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Tremorolytic effects of adenosine A_{2A} antagonists: implications for parkinsonism

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TABLE OF CONTENTS

1. ABSTRACT

Drug-induced tremulous jaw movements in rats have been used as a model of parkinsonian tremor. Because adenosine A_{2A} antagonists have antiparkinsonian effects, the present experiments were conducted to study the ability of adenosine A_{2A} antagonism to reverse the tremulous jaw movements produced by the antipsychotic drugs pimozide, haloperidol and reserpine. In one group of studies, rats received daily injections of the dopamine antagonist pimozide, and on day 8 they received injections of pimozide plus various doses of the A_{2A} antagonists KW 6002 or MSX-3. KW 6002 and MSX-3 suppressed pimozide-induced tremulous jaw movements, reduced catalepsy, and increased locomotion. MSX-3 also suppressed the jaw movements induced by haloperidol and reserpine. In addition, local injections of MSX-3 into the ventrolateral neostriatum suppressed pimozide-induced tremulous jaw movements. Thus, adenosine A_{2A} antagonism can reverse the tremulous movements induced by antipsychotic drugs, which is consistent with the hypothesis that antagonism of adenosine A_{2A} receptors can result in antiparkinsonian effects. Adenosine A_{2A} antagonists may be useful for their tremorolytic effects, and may help in treating both idiopathic and antipsychotic-induced parkinsonian symptoms.

2. INTRODUCTION

The motor symptoms of idiopathic Parkinson's disease, as well as drug-induced parkinsonism, result from a wide array of neurochemical interactions that occur in the circuitry of the basal ganglia. Depletion of striatal dopamine (DA) is widely seen as the immediate cause leading to the development of idiopathic Parkinson´s disease (1), and drug-induced parkinsonism often develops with the blockade of DA transmission produced by antipsychotic drugs (2). Yet despite the enormous literature linking DA systems to the regulation of parkinsonian symptoms, it is nevertheless true that DA transmission in the basal ganglia takes place amidst the context of numerous interactions between several transmitters (3-5). Research on these interactions offers avenues for exploring the mechanisms underlying the production of parkinsonian symptoms, as well as the potential for developing novel non-dopaminergic therapies. DA and acetylcholine systems interact in the regulation of parkinsonian symptoms, and muscarinic antagonists have been used for several years to treat parkinsonian symptoms (6-9). GABA and glutamate in basal ganglia output areas such as pallidal structures, subthalamic nucleus and

substantia nigra pars reticulata are known to be involved in the regulation of motor functions related to parkinsonism (10-15). Recent evidence also has indicated that brain adenosine neurons play an important role in regulating the functions of the basal ganglia (16-19). The adenosine A_{2A} receptor subtype is expressed to a high degree in the neostriatum (19,20), and in the striatum these receptors are largely expressed on enkephalin-positive striatopallidal neurons, which also tend to contain DA D2 receptors (19-24). Antagonism of adenosine A_{2A} receptors produces motor effects in animal models that are consistent with antiparkinsonian actions (16,25-28), and it has been widely suggested that adenosine A_{2A} antagonists could be used as a non-dopaminergic alternative for the treatment of parkinsonian symptoms (17,18,29-39). For these reasons, it is important to assess the effects of adenosine A_{2A} antagonists in both animal models and human clinical trials. Akinesia/bradykinesia, rigidity, and tremor are the classic motor symptoms of parkinsonism in humans (2,40), and various tests in rodents are used to study motor dysfunctions related to parkinsonism (41,42). Within the last few years, several of these procedures have been employed to study the potential antiparkinsonian effects of adenosine A_{2A} antagonists. In rodents, adenosine A_{2A} antagonists have been shown to reverse effects such as hypolocomotion, catalepsy, and muscle rigidity that are induced by DA antagonists or DA depleting agents (43-46).

Resting tremor is one of the cardinal symptoms of parkinsonism, and there is considerable uncertainty about the neurochemical mechanisms that underlie tremorogenesis (47-50). Although there have been a number of studies investigating the motor effects of adenosine A_{2A} antagonists, very few have focused upon tremor. The initial studies, which employed non-selective adenosine antagonists such as theophylline, yielded mixed results. Mally and Stone (32,33) reported that theophylline could reduce parkinsonian tremor in human patients. However, Kulisevsky *et al.* (51) did not observe a significant effect of theophylline. A more recent study (52) reported that the selective adenosine A_{2A} antagonist KW6002 enhanced the antiparkinsonian effects of a low dose of L-DOPA, and that resting tremor was particularly responsive to this treatment. Clearly, it is important to investigate the pharmacology and neurochemistry of tremor, and studies employing animal models are a critical aspect of this research strategy (49). For several years, there were few reports of parkinsonian tremor in rodents (42,49). Nevertheless, one of the rodent behavioral procedures that has emerged within the last few years as a model of parkinsonian resting tremor is drug-induced tremulous jaw movements (i.e., TJMs; see refs. 8,9,42,53-55). TJMs are rapid vertical deflections of the lower jaw that resemble chewing but are not directed at any stimulus (8). Studies using slow-motion or freeze-frame video analyses, as well as electromyographic methods, have shown that these movements occur largely in the 3-7 Hz frequency range that also is characteristic of parkinsonian resting tremor (8,53,56-59). TJMs can be induced by striatal DA depletions (57,60) and by centrally-acting cholinomimetic drugs (54,56,61). They also are induced by typical antipsychotics such as haloperidol (62,63), pimozide (53), and reserpine (59,64,65), but they generally are not induced by atypical antipsychotics (63). Although chronic administration of antipsychotic drugs can result in oral movements that may be related to other movement disorders, such as tardive dyskinesia, considerable evidence indicates that the chewing-like jaw movements induced by acute or subchronic administration of typical antipsychotic drugs share many characteristics with parkinsonian symptoms (8,53,59,62,65-69). TJMs have been used as a rodent model of parkinsonian tremor for assessing antiparkinsonian drugs with various pharmacological profiles, including L-DOPA, DA agonists, and muscarinic antagonists (8,54,62,64,66,70). The adenosine A_{2A} antagonist KF17837 (10.0-20.0 mg/kg) was recently shown to suppress haloperidol-induced TJMs (43), and the TJMs induced by the anticholinesterase tacrine were reduced by systemic or intrastriatal injections of the adenosine A_{2A} antagonists SCH 58261 and SCH BT2 (27).

In the present experiments, the potential antiparkinsonian effects of the selective adenosine A_{2A} antagonist MSX-3 were assessed by using acute or subchronic administration of antipsychotic drugs to induce TJMs. MSX-3 is a water-soluble phosphate prodrug that is rapidly cleaved by phosphatases *in vivo* to yield the physiologically active compound MSX-2 (71,72). Previous results have demonstrated that MSX-3 can reverse haloperidol-induced catalepsy (73) and suppression of locomotion (74), but no published studies have examined the effects of MSX-3 on tremulous movements. In the first group of studies, pimozide (Orap) was used to induce motor impairments. Pimozide is a typical antipsychotic drug of the diphenylbutylpiperidine class, which has been shown to produce motor side effects, including tremor, in patients with schizophrenia (75,76,77), and to worsen motor signs in patients with Parkinson's disease (78). Moreover, pimozide has been reported to be more likely to produce parkinsonian tremor compared to other typical antipsychotics (77). A recent paper demonstrated that pimozide could induce TJMs with acute or subchronic administration (i.e., 1, 7 or 13 days of injections) at doses up to 1.0 mg/kg (53). Based upon these previous experiments, the first group of studies assessed the ability of adenosine A_{2A} antagonism to suppress tremulous movements and increase motor activity in pimozide-treated rats. Experiment 1 established the behavioral procedure by assessing the effects of pimozide on a motor test battery. For this study, pimozide (1.0 mg/kg) or vehicle were administered for 8 consecutive days, and after the day 8 injections rats were assessed in the test battery, in which TJMs, catalepsy using the bar test, and locomotion in small stabilimeter cages, were recorded. In experiment 2, effects of the selective adenosine A_{2A} antagonist KW 6002 on pimozide-induced motor impairments were assessed. KW 6002 (Istradefylline) was used to provide positive control, because this drug is a well characterized compound that is currently undergoing clinical assessment in parkinsonian patients (30). Experiment 3 studied the ability of the adenosine A_{2A} antagonist MSX-3 to reverse the motor effects produced by subchronic pimozide administration. Because the focus of the present work was on models related to tremor, experiments 4-6 only employed measures

of jaw movement activity. Experiments 4 and 5 assessed the ability of MSX-3 to reverse the TJMs induced by subchronic administration of the DA antagonist haloperidol and acute treatment with the DA depleting agent reserpine. The final study (experiment 6) investigated the effects of intracranial injections of MSX-3 into the ventrolateral neostriatum (VLS), in order to determine if local injections of an adenosine A_{2A} antagonist could reverse the TJMs induced by pimozide. The VLS was chosen because this striatal subregion, which is the rodent homologue of the ventral putamen, has been strongly implicated in the control of TJM activity (8,27,56-58,61,79,80).

3. MATERIALS AND METHODS

3.1. Animals

A total of 283 male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) with no prior drug experience were used in the present experiments. The rats weighed 315-480 g during the course of the experiment and had *ad libitum* access to lab chow and water. The rats 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 hrs). These studies were conducted according to University of Connecticut and NIH guidelines for animal care and use.

3.2. Drugs

Pimozide, haloperidol and reserpine were purchased from Sigma Aldrich Chemical (St. Louis, MO), and were dissolved in warm 0.3% tartaric acid. MSX-3 ((E)-phosphoric acid mono-[3-[8-[2-(3-methoxyphenyl)vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl] ester) was synthesized at the Pharmazeutisches Institut (Universität Bonn; Bonn, Germany; see Hockemeyer *et al.* 2004). MSX-3 was dissolved in 0.9% saline. The pH of the MSX-3 solution was adjusted by adding 1.0 N NaOH until the drug was completely in solution after conversion to its disodium salt (pH 7.1 – 7.4). The pH of the saline vehicle control was adjusted accordingly to obtain a similar pH. KW 6002 was generously donated by Lundbeck Pharmaceuticals (Copenhagen, Denmark), and was dissolved in DMSO and Tween-80 mixed with 0.9% saline (10:10:80 % mixture).

3.3. Selection of Doses and Treatment Procedures

The dose and time course for the subchronic 1.0 mg/kg (IP) pimozide treatment procedure selected for the present studies were based upon previously published experiments (53). The dose and time course for the 14-day haloperidol (0.5 mg/kg IP) procedure for induction of TJMs was the same as that used in several other studies (15,43,63). The procedure of screening animals by assessing them for TJMs the day before the drug challenge day (i.e., observing on day 7 in the pimozide experiments, and day 13 in the haloperidol experiment) was the same as that used in a previous study (15). 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., < 8%) failed to show a substantial jaw movement response to pimozide (i.e., < 15 TJMs) on day 7. Reserpine-induced TJMs have been reported to occur after acute administration in previous papers (59,64,65), and the specific repeated-measures procedure used in the present study (i.e., 1.25 mg/kg, injections every two weeks) was determined based upon these published studies and also upon extensive pilot work. Doses of MSX-3 used in experiments 2-3 were selected based upon pilot data, and on previous studies with locomotion (74). Because pilot studies indicated that higher doses of MSX-3 would be necessary for blocking reserpine-induced TJMs, a higher dose progression was used for experiment 5. The doses range used for KW 6002 was based upon pilot studies.

3.4. Behavioral procedures

3.4.1. Overview

For experiments 1-3, rats were tested in a battery that included assessments of pimozide-induced tremulous jaw movements, catalepsy and suppression of locomotion. Experiments 4, 5 and 6 only included measurements of tremulous jaw movements, which in experiment 4 and 5 were induced by haloperidol and reserpine, respectively. Experiment 6 studied the effects of intrastriatal injections of MSX-3 on pimozide-induced jaw movements.

3.4.2. Tremulous jaw movements (TJMs)

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. TJMs were defined as rapid vertical deflections of the lower jaw that resembled chewing but were not directed at any particular stimulus (8). 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 $r = 0.93$ ($p < 0.05$) using these methods.

3.4.3. Behavioral procedures: catalepsy

Catalepsy was tested by placing both forelimbs of the rat on to a stationary horizontal metal bar, raised 12.5 cm above a wooden platform, and then allowing the rat to stabilize itself with the hindpaws resting on the platform. Latency for the subject to cease having both forelimbs on the metal bar was timed. Three trials were conducted and the latencies for each trial were averaged.

3.4.4. Behavioral procedures: locomotor activity

Locomotor activity was assessed by placing the rats in an automated locomotor activity chamber (28 X 28 X 28 cm) enclosed in a sound-proof casing. Two moveable wire-mesh panels, elevated 6 cm above the chamber bottom, constituted the floor of each activity chamber. A center rod between the two panels (panel 25 X 12 cm) allowed for the deflection of one of the four quadrants (a quadrant was defined as half a panel), and movement of the animal resulted in the closure of a microswitch fixed to the outside of the activity chamber. Each depression in a quadrant 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.

3.4.5. Surgical and intracranial injection procedures

Rats were anesthetized with injection of a 1.0 ml/kg IP solution of ketamine/xylazine (recipe: 0.75 ml of a 20 mg/ml solution of xylazine added to a 10.0 ml bottle of a 100 mg/ml ketamine solution). Guide cannulae (25 ga extra-thin wall stainless steel tubing, Small Parts) were bilaterally implanted 1.0 mm dorsal to the target site for the VLS at the following stereotaxic coordinates: AP + 1.4 mm, ML ± 3.8 mm, DV - 6.2 mm; incisor bar 5 mm above the interaural line). The dorsal/ventral coordinate was slightly adjusted based upon weight, such that 1.0-2.0 additional mm were added for rats weighing over 350 g. Operated rats were housed singly after surgery, and were allowed 7-10 days recovery before testing. Stainless steel stylets were kept inside the guide cannulae to maintain their integrity prior to injection. On the drug test day, the intracranial injections were made via 30-gauge stainless-steel injectors extending 1.0 mm below the guide cannulae. The injectors were attached to 10-µl Hamilton syringes by PE-10 tubing. All injections were made at a volume of 1.0 µl per side (at a rate of 0.5 µl/min for 1 min). Injectors were left in place for 1 min after the infusion to allow for diffusion of the drug. After experiment 6 was completed, all animals were intracardially perfused with 0.9% saline, followed by 3.7 % formalin. Brains were stored refrigerated in a formalin solution several days, and were cryoprotected with sucrose-formalin before slicing on a cryostat. The placements of the injectors were verified histologically by slicing consecutive 50 micron sections through the relevant brain areas. Sections were mounted on slides and stained with cresyl violet, and all slides were viewed microscopically to assess accuracy of implantation. Any animal with improper placement in either hemisphere (i.e., not in the target area of the VLS, or significant damage around the injection site, was not included in the statistical analyses of behavioral data.

3.5. Experiments

3.5.1. Experiment 1: Effects of pimozide on TJMs, catalepsy and locomotion

A group of 18 rats was used to assess the effects of pimozide injections on jaw movement activity, catalepsy and locomotor activity. All rats received an IP injection of either tartaric acid vehicle (n=9) or 1.0 mg/kg pimozide (n=9) IP for 8 consecutive days. On day 7 of the subchronic injections rats were assessed for the induction of TJMs. Three hrs and 50 min following their daily pimozide or vehicle injections, animals were place in the Plexiglas observation chamber and allowed to habituate for 10 min. Immediately following habituation, TJMs were counted for 5 min as described above. On day 8, rats were assessed in the neurological battery for TJMs, catalepsy, and locomotor activity. They were placed in the TJM observation chamber 3 hr 50 min after IP injection, were habituated in the chamber for 10 min, and then were assessed for TJMs in a 5-min observation session. The cessation of the TJM observation session was followed immediately by assessment of catalepsy, which was tested as described above. After the catalepsy test, locomotor activity was then measured by placing the rats in the locomotor activity chamber and recording the total number of activity counts for a 10 min session, as described above.

3.5.2. Experiment 2: Effects of KW 6002 on pimozide-induced TJMs, catalepsy and suppression of locomotion

A group of 48 rats was used to assess the effects of KW 6002 on pimozide-induced jaw movement activity, catalepsy and suppression of locomotor activity. All rats received IP injections of 1.0 mg/kg pimozide each day, and were tested using behavioral procedures similar to those used in experiment 1. Rats were tested for TJMs on day 7 of the subchronic injections as described above, and only the rats that had > 15 jaw movements on day 7 were used for the day 8 drug challenge test. For the day 8 behavioral test, all rats were treated with 1.0 mg/kg pimozide, and were randomly assigned to receive one of the following doses of KW 6002 or vehicle on day 8: saline vehicle control, 1.25 mg/kg, 2.5 mg/kg, 5.0 mg/kg, and 10.0 mg/kg KW 6002 (n = 9-10 for each group, total n=48). Three hr and 40 min following their daily pimozide injection on day 8, rats received an IP injection of KW 6002, according to the previously assigned doses. TJMs, catalepsy and locomotion were assessed in the same manner as described above for experiment 1.

3.5.3. Experiment 3: Effects of MSX-3 on pimozide-induced TJMs, catalepsy and suppression of locomotion

A group of 50 rats was used to assess the effects of MSX-3 on pimozide-induced jaw movement activity, catalepsy and suppression of locomotor activity. All rats received IP injections of 1.0 mg/kg pimozide each day, and were tested using behavioral procedures similar to those used in experiment 1. Rats were tested for TJMs on day 7 of the subchronic injections as described above, and only the rats that had > 15 jaw movements on day 7 were used for the day 8 drug challenge test. For the day 8 behavioral test, all rats were treated with 1.0 mg/kg pimozide, and were randomly assigned to receive one of the following doses of MSX-3 or vehicle on day 8: saline vehicle control, 1.25 mg/kg, 2.5 mg/kg, 5.0 mg/kg, and 10.0 mg/kg MSX-3 (n = 10 for each group; total n= 50). Three hr and 40 min following their daily pimozide injection on day 8, rats received an IP injection of MSX-3, according to the previously assigned doses. TJMs, catalepsy and locomotion were assessed in the same manner as described above for experiments 1-2.

3.5.4. Experiment 4: Effects of MSX-3 on haloperidol-induced TJMs

A group of 104 rats was used to assess the effects of MSX-3 on haloperidol-induced jaw movement activity. All rats received an IP injection of 0.5 mg/kg haloperidol for 14 consecutive days. On day 13 of the subchronic injections rats were assessed for the induction of TJMs in a 5-min session that followed a 10-min habituation period, which was initiated 40 min after their daily haloperidol injections. Only the rats that had $\geq$ 15 jaw movements on day 13 were used for the day 14 drug challenge test. For the day 14 behavioral test, all rats were treated with 0.5 mg/kg haloperidol, and were randomly assigned to receive one of the following doses of MSX-3 on day 14: saline

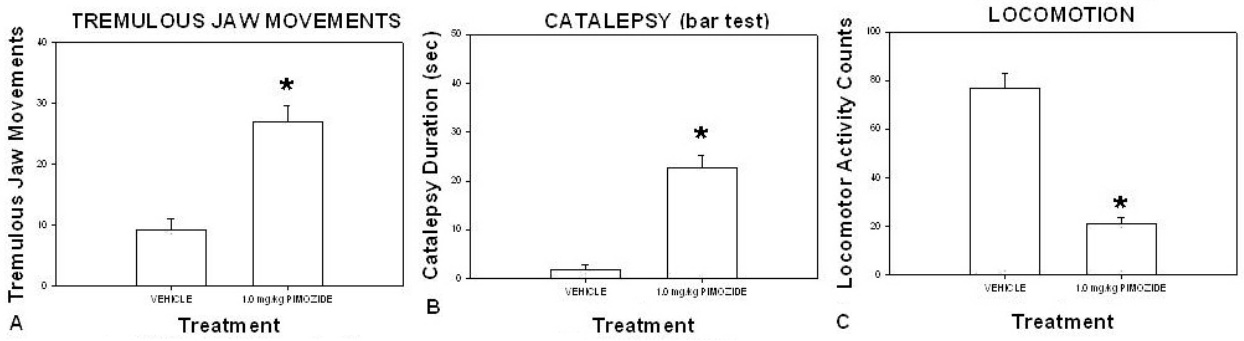

Figure 1. Effect of pimozide on tremulous jaw movements (TJMs), catalepsy and locomotion. A. Mean (± SEM) number of individual jaw movements (per 5 min observation period) after injection of tartaric acid vehicle or 1.0 mg/kg pimozide. B. Mean (± SEM) catalepsy response (in seconds) after injection of tartaric acid vehicle or 1.0 mg/kg pimozide. C. Mean (± SEM) number of locomotor counts after injection of tartaric acid vehicle or 1.0 mg/kg pimozide. * $p < 0.05$, different from vehicle.

vehicle control, 0.625, 1.25, 2.5, 5.0, and 10.0 mg/kg MSX-3 (n = 15-22 for each group; total n=104). Rats received an IP injection of MSX-3 or saline 30 min following their daily haloperidol injection on day 14. TJMs were assessed in the same manner as described above.

3.5.5. Experiment 5: Effects of MSX-3 on reserpine-induced TJMs

For the reserpine experiment, rats were tested in a repeated measures design, receiving drug treatments once every two weeks until each rat had received all treatments in a randomly varied order. In order to induce TJMs, all rats received an IP injection of 1.25 mg/kg reserpine 90 min prior to the onset of the behavioral testing; rats (n = 12) were assessed for the induction of TJMs in a 5-min session that followed a 10-min habituation period, which was initiated 80 min after the reserpine injections. On the day of the behavioral test, all rats were treated with 1.25 mg/kg reserpine, and also received one of the following treatments: saline vehicle control, 10.0 mg/kg, and 20.0 mg/kg MSX-3. Rats received an IP injection of MSX-3 or saline 70 min following their reserpine injection, and TJMs were assessed in the same manner as described above.

3.5.6. Experiment 6: Effects of ventrolateral neostriatal injections of MSX-3 on pimozide-induced TJMs

Separate groups of rats were used to test each dose of MSX-3, and a total of **XX** rats were used. Rats received bilateral implantations with stainless steel guide cannulae in the VLS as described above. After 7-10 days of recovery, rats received daily i.p. injections of 1.0 mg/kg pimozide for 7 consecutive days. On day 8, the rats were given i.p. injections of 1.0 mg/kg pimozide 3 hours and 50 min before being placed in the chamber. Immediately before being placed in the chamber, animals received bilateral intracranial injections of one of the following doses of MSX-3 or saline: saline vehicle, 2.5 µg, 5.0 µg, 10.0 µg MSX-3 per side, as described above (n=10-17 per group; total n=51). Animals were then placed in the observation chamber, allowed to habituate for 10 min, and were then assessed for TJM activity during the period 10-15 min after intracranial injection.

3.6. Data analyses

The behavioral data for experiments 1-4 and 6 were analyzed using a between-groups analysis of variance (ANOVA). For experiments 1-3, an average latency for three catalepsy trials was calculated and then used in the ANOVA analysis. The results of experiment 5 were analyzed using repeated measures ANOVA. A computerized statistical program (SPSS 10.1 for Windows) was used to perform these analyses. For all experiments, planned comparisons using the overall error term were used to assess the differences between each dose and the control condition, which kept the total number of comparisons to the number of conditions minus one (81).

4. RESULTS

4.1. Experiment 1: Effects of Pimozide on TJMs, catalepsy and locomotion

The day 7 test results showed that pimozide significantly increased TJMs relative to saline treatment (mean ± SEM, vehicle: 9.5 ± 1.8; 1.0 mg/kg pimozide: 20.3 ± 3.1; $F(1,16)= 8.7$, $p < 0.01$). For the day 8 test, 1.0 mg/kg pimozide produced significant changes on all three behavioral measures compared to control injections of tartaric acid vehicle (Figure 1A-C). Pimozide produced a significant induction of TJMs relative to vehicle administration (Figure 1A; $F(1,16) = 29.4$, $p < 0.01$). In addition, pimozide administration significantly induced catalepsy (Figure 1B; $F(1,16) = 60.7$, $p < 0.01$) and suppressed locomotion (Figure 1C; $F(1,16) = 66.8$, $p < 0.01$).

4.2 Experiment 2: Effects of KW 6002 on pimozide-induced TJMs, catalepsy and suppression of locomotion

Co-administration of KW 6002 significantly reversed the motor effects of pimozide (Figure 2A-C). Analysis of the TJM data from the day 8 test revealed a significant effect of KW 6002 on pimozide-induced TJMs ($F(4,43) = 6.62$, $p<0.001$; Figure 2A), with all doses of KW 6002 plus pimozide differing significantly from the pimozide plus vehicle control ($p < 0.05$). There also was a significant effect of KW 6002 on the reversal of pimozide-induced catalepsy ($F(4,43) = 4.1$, $p<0.01$; Figure 2B). All doses of KW 6002 plus pimozide differed significantly from the pimozide plus vehicle control condition ($p < 0.05$). In addition, KW 6002 significantly increased locomotion in pimozide-treated rats ($F(4,42) = 4.5$, $p<0.01$;

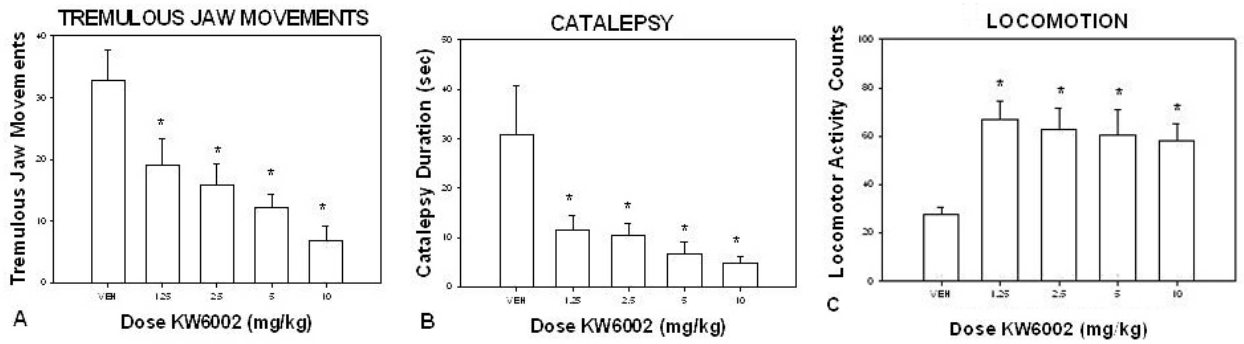

Figure 2. Effect of KW 6002 on pimozide-induced tremulous jaw movements (TJMs), catalepsy, and suppression of locomotion. A. Mean (± SEM) number of individual jaw movements (per 5 min observation period) after injection of tartaric acid vehicle plus1.0 mg/kg pimozide or pimozide plus various doses of KW 6002. B. Mean (± SEM) catalepsy response (in seconds) after injection of tartaric acid vehicle plus1.0 mg/kg pimozide or pimozide plus various doses of KW 6002. C. Mean (± SEM) number of locomotor counts after injection of tartaric acid vehicle plus1.0 mg/kg pimozide or pimozide plus various doses of KW 6002. * $p < 0.05$, different from vehicle plus pimozide.

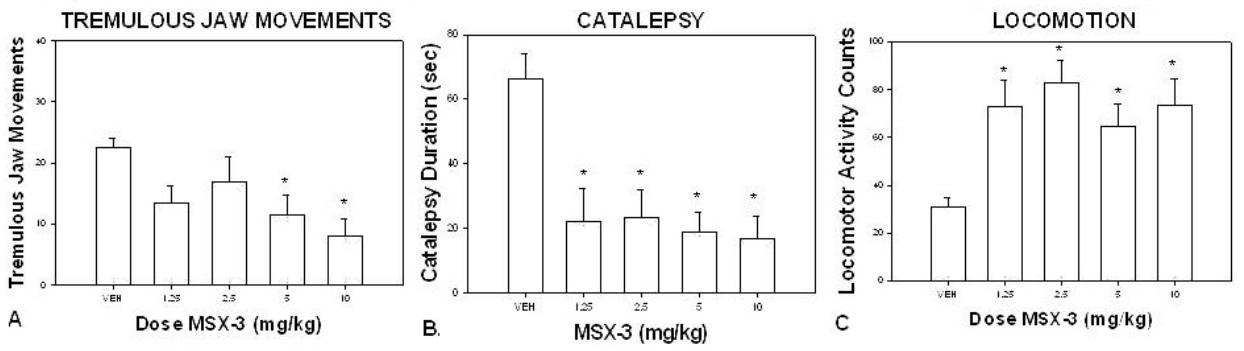

Figure 3. Effect of MSX-3 on pimozide-induced tremulous jaw movements (TJMs), catalepsy, and suppression of locomotion. A. Mean (± SEM) number of individual jaw movements (per 5 min observation period) after injection of tartaric acid vehicle plus1.0 mg/kg pimozide or pimozide plus various doses of MSX-3. B. Mean (± SEM) catalepsy response (in seconds) after injection of tartaric acid vehicle plus1.0 mg/kg pimozide or pimozide plus various doses of MSX-3. C. Mean (± SEM) number of locomotor counts after injection of tartaric acid vehicle plus1.0 mg/kg pimozide or pimozide plus various doses of MSX-3. * $p < 0.05$, different from vehicle plus pimozide.

Figure 2C). All doses of KW 6002 plus pimozide differed significantly from the pimozide plus vehicle control ($p<0.05$).

4.3 Experiment 3: Effects of MSX-3 on pimozide-induced TJMs, catalepsy and suppression of locomotion

The motor effects of pimozide also were reversed by co-administration of MSX-3 (Figure 3A-C). Analysis of the day 8 TJM data demonstrated a significant effect of MSX-3 on the TJMs induced by pimozide (see Figure 3A; $F(4,45) = 3.184$, $p<0.05$), with the 5.0 mg/kg and 10.0 mg/kg doses of MSX-3 plus pimozide differing significantly from the vehicle plus pimozide control ($p<0.05$). There also was a significant effect of MSX-3 in terms of the reversal of pimozide-induced catalepsy (Figure 3B; $F(4,45) = 6.613$, $p<0.001$). All doses of MSX-3 plus pimozide differed significantly from the vehicle plus pimozide control condition ($p<0.05$). A significant effect of MSX-3 also was shown for enhancement of locomotion in pimozide-treated rats (Figure 3C; $F(4,45) = 4.583$, $p<0.005$). All doses of MSX-3 plus pimozide significantly differed from the vehicle plus pimozide control ($p<0.05$).

4.4 Experiments 4 and 5: Effects of MSX-3 on haloperidol and reserpine-induced TJMs

Experiments 4 and 5 focused upon the effects of MSX-3 on the TJMs induced by haloperidol and reserpine. Co-administration of MSX-3 with these drugs resulted in reduced levels of TJM activity (Figure 4A-B). Analysis of the day 14 TJM data demonstrated a significant effect of MSX-3 on haloperidol-induced TJMs ($F(5,98) = 4.64$, $p<0.05$), with the 1.25, 2.5, 5.0 and 10.0 mg/kg doses of MSX-3 plus haloperidol differing significantly from the vehicle plus haloperidol control ($p<0.05$; Figure 4A). In addition, MSX-3 significantly reduced reserpine-induced TJMs ($F(2,11) = 11.4$, $p < 0.01$). Both the 10.0 and 20.0 mg/kg doses of MSX-3 plus reserpine significantly suppressed TJMs relative to vehicle plus reserpine ($p<0.05$; Figure 4B).

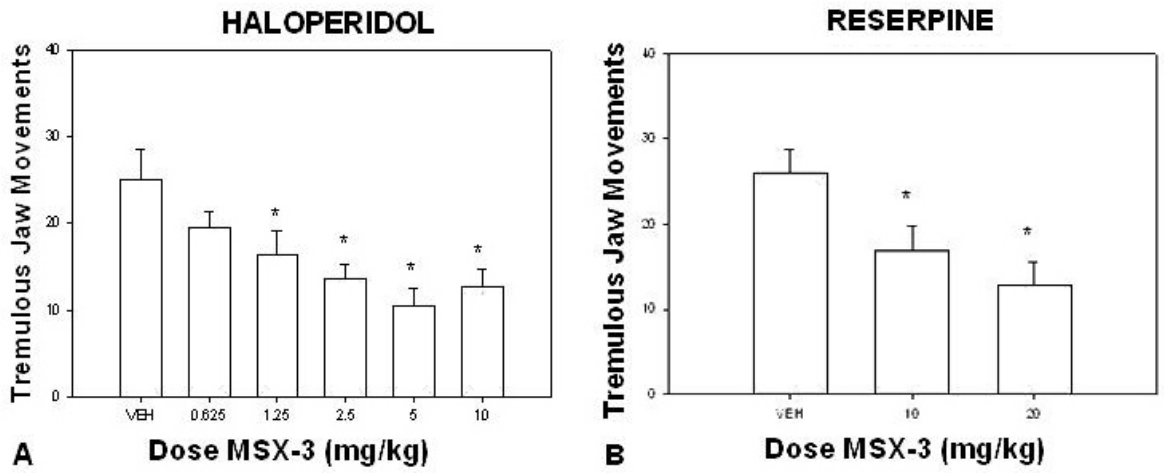

Figure 4. Effect of MSX-3 on haloperidol and reserpine-induced tremulous jaw movements (TJMs). A. Mean (± SEM) number of individual jaw movements (per 5 min observation period) after injection of tartaric acid vehicle plus 0.5 mg/kg haloperidol or haloperidol plus various doses of MSX-3. B. Mean (± SEM) number of individual jaw movements (per 5 min observation period) after injection of tartaric acid vehicle plus 1.25 mg/kg reserpine or reserpine plus various doses of MSX-3. * $p < 0.05$, different from vehicle plus pimozide.

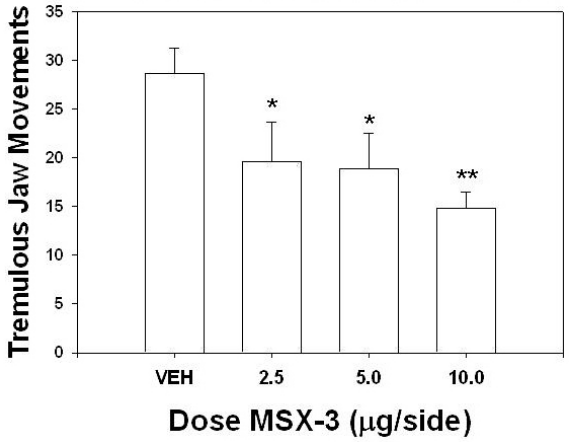

Figure 5. Effect of intrastriatal injections of MSX-3 into the VLS target site on pimozide-induced tremulous jaw movements (TJMs). Mean (± SEM) number of individual jaw movements (per 5 min observation period) after injection of saline vehicle plus1.0 mg/kg pimozide, or pimozide plus various doses of MSX-3. * $p < 0.05$, different from vehicle plus pimozide, ** $p < 0.01$, different from vehicle plus pimozide.

4.5. Experiment 6: Effects of ventrolateral neostriatal injections of MSX-3 on pimozide-induced TJMs

Analysis of the TJM data from Day 8 (i.e., the drug challenge day) in experiment 6 demonstrated that intrastriatal injections of the adenosine A2A antagonist MSX-3 into the VLS produced an overall suppression of the jaw movement activity induced by pimozide (see Figure 5; $F(3,47=4.69$, $p <0.01$). Planned comparisons indicated that the 2.5 and 5.0 μg doses of MSX-3 plus pimozide significantly suppressed jaw movements relative to the vehicle control plus pimozide group at the $p = 0.05$ level, while the 10.0 μg dose of MSX-3 plus pimozide significantly differed from the vehicle control plus pimozide group at the 0.01 probability level. Figure 6 shows the histological results from a representative animal that received 10.0 μg dose of MSX-3 plus pimozide.

5. DISCUSSION

The present studies demonstrate that systemic administration of adenosine A_{2A} receptor antagonists is capable of reversing the TJMs induced by different types of antipsychotic drugs, including the diphenylbutylpiperidine DA antagonist pimozide, the butyrophenone DA antagonist haloperidol, and the DA depleting agent reserpine. The well characterized adenosine A_{2A} antagonist, KW 6002, suppressed the TJMs induced by pimozide (Experiment 2). Moreover, MSX-3 suppressed the TJMs induced by pimozide, haloperidol and reserpine (Experiments 3-5). The suppression of pimozide-induced TJMs that was produced by KW 6002 and MSX-3 occurred in roughly the same dose range that also reversed the effects of pimozide on catalepsy and locomotion (Experiments 2-3). It appears

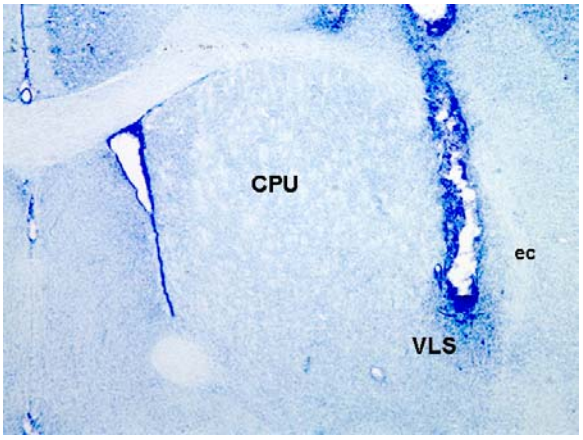

Figure 6. Photomicrograph of a Nissl-stained coronal section through the neostriatum of a representative rat that received 10.0 µg MSX-3 per side in the VLS plus a systemic injection of 1.0 mg/kg pimozide. CPU- caudate putamen; VLS- ventrolateral neostriatum; ec- external capsule.

that higher doses of MSX-3 were required to reverse reserpine-induced TJMs compared to those induced by DA antagonists, which may be due to the fact that reserpine acts to deplete DA rather than block DA receptors. The present results are consistent with recent papers showing that systemic administration of the adenosine A_{2A} antagonist KF17837 could suppress the TJMs induced by haloperidol (43), and that the TJMs induced by the anticholinesterase tacrine were reduced by systemic or intrastriatal injections of the adenosine A_{2A} antagonists SCH 58261 and SCH BT2 (27). Furthermore, the present studies demonstrated that direct injections of MSX-3 into the ventrolateral region of the neostriatum (i.e., the VLS) also were capable of suppressing the tremulous oral movements induced by pimozide (experiment 6). Together with these previous studies, the present results demonstrate that a broad range of adenosine A_{2A} antagonists are capable of showing tremorolytic effects in the TJM model, and are consistent with the hypothesis that adenosine A_{2A} antagonists can produce antiparkinsonian effects in animal models. These findings also are consistent with human clinical data indicating that theophylline and KW 6002 can suppress parkinsonian tremor (32,52).

These results obtained with adenosine A_{2A} antagonists are comparable to other findings demonstrating that the TJM model can be used to assess the effects of antiparkinsonian drugs from a variety of different classes. Cholinomimetic-induced TJMs can be suppressed by several different types of drugs that stimulate DA transmission, including L-DOPA and DA agonists with different selectivity profiles, such as apomorphine, bromocriptine, pergolide, ropinirole, SKF 82958 and CY 208 243 (54,70,80). Furthermore, the potency of various dopaminergic drugs for suppressing cholinomimetic-induced TJMs is highly correlated with the clinical potency of these drugs for suppression of parkinsonian tremor in humans (54). The atypical antipsychotic drug clozapine, which is known to be antitremorogenic in human parkinsonian patients (82-86), has been shown repeatedly to suppress cholinomimetic-induced TJMs (63,87,88). These movements also are suppressed by the atypical antipsychotic quetiapine (89), which shares many neurochemical and behavioral characteristics with clozapine. In addition, antiparkinsonian anticholinergic drugs such as scopolamine, atropine, benztropine, and trihexyphenidyl have been shown repeatedly to suppress the TJMs induced either by cholinomimetic drugs (58,70) or by antipsychotic drugs such as haloperidol, reserpine or pimozide (59,62,66). Recent studies indicate that the suppression of pimozide-induced TJMs produced by adenosine A_{2A} antagonists is comparable to the magnitude of the effect produced by the anticholinergic drugs atropine and tropicamide (66). Although anticholinergic drugs have little or no consistent effect on tardive dyskinesia, it is important to emphasize that muscarinic antagonists often are used to suppress the parkinsonian side effects produced by antipsychotic drugs (8,66,90,91). However, treatment of the parkinsonian side effects of antipsychotic drugs with muscarinic antagonists can result in undesirable outcomes such as autonomic dysfunctions and cognitive impairments (66,91). The present results, in combination with previous data (e.g. 43), suggest that adenosine A_{2A} antagonists could be used to treat the parkinsonian side effects induced by typical antipsychotic drugs. Moreover, it is reasonable to suggest that a drug that had the ability to block both DA D2 receptors and adenosine A_{2A} receptors could have the profile of an atypical antipsychotic drug (43), provided that adenosine A_{2A} antagonism does not lessen the antipsychotic effect of D2 antagonism (92,93,94).

As well as studying the pharmacological interactions regulating tremulous movements, Experiments 1-3 also employed a battery of motor tests that included measures of catalepsy and locomotion. As expected, Experiment 1 demonstrated that pimozide administration (1.0 mg/kg IP) was characterized by the induction of TJMs and catalepsy, and also by the suppression of locomotor activity. The adenosine A_{2A} antagonists KW 6002 and MSX-3 reversed all of these effects of pimozide in Experiments 2 and 3. The effects of KW 6002 and MSX-3 on locomotion appear to be more potent than the effects of these drugs on TJMs, as the locomotion effect appeared to be maximal even at the lowest doses tested. Thus, in order to fully characterize the locomotor effects of KW 6002 and MSX-3, lower doses would have to be used. The test battery developed for these studies appears to be useful for providing multiple measures of motor function in the same groups of animals. The measures that were chosen (i.e., TJMs, catalepsy and locomotion) offer indices of different aspects of motor function, which could be related to distinct symptoms of parkinsonism. For example, although TJMs share characteristics with parkinsonian tremor (8,53,54), catalepsy and locomotion appear to be more closely related to akinesia/bradykinesia (42,95). Thus, adenosine A_{2A} antagonists are capable of reversing motor dysfunctions that are related to distinct aspects of motor control.

Although drug-induced decreases in TJMs can be accompanied by changes in other behaviors, such as locomotion and catalepsy, there is considerable evidence

indicating that the modulation of TJM activity is not simply an artifact of changes in gross locomotion or rearing (60,62). In fact, the brain mechanisms regulating locomotion are distinct from those that are involved in the generation of tremulous movements such as TJMs. Recent research has indicated that the nucleus accumbens, which is known to be involved in locomotor activity (92,96,97,98), is an important brain area at which adenosine A_{2A} antagonists can reverse the suppressive effects of DA antagonism on locomotion (74). Injections of MSX-3 into the nucleus accumbens core were capable of reversing the suppression of locomotion induced by haloperidol, while injections into nucleus accumbens shell or VLS were ineffective (74). Previous work has suggested minimal involvement of the VLS in locomotor function; depletions of DA in the VLS by local infusions of 6-hydroxydopamine failed to suppress locomotion (60,97), and intracranial injections of amphetamine into the VLS were reported to have no effect on locomotor activity (98). Yet despite the fact that the VLS does not appear to be an important site for the regulation of locomotion, neurochemical interactions in this striatal region are important for control of TJM activity (8,27,56-58,61,79,80). Anatomical evidence indicates that the VLS is the rat homologue of the ventral putamen, and that the lateral striatum of the rat, like the primate putamen, has some degree of somatotopic organization (8). Various lines of evidence indicate that the VLS of the rat appears to be particularly important for head, orofacial and forepaw motor control (8). Previous work has shown that the VLS also is an important site at which DA depletions and cholinomimetics can induce TJMs (8,27,56-58,61,79,80). More recently, it was demonstrated that the jaw movements induced by the anticholinesterase tacrine could be reversed by infusions of the adenosine A_{2A} antagonist SCH BT2 directly into the VLS (27). Nevertheless, in view of data indicating that the TJMs induced by cholinomimetics and DA antagonists can demonstrate different neurochemical and anatomical characteristics (15), it is important to assess the effects of local injections of adenosine A_{2A} antagonists into the VLS on the jaw movements induced by DA antagonists. In this regard, it is worth emphasizing that local injections of MSX-3 into the VLS were able to suppress the TJMs induced by administration of pimozide in experiment 6. Future studies should investigate this more fully by directly comparing the tremorolytic effects of local administration of adenosine A_{2A} antagonists into the VLS with injections into other striatal subregions. Nevertheless, together with the findings reviewed above, the present results support the concept that different effects of antiparkinsonian drugs (i.e., increases in locomotion, decreases in rigidity or tremor) are related to actions on distinct striatal subcircuits.

In summary, adenosine A_{2A} antagonism can reverse the induction of oral tremor and catalepsy, as well as the suppression of locomotion, that is induced by antipsychotic drugs. These data are consistent with the hypothesis that adenosine A_{2A} antagonists can produce antiparkinsonian actions in animal models, and additionally they suggest that adenosine A_{2A} antagonists could be useful for ameliorating the parkinsonian side effects of typical antipsychotics. The test battery developed for these studies may have utility for screening novel adenosine A_{2A} antagonists for potential antiparkinsonian actions on multiple tasks. Furthermore, studies with local intrastriatal injections indicated that the ventrolateral subregion of the neostriatum is a site of action at which adenosine A_{2A} antagonism can reverse the tremorogenic effects produced by DA antagonism.

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