated pimozide-induced tremulous jaw movements and also reduced pimozide-induced increases in c-Fos expression in ventrolateral neostriatum (Betz et al., 2009). Furthermore, ventrolateral neostriatal injections of adenosine antagonists also reduced the tremulous jaw movements induced by cholinomimetic drugs (Simola et al., 2004, 2006; Tronci et al., 2007). Nevertheless, in view of the literature demonstrating that different striatal subregions subserve distinct motor functions (e.g. Ishiwari et al., 2007), it is likely that striatal subregions other than ventrolateral neostriatum are the primary locus at which DA D2 and adenosine A_{2A} receptors interact to regulate catalepsy and locomotion. Hauber et al. (2001) showed that local injections of MSX-3 into medial neostriatum attenuated the catalepsy induced by local injection of DA antagonists. More recently, Ishiwari et al. (2007) demonstrated that the locomotor suppression induced by haloperidol could be reversed by injections of MSX-3 into the nucleus accumbens core, but not into the accumbens shell or ventrolateral neostriatum. Thus, it appears that DA D2 and adenosine A_{2A} receptors interact throughout the striatal complex, but the functional significance of this interaction differs across these distinct subregions (Ishiwari et al., 2007).

In the second group of experiments, Lu AA47070 was able to reverse the behavioral effects of the DA D2 antagonist haloperidol on performance of the concurrent FR5/chow intake choice task. Haloperidol (0.1 mg/kg) substantially altered the relative allocation of choice behavior in rats performing on this task, significantly decreasing lever pressing and increasing chow intake, which is consistent with several previous studies (Salamone et al., 1991, 2002, 2009a,b; Farrar et al., 2007). The concurrent FR5/chow feeding choice task has been extensively studied, and considerable evidence indicates that the shift from lever pressing to chow intake that is induced by DA antagonism or accumbens DA depletions is not due to effects on appetite or food preference, and is not related to the type of forepaw motor control deficits that are seen after ventrolateral neostriatal DA depletions (Salamone et al., 1991, 1993, 2002, 2007, 2009a,b; Cousins et al., 1994; Koch et al., 2000; Nowend et al., 2001; Sink et al., 2008). Thus, the effects of haloperidol on this task have typically been interpreted in terms of actions on processes such as behavioral activation and effort-related choice behavior (e.g., Salamone et al., 2003, 2005a, b, 2006, 2007, 2009a,b); low doses of DA antagonists or interference with accumbens DA transmission appear to suppress lever pressing but leave rats directed toward the acquisition and consumption of food, and therefore rats treated with DA antagonists select a different path to obtain food (i.e., consumption of the less preferred chow). In

recent studies, the adenosine A_{2A} antagonists MSX-3 and istradefylline also were shown to be capable of reversing the shifts in effort-related choice behavior induced by DA antagonism in rats responding on either the operant FR5/chow feeding task (Farrar et al., 2007, 2010; Salamone et al., 2009a,b; Worden et al., 2009; Nunes et al., 2010) or the T-maze barrier climbing task (Mott et al., 2009; Pardo et al., 2010). The ability of adenosine antagonists to reverse the effects of DA antagonism appears to be related to the subtype of adenosine receptor being blocked; although MSX-3, istradefylline, and Lu AA47070 have all been shown to reverse the effects of DA antagonists on effort-related choice behavior, the A_1 antagonists DPCPX and CPT were ineffective (Salamone et al., 2009a,b; Nunes et al., 2010). Moreover, the ability of adenosine A_{2A} antagonists to reverse the effects of D2 antagonists in rats responding on the concurrent lever pressing/chow feeding task appears to be related to actions on the nucleus accumbens (Farrar et al., 2010).

Across these different tests, Lu AA47070 appears to be about 2–3 times less potent than MSX-3, and about 10 times less potent than istradefylline (Farrar et al., 2007; Salamone et al., 2008a, 2009a,b; Worden et al., 2009). However, in terms of efficacy, Lu AA47070 is comparable to these other compounds. In addition, it is evident that higher doses of Lu AA47070 are needed to suppress tremulous jaw movements compared to all the other measures, including the reversal of the operant effects of haloperidol. This is consistent with previous reports using other adenosine A_{2A} antagonists (Farrar et al., 2007; Salamone et al., 2008a, 2009a,b), and could reflect the fact that tremor is one of the most difficult parkinsonian symptoms to treat, even by L-DOPA. Nevertheless, the fact that tremulous jaw movements are sensitive to adenosine A_{2A} antagonists (Correa et al., 2004; Salamone et al., 2008a; Collins et al., 2010b, 2011; present studies) may suggest that drugs such as Lu AA47070 could be useful for their tremorolytic effects in humans. Overall, the results of the current experiments suggest that Lu AA47070 and other adenosine A_{2A} antagonists are capable of producing antiparkinsonian effects in animal models. This lends further support for their use as a novel non-dopaminergic therapy for the treatment of idiopathic or drug-induced parkinsonism. Furthermore, the present results may be relevant for the development of treatments for depression. Previous studies have demonstrated that adenosine A_{2A} antagonists are effective in models of depression (El Yacoubi et al., 2003; Hodgson et al., 2009; Hanff et al., 2010). The effects of adenosine A_{2A} antagonists on effort-related choice behavior could indicate that these drugs may alleviate motivational symptoms such as psychomotor slowing, anergia or fatigue that often are seen in patients with depression, parkinsonism and other disorders (Farrar et al., 2007; Salamone et al., 2006, 2007, 2009a,b, 2010).

Disclosure/conflict of interest

The PI (J.S.) has received financial support from Merck Serono and Pfizer in the last 3 years. There are no personal financial holdings that could be perceived as contributing a potential conflict of interest.

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