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Oral tremor induced by galantamine in rats: A model of the parkinsonian side effects of cholinomimetics used to treat Alzheimer's disease

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ABSTRACT

Anticholinesterases are the most common treatment for Alzheimer's disease, and, in recent years, a new group of cholinesterase inhibitors (i.e. rivastigmine, galantamine, and donepezil) has become available. Although these drugs improve cognitive symptoms, they also can induce or exacerbate parkinsonian symptoms, including tremor. The present studies were conducted to determine if galantamine induces tremulous jaw movements, a rodent model of parkinsonian tremor, and to investigate whether these oral motor impairments can be reversed by co-administration of adenosine A_{2A} antagonists. The first experiment demonstrated that systemic injections of galantamine (0.75–6.0 mg/kg I.P.) induced a dose-related increase in tremulous jaw movements in rats. In a second study, co-administration of the muscarinic antagonist scopolamine (0.0156–0.25 mg/kg I.P.) produced a dose dependent suppression of tremulous jaw movements induced by a 3.0 mg/kg dose of galantamine, indicating that galantamine induces these tremulous oral movements through actions on muscarinic acetylcholine receptors. In two additional studies, analyses of freeze-frame video and electromyographic activity recorded from the lateral temporalis muscle indicated that the local frequency of these galantamine-induced jaw movements occurs in the 3–7 Hz frequency range that is characteristic of parkinsonian tremor. In the final experiment, the adenosine A_{2A} antagonist MSX-3 significantly attenuated the tremulous jaw movements induced by the 3.0 mg/kg dose of galantamine, which is consistent with the hypothesis that co-administration of adenosine A_{2A} antagonists may be beneficial in reducing parkinsonian motor impairments induced by anticholinesterase treatment.

1. Introduction

Alzheimer's disease affects an estimated 15 million people worldwide and accounts for approximately 50–60% of cases of dementia in people over 65 years of age (Francis et al., 1999; van Marum, 2008;). Characterization of dementia-related cholinergic dysfunctions, including reductions in choline acetyltransferase activity and acetylcholine synthesis (Bowen et al., 1976; Davies and Maloney, 1976; Perry et al., 1977), lowered levels of choline uptake (Rylett et al., 1983) and acetylcholine release (Nilsson et al., 1986), and loss of cholinergic cell bodies from the nucleus basalis of Meynert (Whitehouse et al., 1982), led to the hypothesis that cholinomimetic drugs could be useful for treating the cognitive symptoms of Alzheimer's disease and related disorders (Bartus et al., 1982; Salamone, 1986). Anticholinesterases are used to improve cognitive function in Alzheimer's disease patients (see Birks, 2006; van Marum, 2008 for review). Yet despite their positive therapeutic effects, anticholinesterases also have been shown to induce parkinsonian symptoms, including tremor, in human patients. It was reported several years ago that tacrine, which was the first anticholinesterase that was approved for treatment of Alzheimer's disease, could produce parkinsonian side effects including tremor (Ott and Lannon, 1992; Cabeza-Alvarez et al., 1999). More recently, the anticholinesterases donepezil, rivastigmine and galantamine were developed as alternatives that have largely replaced tacrine, yet tremor has been reported to occur in response to each of them (Shea et al., 1998; Arai, 2000; Aarsland et al., 2003; Emre et al., 2004; Gurevich et al., 2006; McCain et al., 2007; Litvinenko et al., 2007, 2008; Song et al., 2008).

Animal research on the tremor induced by anticholinesterases could yield insights into the neurochemical processes underlying cholinomimetic-induced parkinsonian tremor in humans, and could lead to the development of novel treatments. One rodent model of parkinsonian tremor that has undergone extensive validation is the tremulous jaw movement model (Salamone et al., 1998, 2005, 2008b; Cenci et al., 2002; Simola et al., 2004, 2006; Miwa, 2007; Miwa et al., 2008, 2009). Tremulous jaw movements are defined as repetitive vertical deflections of the lower jaw that resemble chewing but are not

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directed at a particular stimulus (Salamone et al., 1998). These movements can be induced by many conditions that are associated with parkinsonism, including neurotoxic or pharmacological depletion of striatal dopamine (DA; Jicha and Salamone, 1991; Baskin and Salamone, 1993; Steinpreis and Salamone, 1993; Rodriguez Diaz et al., 2001; Salamone et al., 2008a; 2008b), and acute or subchronic administration of DA antagonists (Steinpreis et al., 1993; Trevitt et al., 1998; Wisniecki et al., 2003; Ishiwari et al., 2005; Betz et al., 2007; Salamone et al., 2008a, 2008b). Tremulous jaw movements also are induced by cholinomimetic drugs, including muscarinic agonists such as pilocarpine, arecoline and oxotremorine (Salamone et al., 1986, 1990, 2001; Mayorga et al. 1999; Collins et al., 2010a), and the anticholinesterases physostigmine and tacrine (Mayorga et al., 1997; Cousins et al. 1999; Simola et al., 2004, 2006). As shown by studies using videotape analyses or electromyographic (EMG) methods, tremulous jaw movements occur largely within the 3–7 Hz frequency range that is characteristic of parkinsonian resting tremor (Cousins et al., 1998; Finn et al., 1997; Mayorga et al., 1997; Ishiwari et al., 2005; Collins et al., 2010a). Tremulous jaw movements also can be attenuated by several classes of antiparkinsonian drugs, including DA agonists, anticholinergics, and adenosine A_{2A} antagonists (Baskin and Salamone, 1993; Steinpreis et al., 1993; Cousins et al., 1997; Salamone et al., 1998, 2005, 2008ab; Correa et al., 2004; Simola et al., 2004, 2006; Betz et al., 2007, 2009; Tronci et al., 2007; Collins et al., 2010a; Pinna et al., 2010).

The present experiments investigated the ability of the anticholinesterase galantamine to induce parkinsonian tremor using the tremulous jaw movement model in rats. Galantamine (Reminyl) has been shown to induce and worsen tremor in human patients (Aarsland et al., 2003; Schrauwen and Ghaemi, 2006; Litvinenko et al., 2008; Grace et al., 2009). The first experiment studied the ability of galantamine (0.75 mg/kg–6.0 mg/kg) to induce tremulous jaw movements. A second study was conducted to investigate the ability of the muscarinic antagonist scopolamine to block the oral movements induced by 3.0 mg/kg galantamine in order to determine whether the tremorogenic effects of galantamine were occurring through actions on muscarinic acetylcholine (ACh) receptors. In the third and fourth studies, the local frequency range of the tremulous jaw movement "bursts" induced by galantamine administration was characterized using freeze-frame videotape analysis and EMG recording of the lateral temporalis muscle, which is the jaw-closing muscle most closely associated with tremulous jaw movement activity (Cousins et al. 1998; Collins et al., 2010a). In the final experiment, the ability of the adenosine A_{2A} antagonist MSX-3 to reverse galantamine-induced tremulous jaw movements was investigated. Adenosine A_{2A} receptors are expressed to a high degree in the neostriatum, particularly on the enkephalin-positive striatopallidal neurons (Schiffman et al., 1991; Fink et al., 1992; Rosin et al., 1998; Svenningsson et al., 1999; Fuxe et al., 2007), and adenosine A_{2A} antagonists are being assessed for their potential antiparkinsonian actions (Ferré et al., 2008; LeWitt et al., 2008; Jenner et al., 2009; Salamone, 2010). MSX-3 is a well characterized (Salamone et al., 2008a; Collins et al., 2010a,b) pro-drug that is converted in vivo into the active adenosine A_{2A} antagonist MSX-2. Based upon previous findings that adenosine A_{2A} antagonists are capable of attenuating the tremulous jaw movements induced by DA antagonists (Correa et al., 2004; Betz et al., 2009; Salamone et al., 2008a), the muscarinic agonist pilocarpine (Collins et al., 2010a), and the anticholinesterase tacrine (Simola et al., 2004; Tronci et al., 2007), it was hypothesized that MSX-3 would be able to reverse galantamine-induced tremulous jaw movements.

2. Materials and methods

2.1. Animals

A total of 35 male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) with no prior drug experience were used. The rats weighed 350–500 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. Pharmacological agents

The acetylcholinesterase inhibitor galantamine hydrobromide ((4a*S*,6*R*,8a*S*)-5,6,9,10,11,12-hexahydro-3-methoxy-11-methyl-4a*H*-[1]benzofuro[3a,3,2-*ef*] [2] benzazepin-6-ol) was obtained from Tocris Bioscience (Bristol, UK). Galantamine was dissolved in 0.9% saline. The muscarinic antagonist scopolamine hydrobromide was purchased from Sigma Aldrich Chemical (St. Louis, MO). Scopolamine was dissolved in 0.9% saline. 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 Sauer et al., 2000; 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). MSX-3 is a pro-drug of the active adenosine A_{2A} antagonist, MSX-2. All injections were intraperitoneal (IP), in a total volume of 1.0 mL/kg.

2.3. Selection of doses

The doses of galantamine used in the first experiment were similar to those used in previous behavioral experiments in rodents (Sweeney et al., 1990; Van Dam et al., 2005; Hohnadel et al., 2007; Muthuraju et al., 2009; Yano et al., 2009) and were based upon extensive pilot work. The specific dose of 3.0 mg/kg galantamine used in experiments 2–6 was based upon the results of the first experiment, and was consistent with a dose used commonly in other behavioral studies (Woodruff-Pak et al., 2001, 2007; Woodruff-Pak and Santos, 2000; Sharp et al., 2004; Hernandez et al., 2006; Hohnadel et al., 2007; Muthuraju et al., 2009; Yano et al., 2009). The doses of scopolamine used in the second experiment were selected based upon previous studies reporting that this dose range is behaviorally effective in the tremulous jaw movement model, operant choice tasks, and cognitive tasks in rodents (Warburton and Brown, 1971; Mayorga et al., 1997; Presburger and Robinson, 1999; McLaughlin et al., 2005). Doses of MSX-3 used in the final experiment were previously shown to reverse tremulous jaw movements and locomotor deficits induced by administration of DA antagonists and muscarinic agonists in rodents (Salamone et al., 2008a; Collins et al., 2010a, 2010b).

2.4. Behavioral procedures

2.4.1. 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 $r = 0.98$ ($p < 0.05$) using these methods.

2.4.2. EMG electrode implantation, recording, and analysis of tremulous jaw movements

Rats were anesthetized with a ketamine/xylazine cocktail, and two electrodes of linearly spaced 50.0 μm tungsten wire (California Fine

Wire, Grover Beach, CA) were surgically implanted approximately 1.0 mm deep with a 27-gauge needle into each lateral temporalis muscle (for a total of four electrodes). Previous research has demonstrated that the lateral temporalis muscle is the jaw muscle that shows activity most closely related to tremulous jaw movements (Cousins et al., 1998). All electrodes were then attached to a female pin (Omnetics, Minneapolis, MN) secured in a rectangular five by four pin array. Two stainless steel watch screws served as indifferent and ground electrodes. The ensemble was fastened to the skull with two additional screws and cranioplastic cement. Following electrode implantation, rats were allowed one week to recover, after which recording sessions began. On the test day, rats received an IP injection of 3.0 mg/kg galantamine. Twenty minutes later, recordings were performed for 15 min. During the recording session, the animals were connected to the recording apparatus by a multi-wire cable that was attached to a pulley system in the ceiling. All recordings were performed using the Cheetah 16 recording system and Cheetah Data Acquisition Software (Neuralynx, Bozeman, MT). During the recording session, a trained observer recorded tremulous jaw movements. At the conclusion of the recording session, data were examined using the Neuraview program (Neuralynx, Bozeman, MT), which allowed for the simultaneous viewing of EMG traces and tremulous jaw movement event recordings. Traces were then imported into Matlab 7.4 (Mathworks Inc., Natick, MA), bandpass filtered between 500 and 1500 Hz, and plotted graphically.

2.5. Experimental procedures

2.5.1. Experiment 1: induction of tremulous jaw movements by the anticholinesterase galantamine

A group of 8 rats was used to assess the effect of galantamine (0.75–6.0 mg/kg) on the induction of tremulous jaw movements. All rats received IP injections of 1.0 mL/kg saline or 0.75 mg/kg, 1.5 mg/kg, 3.0 mg/kg, or 6.0 mg/kg galantamine in a within-groups design, with all rats receiving all drug treatments in a randomly varied order (one treatment per week; no treatment sequences were repeated). Ten min after IP injection, rats were placed in the Plexiglas observation chamber and allowed 10 min to habituate. Following this habituation period, tremulous jaw movements were counted for 15 min, with the observation period divided into three separate 5-minute epochs (following the procedure of Collins et al., 2010a). Jaw movements were recorded for each of the epochs, after which both the total number of jaw movements for the observation period and the average number of jaw movements per 5 min epoch was calculated.

2.5.2. Experiment 2: blockade of galantamine-induced tremulous jaw movements by the muscarinic antagonist scopolamine

A group of 10 rats was used to assess the effects of scopolamine (0.0156–0.25 mg/kg IP) on the tremulous jaw movements induced by IP administration of 3.0 mg/kg galantamine. A within-groups design was utilized for this study, with all rats receiving all combined drug treatments in a randomly varied order (one treatment per week; no treatment sequences were repeated). On the test day each week, all rats first received an IP injection of either1.0 ml/kg saline or 0.0156 mg/kg, 0.03125 mg/kg, 0.0625 mg/kg, 0.125 mg/kg or 0.25 mg/kg scopolamine. Thirty minutes after scopolamine injection, each rat was given an IP injection of 3.0 mg/kg galantamine to yield the following combined treatment conditions: 3.0 mg/kg galantamine + saline vehicle, 3.0 mg/kg galantamine + 0.0156 mg/kg scopolamine, 3.0 mg/kg galantamine + 0.03125 mg/kg scopolamine, 3.0 mg/kg galantamine + 0.0625 mg/kg scopolamine, 3.0 mg/kg galantamine + 0.125 mg/kg scopolamine, or 3.0 mg/kg galantamine + 0.25 mg/kg scopolamine. Ten minutes after galantamine injection, rats were placed in the Plexiglas observation chamber and allowed 10 min to habituate, after which tremulous jaw movements were counted for 15 min, following the same procedure outlined above.

2.5.3. Experiment 3: freeze-frame video analysis of local frequency of the tremulous jaw movements induced by galantamine

Five rats received an injection of 3.0 mg/kg galantamine. Twenty minutes later, rats were placed in a clear Plexiglas tube (9 cm in diameter) so that a consistent view of the orofacial area could be achieved. After habituating for 10 min, each rat was videotaped for 15 min using a FlipVideo UltraHD (Cisco Systems, Farmington, CT). The sections of these video files that allowed for clear observation of the orofacial area were then subjected to a freeze-frame analysis (1 frame = 1/30 s), in which the observer went frame-by-frame through each burst of jaw movements (i.e., each group of at least two jaw movements that were within 1.0 s of each other). The observer recorded the inter-movement interval for each jaw movement within these bursts, which was defined as the number of frames between each point at which the jaw was fully closed during successive jaw movements. This information was then used to determine the local frequency within bursts of jaw movements.

2.5.4. Experiment 4: Local Frequency analysis of EMG activity recorded from the lateral temporalis muscle during galantamine-induced tremulous jaw movements

Four rats were implanted bilaterally in the lateral temporalis muscle with tungsten wire electrodes (two electrodes in each muscle for a total of four electrodes) using the procedure described above. Following a one week recovery period, rats were administered an IP injection of 3.0 mg/kg galantamine. Ten minutes later, rats were connected to the recording apparatus via a multiwire cable and placed into an elevated Plexiglas chamber for a 10 min habituation period. Following this 10 min period, recordings of EMG activity were performed as described above. Following identification of individual tremor epochs (e.g., the periods of time during which bursts of tremor were shown), a spectrum of each epoch was calculated using the multi-taper spectral estimation method (Mitra and Bokil, 2008a, 2008b). All spectral analyses were performed using the Spectral Analysis Toolbox of Chronux (http://chronux.org/).

2.5.5. Experiment 5: ability of the adenosine A_{2A} antagonist MSX-3 to attenuate galantamine-induced tremulous jaw movements

A group of 8 rats was used to assess the effects of the adenosine A_{2A} antagonist MSX-3 (1.25–10.0 mg/kg) on the tremulous jaw movements induced by administration of 3.0 mg/kg galantamine. A within-groups design was utilized for this study, with all rats receiving all drug treatments in a randomly varied order (one treatment per week; no treatment sequences were repeated). On test day each week, all rats received an IP injection of 3.0 mg/kg galantamine. Concurrently, each rat was given an IP injection of either 1.0 mL/kg saline or 1.25 mg/kg, 2.5 mg/kg, 5.0 mg/kg, or 10.0 mg/kg MSX-3, to yield the following combined treatment conditions: 3.0 mg/kg galantamine + saline vehicle, 3.0 mg/kg galantamine + 1.25 mg/kg MSX-3, 3.0 mg/kg galantamine + 2.5 mg/kg MSX-3, 3.0 mg/kg galantamine + 5.0 mg/kg MSX03, and 3.0 mg/kg galantamine + 10.0 mg/kg MSX-3. Ten minutes after injections, rats were placed in the Plexiglas observation chamber and allowed 10 min to habituate, after which tremulous jaw movements were counted for 15 min as described above.

2.6. Data analyses

The behavioral data for all experiments were analyzed using a repeated measures analysis of variance (ANOVA). Average tremulous jaw movements over the three 5-min observation epochs were calculated and then used in the ANOVA calculations. A computerized statistical program (SPSS 12.0 for Windows) was used to perform these 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). All spectral analyses

were performed using the Spectral Analysis Toolbox of Chronux (http://chronux.org/; Mitra and Bokil, 2008a, 2008b).

3. Results

3.1. Experiment 1: induction of tremulous jaw movements by the anticholinesterase galantamine

Fig. 1A shows the effects of injections of galantamine (0.75–6.0 mg/kg) on tremulous jaw movement activity. Repeated measures ANOVA revealed that there was a significant overall effect of drug treatment on tremulous jaw movement activity ($F(4,28)=25.796$; $p<0.001$). Planned comparisons showed that the 1.5 mg/kg ($p<0.01$), 3.0 mg/kg ($p<0.001$) and 6.0 mg/kg ($p<0.001$) doses of galantamine all significantly induced tremulous jaw movements ($p<0.001$) compared to rats treated with saline vehicle.

3.2. Experiment 2: blockade of galantamine-induced tremulous jaw movements by the muscarinic antagonist scopolamine

Fig. 1B illustrates that administration of the muscarinic antagonist scopolamine blocked the tremulous jaw movements induced by 3.0 mg/kg galantamine. Repeated measures ANOVA revealed that there was a significant overall effect of scopolamine treatment on the induction of tremulous jaw movement activity by 3.0 mg/kg galantamine ($F(5,45)=136.2$; $p<0.001$). Planned comparisons showed that all 5 doses of scopolamine (0.0156 mg/kg; 0.03125 mg/kg; 0.0625 mg/kg; 0.125 mg/kg; and 0.25 mg/kg) were capable of significantly reducing the jaw movements induced by 3.0 mg/kg galantamine (different from galantamine plus saline, $p<0.001$). Orthogonal analysis of trend showed that there was a significant linear dose-related trend ($F(1,9)=202.2$, $P<0.001$) and a significant dose-related quadratic trend ($F(1,9)=250.4$, $p<0.001$).

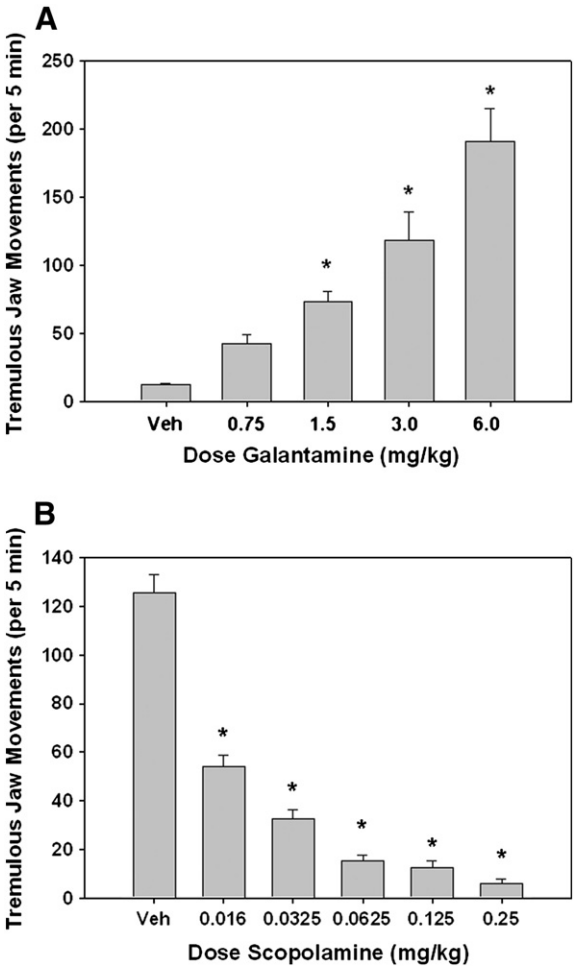

Fig. 1. A. Effects of different doses of galantamine (IP) on tremulous jaw movements. Mean (± SEM) number of jaw movements in rats treated with either saline vehicle (Veh) or galantamine. *significant difference from vehicle control ($p<0.05$). B. Effect of the muscarinic antagonist scopolamine on galantamine-induced tremulous jaw movements. All rats received an IP injection of 3.0 mg/kg galantamine. Mean (± SEM) number of jaw movements in rats treated with galantamine plus vehicle (Veh), and galantamine plus various doses of scopolamine (0.01625–0.25 mg/kg IP). *significant difference from galantamine plus vehicle (Veh) control ($p<0.05$).

3.3. Experiment 3: freeze-frame video analysis of local frequency of the tremulous jaw movements induced by galantamine

Fig. 2 displays the results of the freeze-frame analyses of videotaped samples of galantamine-induced jaw movement activity. A total of 753 jaw movements were analyzed. 65.78% of these jaw movements took place within "bursts," defined as a group of at least two jaw movements that were within 1.0 s of each other. Data are shown as the number of inter-movement intervals between movements in each burst, defined as the number of 1/30 s frames between each point at which the jaw was fully closed during successive jaw movements. To interpret these data in terms of frequencies (i.e. jaw movements per second), the reciprocal of the inter-movement interval was calculated (e.g. 5/30 frames per second corresponds to 6 Hz; 6/30 frames per second to 5 Hz, etc.) The vast majority (96.95%) of the jaw movement activity within bursts took place in the 3.0–7.5 Hz frequency range, with a peak in activity in the 5–6 Hz range. Repeated measures ANOVA revealed that the number of inter-movement intervals showed an overall significant difference across the different interval bins ($F(29,116)=6.684$; $p<0.001$, $R^2=0.626$).

3.4. Experiment 4: local frequency analysis of EMG activity recorded from the lateral temporalis muscle during galantamine-induced tremulous jaw movements

Fig. 3 displays representative 2-s traces recorded from the lateral temporalis muscle of an animal that received an injection of 3.0 mg/kg galantamine. The top panel shows raw EMG and spectral analysis data

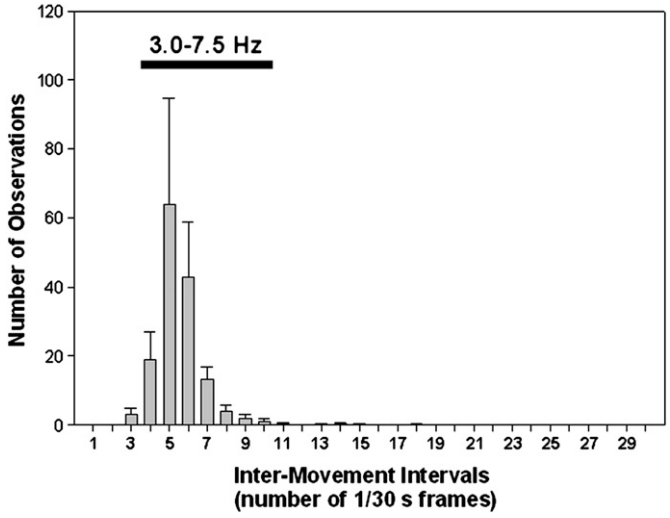

Fig. 2. This figure shows the results of the freeze-frame analysis of inter-movement intervals for the galantamine-induced tremulous jaw movements in 5 representative rats. Distribution of the mean (±SEM) number of inter-movement intervals within each 1/30 s time bin is shown. Bar indicates the inter-movement times that correspond to the 3.0–7.5 Hz frequency range (i.e., 4/30 = 7.5 Hz, 10/30 = 3.0 Hz).

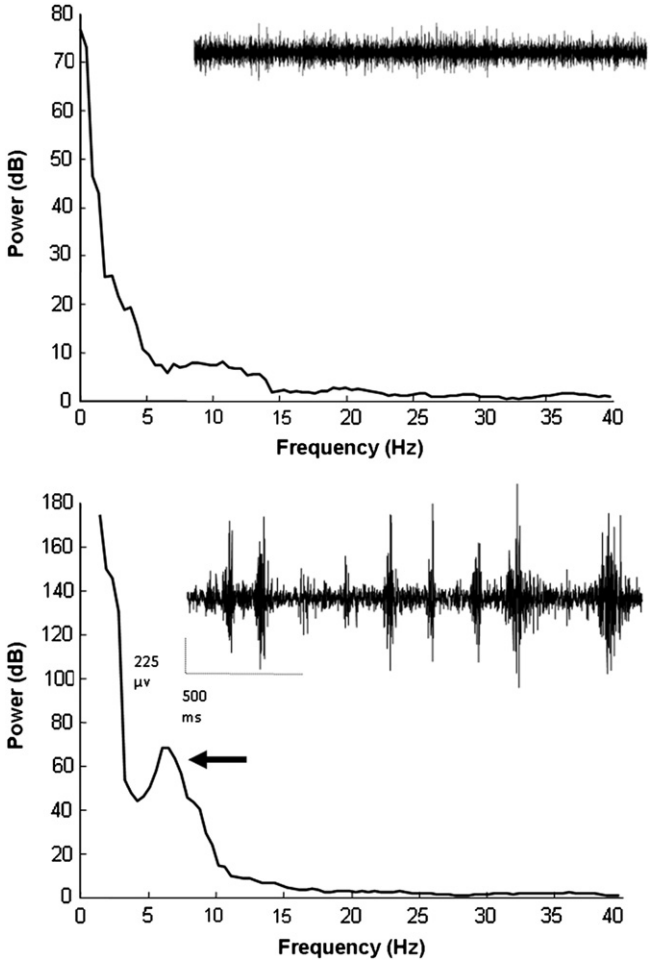

Fig. 3. Raw EMG traces obtained from the temporalis muscle, and the corresponding spectral analyses. Top. Representative raw EMG trace (2 s sweep) from the temporalis muscle of a rat that received 3.0 mg/kg galantamine, during which no tremulous jaw movements were shown. During this period of quiescence (i.e. when no jaw movements were occurring), spectral analysis of the trace using multi-taper spectral estimation showed no peak in the 3–7 Hz frequency range (only inherent noise in the recording system at frequencies less than 1 Hz, and an exponential decay at higher frequencies). Bottom. Representative raw EMG trace (2 s sweep) from the temporalis muscle of a rat that received 3.0 mg/kg galantamine, during which the rat showed a large burst of multiple jaw movements recorded by the observer. The temporalis muscle is a jaw closing muscle, and this burst of activity in the 5–6 Hz frequency range corresponded with the jaw closing phase of each jaw movement. Spectral analysis of this trace using multi-taper spectral estimation carried out using the Spectral Analysis Toolbox of the Chronux software package indicated a peak in power at the 5–6 Hz frequency (arrow).

for a trace in which there were no tremulous jaw movements. During this period without jaw movements, there was no peak in the 3–7 Hz range. The bottom panel shows a raw 2-s EMG trace during which the rat showed a large burst of tremulous jaw movement activity. As the trace illustrates, these movements were accompanied by jaw muscle activity in the 5–6 Hz frequency range. Furthermore, spectral analysis of this trace using the multi-taper spectral estimation method demonstrated that the peak in power of the tremor epoch occurred at 5–6 Hz. These findings are consistent with freeze frame videotape analyses performed in the third experiment.

3.5. Experiments 5: ability of the adenosine A_{2A} antagonist MSX-3 to attenuate the tremulous jaw movements induced by galantamine

Co-administration of the adenosine A_{2A} antagonist MSX-3 attenuated the tremulous jaw movements induced by a dose of 3.0 mg/kg galantamine (Fig. 4). Repeated measures ANOVA revealed that there was a significant overall effect of MSX-3 treatment on the induction of tremulous jaw movement activity by 3.0 mg/kg galantamine ($F(4,28) = 5.364$; $p<0.01$). Planned comparisons showed that the 5.0 and 10.0 mg/kg doses of MSX-3 were capable of significantly reducing the tremulous jaw movements induced by 3.0 mg/kg galantamine (i.e., compared to galantamine plus saline; $p<0.01$).

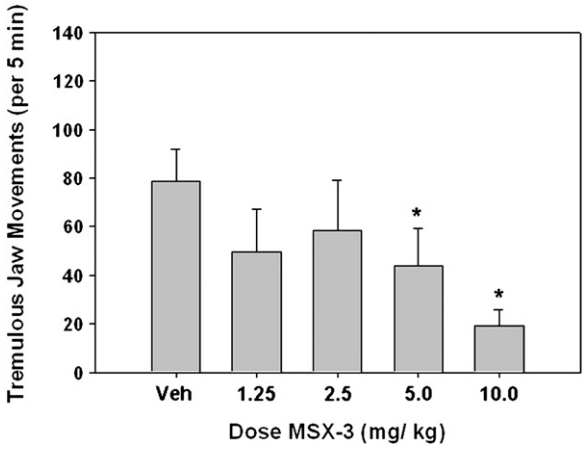

Fig. 4. Effect of the adenosine A_{2A} antagonist MSX-3 on the tremulous jaw movements induced by 3.0 mg/kg galantamine IP. Mean (±SEM) number of jaw movements in rats treated with galantamine plus vehicle (Veh), and galantamine plus various doses (1.25–10.0 mg/kg) of MSX-3. *significant difference from galantamine plus vehicle (Veh) control ($p<0.05$).

4. Discussion

The present studies investigated the ability of the anticholinesterase galantamine to induce tremulous jaw movements, which is a widely used rodent model of parkinsonian tremor (Salamone et al., 1998, 2005, 2008b; Simola et al., 2004, 2006; Miwa et al., 2008, 2009; Kasture et al., 2009; Trevitt et al., 2009; Delattre et al., 2010). As shown in Fig. 1A, galantamine induced tremulous jaw movements at a dose range of 1.5–6.0 mg/kg. This finding is consistent with previous studies showing that another anticholinesterase, tacrine (Cognex), also is able to induce tremulous jaw movements in rats (Carriero et al. 1997; Mayorga et al., 1997; Finn et al., 1997; Cousins et al., 1997, 1998; Trevitt et al. 1997, 1999; Trevitt et al., 2009; Simola et al., 2004; Betz et al., 2005, 2007; Tronci et al., 2007; Miwa et al., 2008, 2009; Vanover et al., 2008). Local frequency analysis of the galantamine-induced jaw movements using both freeze frame video analysis (Fig. 2) and EMG activity recorded from the lateral temporalis muscle (Fig. 3) indicated that the tremulous jaw movements induced by 3.0 mg/kg galantamine occurred in the 3–7 Hz frequency range, with a peak frequency in the vicinity of 5–6 Hz. Although previous research in this area focused upon visual inspection of raw EMG traces (Cousins et al., 1998; Collins et al., 2010a), this is the first study to characterize the local frequency of tremulous jaw movements using quantitative spectral analysis of EMG data. Spectral analysis of an individual identified tremor burst epoch demonstrated that a peak in power occurred at 5–6 Hz, a phenomenon that was not present in the spectrum when tremor was not occurring (Fig. 3). This frequency range is consistent with the overall finding that the local frequency of the tremulous jaw movements induced by DA depletion, D2 antagonism, and administration of other cholinomimetic drugs (e.g. tacrine) is in the 3–7 Hz range (Salamone and Baskin, 1996; Finn et al., 1997; Mayorga et al., 1997; Cousins et al., 1998; Ishiwari et al., 2005; Collins et al., 2010a). Moreover, this 3–7 Hz frequency range is consistent with that reported during resting tremor in parkinsonian patients, and is distinct from that seen in dyskinesias (1–2 Hz) and postural tremor (8–12 Hz) (Findley et al., 1981; Marsden, 1984; Deuschl

et al., 2000, 1996; Dueschl, 1999; Spieker et al., 1997). Galantamine belongs to a later generation of anticholinesterases that was introduced after tacrine, and, although earlier studies suggested that galantamine did not worsen motor symptoms (Fuchs et al., 2004), later reports have repeatedly demonstrated that galantamine can induce or exacerbate tremor in humans (Schrauwen and Ghaemi, 2006; Litvinenko et al. 2007; Litvinenko et al., 2008; Grace et al., 2009). Other "second generation" anticholinesterases, including donepezil and rivastigmine, also have been shown to induce tremor in human patients (Bourke and Druckenbrod, 1998; Iwasaki et al., 1988; Kao et al., 1993; McSwain and Forman, 1995; Shea et al., 1998; Arai, 2000; Gurevich et al., 2006; Oertel et al., 2008; Song et al., 2008). Taken together, these results raise possible concerns for the widespread use of cholinesterase inhibitors in clinical populations, and suggest the need for the development of treatment strategies to counteract the motor impairments induced by anticholinesterase therapy.

The muscarinic antagonist scopolamine potently blocked the tremulous jaw movements induced by 3.0 mg/kg of galantamine (Fig. 1B). These results are consistent with previous findings demonstrating that cholinomimetic-induced tremulous jaw movements result from central muscarinic stimulation. The anticholinesterase neostigmine, which provides peripheral muscarinic and nicotinic receptor stimulation but does not pass the blood–brain barrier, did not induce tremulous jaw movements (Rupniak et al. 1983, 1985). Systemic administration of carbachol, which does not penetrate easily into the brain, also failed to induce tremulous jaw movements (Salamone et al., 1986). In addition, Salamone et al. (1986) reported that the tremulous jaw movements induced by the muscarinic agonist pilocarpine were blocked by scopolamine, but not by methylscopolamine, an N-methylated derivative of scopolamine that does not cross the blood brain barrier. Similar findings were reported by Kelley et al. (1989), who observed that atropine blocked physostigmine-induced tremulous jaw movements, but methylatropine did not. Several lines of evidence indicate that cholinomimetic-induced tremulous jaw movements result from stimulation of muscarinic ACh receptors in the neostriatum, specifically in the ventrolateral region. Intracranial injections of either physostigmine or pilocarpine into ventrolateral neostriatum induced tremulous jaw movements, while injections into other striatal subregions were ineffective (Kelley et al., 1989; Salamone et al., 1990). Local injections of scopolamine into the ventrolateral neostriatum blocked the tremulous jaw movements induced by pilocarpine (Salamone et al., 1990) as well as the anticholinesterase tacrine (Mayorga et al., 1997). Tacrine-induced tremulous jaw movements were attenuated by ventrolateral neostriatal injections of hemicholinium-3, which inhibits ACh synthesis by blocking choline uptake, while injections of this drug into overlying neocortex were ineffective (Cousins et al., 1998). In addition, microdialysis studies indicated that the elevation of extracellular ACh in ventrolateral neostriatum that was produced by tacrine administration was correlated with the level of tremulous jaw movement activity induced by this drug (Cousins et al. 1999). This evidence suggests that galantamine may be having its tremorogenic effect through stimulation of central muscarinic receptors within the neostriatum, which contains cholinergic interneurons and expresses several subtypes of muscarinic receptors (Hersch et al., 1994; Ince et al., 1997; Zhou et al., 2003).

The finding that scopolamine was able to block galantamine-induced tremulous jaw movements is consistent with clinical studies demonstrating that muscarinic antagonists are effective treatments for parkinsonian symptoms, including tremor (Marsden et al., 1975; McEvoy, 1983; Shrag et al., 1999; Bain, 2002; Milanov, 2001; Romrell et al., 2003). However, muscarinic antagonists would not be useful as treatments for galantamine-induced tremor in Alzheimer's disease patients because these drugs would also antagonize the cognitive benefits of cholinomimetic drugs. For that reason, the final experiment sought to investigate the ability of the adenosine A_{2A} antagonist MSX-3 to reverse the tremulous jaw movements induced by 3.0 mg/kg galantamine. As seen in Fig. 4, MSX-3 significantly attenuated galantamine-induced tremulous jaw movements. Previous findings using other cholinomimetics and adenosine A_{2A} antagonists have reported similar results. The adenosine A_{2A} antagonists SCH 58261 and MSX-3 reduced the tremulous jaw movements induced by a low dose of the muscarinic agonist pilocarpine (Collins et al., 2010a). Similar findings have been reported using the adenosine A_{2A} antagonists SCH 58251, ST1535, and ANR 94 to reverse the tremulous oral movements induced by a low dose (2.5 mg/kg) of the anticholinesterase tacrine (Simola et al., 2004; Tronci et al., 2007; Pinna et al., 2010). The doses of MSX-3 used in the present study have been shown to produce substantial in vivo occupancy of striatal adenosine A_{2A} receptors (Collins et al., 2010a). In contrast, the adenosine A_1 antagonist DPCPX did not occupy striatal adenosine A_{2A} receptors in vivo, and also did not reverse cholinomimetic induced tremulous jaw movements (Collins et al., 2010a). Together with these previous findings, the present results with the tremulous jaw movement model suggest that selective adenosine A_{2A} antagonists could be useful for attenuating motor side effects such as tremor that are induced by anticholinesterases in humans.

In addition to reversing motor impairments induced by cholinergic stimulation, there is a growing body of recent evidence suggesting that adenosine A_{2A} antagonists may also have utility in treating cognitive dysfunction (see Shen and Chen, 2009 for review). Administration of the adenosine A_{2A} antagonist SCH 58261 has been shown to improve social recognition memory (Prediger et al., 2005a, 2005b) and to improve memory performance in a variety of behavioral tasks (Takahashi et al., 2008). In adenosine A_{2A} knockout mice, spatial recognition memory and novelty exploration were found to be enhanced compared to wild-type mice (Wang et al., 2006). Perhaps most strikingly, in mice with β-amyloid induced memory loss, both pharmacological blockade and genetic knockout of adenosine A_{2A} receptors were found to improve memory (Dall'igna et al., 2007; Cunha et al., 2008). Conversely, stimulation of adenosine A_{2A} receptors, either through adenosine A_{2A} agonist administration or transgenic overexpression of A_{2A} receptors in the cortex, has been shown to impair memory retrieval and performance on spatial working memory tasks (Gimenez-Llort et al., 2002; Pereira et al., 2005). Taken together, these results indicate that co-administration of adenosine A_{2A} receptor antagonists with anticholinesterases may not only improve parkinsonian motor impairments such as tremor that are induced by the administration of these cholinomimetic agents, but may also help to further augment the cognitive enhancement produced by cholinesterase inhibition. Additional studies will seek to more completely characterize the effects of these adenosine A_{2A} antagonists on cognition and motor function, an area of research that is critical as anticholinesterases continue to be used in the treatment of Alzheimer's disease, and in patients with Parkinson's disease who are suffering from dementia.

In summary, the present results indicate that galantamine produces tremulous jaw movements in rats that are similar to those produced by other anticholinesterases, such as tacrine. The results of the present experiments with rats are consistent with human studies suggesting that caution should be used when prescribing anticholinesterases for the treatment of Alzheimer's disease or dementia in parkinsonian patients, as the use of these compounds may induce or exacerbate parkinsonian symptoms such as resting tremor. Galantamine-induced tremulous jaw movements in rats occur in the parkinsonian tremor frequency range, and are blocked by muscarinic receptor antagonism. The ability of the adenosine A_{2A} receptor antagonist MSX-3 to reverse galantamine-induced tremulous jaw movements in rats is consistent with the growing literature indicating that this class of drugs can act on neostriatal adenosine A_{2A} receptors to exert antiparkinsonian actions (Ferré et al., 2001, 2008; Wardas et al., 2003; Simola et al., 2004; Schwarzschild et al., 2006; Salamone et al., 2008a,b; Morelli et al., 2009;

Jenner et al., 2009; Salamone, 2010). Furthermore, the ability of adenosine A_{2A} antagonists to attenuate tremor induced by anticholinesterases is consistent with previous studies showing that parkinsonian tremor in humans can be reduced by the nonselective adenosine antagonist theophylline (Mally and Stone, 1996) and the adenosine A_{2A} antagonist istradefylline (Bara-Jimenez et al., 2003). Future studies should seek to investigate whether administration of adenosine A_{2A} receptor antagonists may improve parkinsonian motor dysfunctions induced by anticholinesterases while also augmenting the cognitive enhancing benefits of these compounds.

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