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European Journal of Pharmacology 364 (1999) 7–11

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Characterization of the muscarinic receptor subtype mediating pilocarpine-induced tremulous jaw movements in rats

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Received 27 October 1998; accepted 30 October 1998

Abstract

Four muscarinic receptor antagonists with varying selectivities for the four pharmacologically-defined muscarinic receptor subtypes (M_1–M_4) were administered into the lateral ventricle to determine their relative potency in reducing tremulous jaw movements induced by i.p. injection of the muscarinic receptor agonist pilocarpine (4.0 mg/kg). All four muscarinic receptor antagonists reduced tremulous jaw movements in a dose-dependent manner, with the following rank order of potency: scopolamine > methoctramine ≥ telenzepine > pirenzepine. This pattern is inconsistent with the rank order of affinity of these agents at the muscarinic M_1 receptor, and is consistent with their rank order of affinity at muscarinic M_2 or M_4 receptors. Because tremulous jaw movements are related to striatal function, and the muscarinic M_4 receptor is more predominant than the muscarinic M_2 receptor as a post-synaptic receptor in striatum, the present results suggest that pilocarpine induces jaw movements due to muscarinic M_4 receptor stimulation. In view of the hypothesized relation between parkinsonism and cholinomimetic-induced jaw movements, these data suggest that a centrally-acting muscarinic M_4 receptor antagonist could be useful as an antiparkinsonian agent.

Keywords: Acetylcholine; Tremor; Parkinson's disease; Motor; Vacuous chewing

1. Introduction

Systemic administration of cholinomimetic drugs induces vacuous or purposeless jaw movements in rats (Rupniak et al., 1983; Salamone et al., 1986, 1990). These movements are defined as rapid, repetitive vertical deflections of the lower jaw that resemble chewing but are not directed at any object (e.g., Mayorga et al., 1996). It has been suggested that cholinomimetic-induced jaw movements in rats share important characteristics with parkinsonian tremor. The jaw movements induced by anticholinesterases such as tacrine and muscarinic agonists such as pilocarpine are tremulous in nature; they occur largely in bursts, with a peak activity in the 3–7 Hz frequency range that is characteristic of parkinsonian tremor (Finn et al., 1996; Mayorga et al., 1996). In addition, cholinomimetic-induced jaw movements are reversed by several antiparkinsonian drugs, including L-dihydroxyphenylalanine, apomorphine, bromocriptine, amantadine and benztropine (Stewart et al., 1988; Cousins et al., 1997).

Considerable evidence indicates that cholinomimetic-induced jaw movements are induced by central muscarinic receptor stimulation (Salamone et al., 1986, 1990; Mayorga et al., 1996). Consistent with the hypothesis that these movements are related to parkinsonism, it has been reported that cholinomimetic-induced jaw movements are mediated by stimulation of muscarinic receptors in the ventrolateral striatum, the rodent homologue of the human ventral putamen (Salamone et al., 1990; Mayorga et al., 1996). However, the muscarinic receptor subtype responsible for the generation of cholinomimetic-induced jaw movements remains uncertain. In view of the fact that AFDX 116 (11(2-diethylamino) methyl-1-1-piperidinyl acetyl-5,11,dihydro 6*H*-pyrido (2,3-*b* 1,4) benzodiazepine-6-one) was more potent than pirenzepine at reducing pilocarpine-induced jaw movements (Stewart et al., 1989), it was previously suggested that these movements were re-

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0014-2999/99/$ - see front matter
PII: S0014-2999(98)00811-5

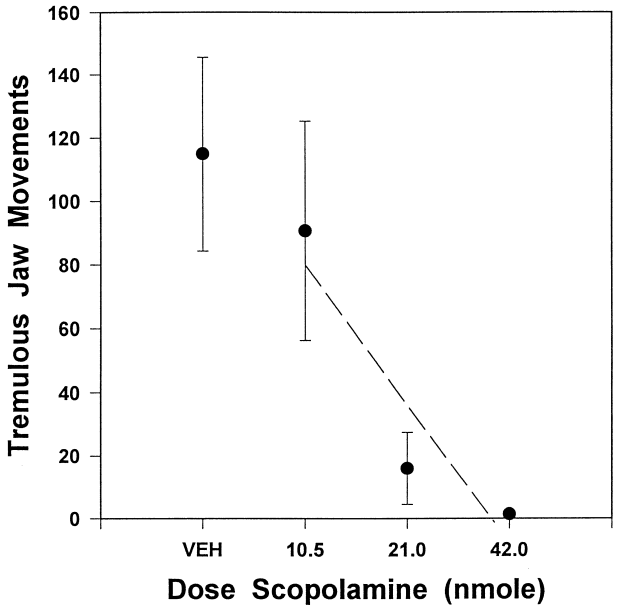

Fig. 1. Dose/response data for the effect of scopolamine on the jaw movements induced by pilocarpine. All rats received i.p. injections of 4.0 mg/kg pilocarpine plus intraventricular injections of vehicle (VEH) or scopolamine. For all figures, mean (±S.E.M.) number of jaw movements per 5 min are shown. Dashed line indicates least-squares regression line.

lated to muscarinic M_2 and not muscarinic M_1 receptor stimulation. Yet, more recent neurochemical research has demonstrated that there are at least five subtypes of muscarinic receptor proteins (m_1–m_5) in the neostriatum (Hersch et al., 1994; Reever et al., 1997), and receptor binding studies with a new generation of selective antagonists have identified four pharmacologically-defined muscarinic receptor subtypes (M_1–M_4; Lazareno et al., 1990). Thus, the present experiments studied the effects of the muscarinic antagonists scopolamine, methoctramine, telenzepine and pirenzepine on pilocarpine-induced jaw movements. These drugs were selected because they show different rank orders of potency for the pharmacologically-defined muscarinic receptor subtypes (Lazareno et al., 1990). Intraventricular injections of these antagonists were used because of the poor central penetrability of pirenzepine and methoctramine; direct intrastriatal injections were not performed due to the low potency of pirenzepine, and because of limitations on the volumes used with direct injections into brain tissue.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (300–350 g) were used for these experiments. The colony was maintained at 23°C, and the colony was on a 12 h light/dark cycle (lights on 0700).

2.2. Drugs

Pilocarpine, scopolamine, methoctramine, telenzepine and pirenzepine all were obtained from RBI (Natick, MA). These drugs were dissolved in 0.9% saline solution for systemic and intraventricular injection.

2.3. Procedures

Rats were anesthetized with sodium pentobarbital (50.0 mg/kg) and implanted unilaterally with stainless steel guide cannulae, with the tip of the cannula resting just above the right lateral ventricle (AP + 0.5, LM + 1.3, DV − 3.0). Rats were allowed to recover for 7 days prior to behavioral testing. Muscarinic antagonists or saline vehicle were administered via an injector set to extend 1.5 mm beyond the tip of the guide cannula into the lateral ventricle. The following doses of each drug were used: scopolamine-vehicle, 4.0, 8.0 and 16.0 μg (10.5, 21.0 and 42.0 nmol; $n = 5$–8 per group); methoctramine-vehicle, 25.0, 50.0 and 100.0 μg (34.0, 68.0 and 136.0 nmol; $n = 5$–10 per group); telenzepine-vehicle, 6.25, 12.5, 25.0, 50.0 and 100.0 μg (14.0, 28.0, 56.0, 112.0 and 224.0 nmol; $n = 6$–10 per group); pirenzepine-vehicle, 25.0, 50.0 and 100.0 μg (59.0, 118 and 236 nmol; $n = 7$–12 per group). The injector was connected to a syringe pump via PE-10 tubing and all injections were made in a 2.0 μl volume at a rate of 1.0 μl/min. The injector was left in place for 1 min to allow for drug diffusion, then rats were immediately injected with 4.0 mg/kg pilocarpine (i.p.)

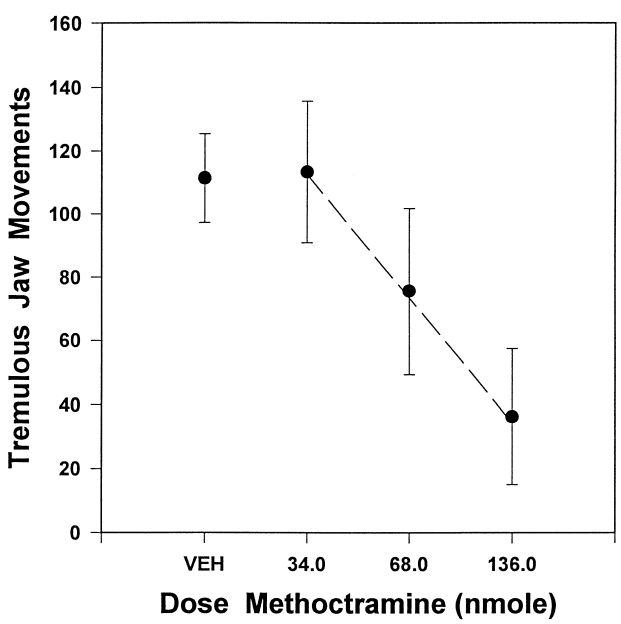

Fig. 2. Dose/response data for the effect of methoctramine on the jaw movements induced by pilocarpine. All rats received i.p. injections of 4.0 mg/kg pilocarpine plus intraventricular injections of vehicle (VEH) or methoctramine. Dashed line indicates least-squares regression line.

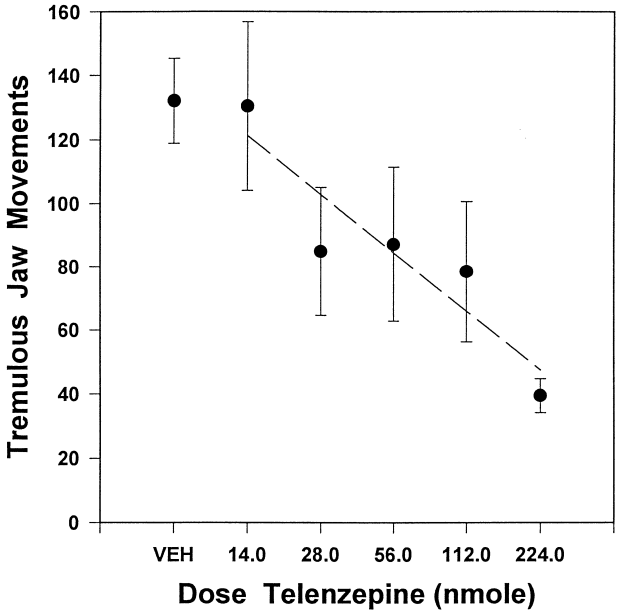

Fig. 3. Dose/response data for the effect of telenzepine on the jaw movements induced by pilocarpine. All rats received i.p. injections of 4.0 mg/kg pilocarpine plus intraventricular injections of vehicle (VEH) or telenzepine. Dashed line indicates least-squares regression line.

and placed into a Plexiglas observation chamber for a 10 min habituation. The number of tremulous jaw movements were counted for a 5-min period (initiated 10 min after pilocarpine administration) by an observer who was unaware of the treatment. Tremulous jaw movements were defined as rapid, repetitive vertical deflections of the lower jaw that resemble chewing but were not directed at any object; each individual deflection of the jaw was counted as a single jaw movement.

2.4. Data analyses

Data for each of the four antagonist experiments were analyzed in two different ways. Raw jaw movement data (number of movements per 5 min) for each drug were analyzed by a means-based analysis of variance (ANOVA), which included the control group, to test for the overall significance of the drug effects. In addition, linear regression analyses were performed to determine ED_{50} values for the suppression of pilocarpine-induced jaw movements by each antagonist. Excluding the control data, the dose values (in nanomoles) for each drug were log transformed, and least-squares regression lines were calculated using the linear regression procedure in Systat 5.0. ED_{50} values were calculated from the regression equations, by solving for the dose that generated a jaw movement value that was 50% of the control mean. ED_{50} values were then converted back to the arithmetic scale by taking the antilogarithm of the log ED_{50}. Standard errors of estimate for the dose axis of each regression were determined, and 95% confidence intervals around the ED_{50} were determined by multiplying the standard error of estimate by the Student's t-value at $P = 0.05$ (two tailed) for the appropriate degrees of freedom value.

3. Results

All four muscarinic receptor antagonists significantly reduced tremulous jaw movements induced by 4.0 mg/kg pilocarpine (Figs. 1–4). Fig. 1 shows the effects of scopolamine. ANOVA demonstrated that there was an overall significant effect of drug treatment ($F(3,30) = 4.8$, $P < 0.02$), and the linear regression was statistically significant ($F(1,14) = 9.2$, $P < 0.01$), demonstrating that the effect of scopolamine was dose-related. Methoctramine produced a significant overall suppressive effect on pilocarpine-induced jaw movements (Fig. 2; $F(3,27) = 3.2$, $P < 0.05$). The linear relation between dose and response was significant ($F(1,20) = 5.2$, $P < 0.05$). In Fig. 3, the results of the telenzepine experiment are shown. There was an overall significant effect of drug treatment ($F(5,47) = 3.0$, $P < 0.02$), and a significant linear relation between dose and response ($F(1,40) = 6.4$, $P < 0.02$). Like the other drugs, pirenzepine also suppressed jaw movements ($F(3,31) = 5.1$, $P < 0.01$), and there was a significant linear dose/response relation ($F(1,24) = 11.4$, $P < 0.002$). Table 1 shows the ED_{50} values for each of the four muscarinic antagonists for the suppression of pilocarpine-induced jaw movements. The rank order of antagonist potency for the reversal of tremulous jaw movement production was scopolamine > methoctramine ≥ telenzepine > pirenzepine.

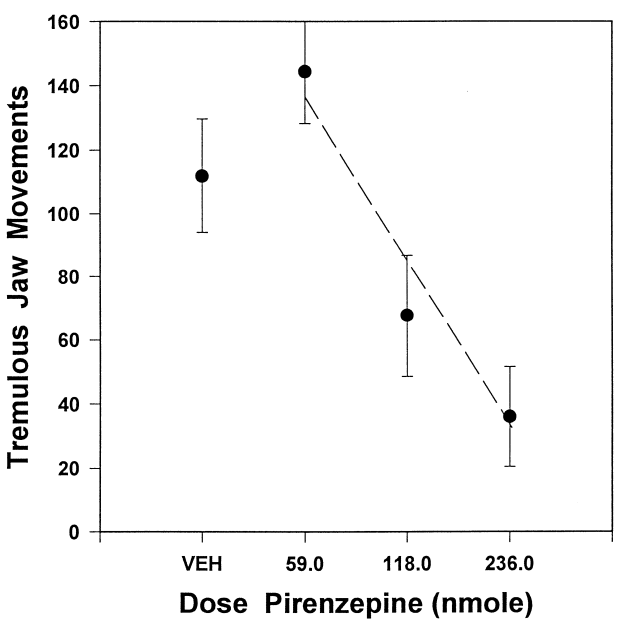

Fig. 4. Dose/response data for the effect of pirenzepine on the jaw movements induced by pilocarpine. All rats received i.p. injections of 4.0 mg/kg pilocarpine plus intraventricular injections of vehicle (VEH) or pirenzepine. Dashed line indicates least-squares regression line.

Table 1
Potency data for suppression of pilocarpine-induced tremulous jaw movements (TJM) by muscarinic antagonists, and affinity data for muscarinic receptor binding

Drug	TJM suppression ED_{50} (nmole) (±95% confidence intervals)	Affinity (pK_i)[a]			
		M_1	M_2	M_3	M_4
Scopolamine	14.4 (±3.4)	9.73	8.85	9.74	9.65
Methoctramine	96.2 (±3.5)	7.60	8.34	6.88	7.81
Telenzepine	104.3 (±4.9)	8.89	7.77	8.12	8.58
Pirenzepine	171.3 (±3.4)	8.02	6.48	7.09	7.47

[a]Affinity data from Lazareno et al. (1990).

Table 1 also shows binding data with these drugs for the four pharmacologically-defined muscarinic receptors as reported by Lazareno et al. (1990).

4. Discussion

Intraventricular injections of all four muscarinic receptor antagonists produced a significant suppression of pilocarpine-induced tremulous jaw movements. The rank order of potency was scopolamine > methoctramine ≥ telenzepine > pirenzepine. Based upon an examination of the binding affinities of these drugs for muscarinic receptors (Table 1), the present results suggest that the pharmacological profile of the muscarinic receptor involved in the production of pilocarpine-induced jaw movements is not of the muscarinic M_1 or M_3 receptor subtypes. If muscarinic M_1 receptor stimulation was the critical mechanism for stimulation of tremulous jaw movements, then methoctramine should have been less potent than telenzepine and pirenzepine for suppression of pilocarpine-induced jaw movements. In fact, methoctramine was more potent than pirenzepine, and approximately the same potency as telenzepine. Thus, in view of the relative potency of methoctramine in suppressing pilocarpine-induced jaw movements, the present results suggest that muscarinic M_2 or M_4 receptor subtypes are involved in the production of jaw movement activity. This observation is consistent with the report of Stewart et al. (1989), who had suggested a non-muscarinic M_1 receptor mechanism for pilocarpine-induced jaw movements at the time when only muscarinic M_1 and M_2 receptors had been distinguished pharmacologically (Stewart et al., 1989).

Although the present results employed intraventricular injections of muscarinic receptor antagonists, considerable evidence indicates that the neostriatum, particularly the ventrolateral striatum, is involved in the production of tremulous jaw movements. Injections of physostigmine or pilocarpine into the ventrolateral striatum induced jaw movement activity, while injections into other brain regions were ineffective (Kelley et al., 1989; Salamone et al., 1990). Local injections of low doses of atropine or scopolamine into ventrolateral striatum suppress cholinomimetic-induced jaw movements (Kelley et al., 1989; Salamone et al., 1990; Mayorga et al., 1996). Inhibition of acetylcholine synthesis by local injections of hemicholinium suppressed tacrine-induced jaw movements when hemicholinium was injected into the ventrolateral striatum, but not into overlying cortex (Cousins et al., in press).

In view of the data indicating that inhibition of striatal muscarinic receptors with non-selective antagonists can suppress jaw movements, it is relevant to note that the muscarinic M_1, M_2, M_3 and M_4 receptors all have been identified in striatum (Waelbroeck et al., 1990). The present data suggest that muscarinic M_1 and M_3 receptors are not involved in the production of jaw movement activity, and that the receptor involved in the production of pilocarpine-induced jaw movements has the pharmacological properties of the muscarinic M_2 or M_4 receptor. Considerable evidence indicates that the muscarinic M_2 receptor is largely a presynaptic receptor in striatum, and is the major autoreceptor on cholinergic terminals in striatum (Hersch et al., 1994; Billard et al., 1995). It is unlikely that blockade of muscarinic M_2 autoreceptors would block pilocarpine-induced jaw movements, because this action increases release of acetylcholine (Billard et al., 1995). Previous work with anticholinesterases indicates that elevations of extracellular acetylcholine act to induce, rather than suppress, jaw movement activity (Carriero et al., 1997; Cousins et al., in press; Mayorga et al., 1996). Thus, although it is possible that antagonism of an M_2 heteroreceptor could block jaw movement activity, it seems very unlikely that antagonism of muscarinic M_2 autoreceptors would do so. The muscarinic m_4 receptor protein, which is thought to correspond to the pharmacological muscarinic M_4 receptor, is abundant in striatum (Hersch et al., 1994). Evidence indicates that, unlike the muscarinic M_2 receptor, the muscarinic M_4 receptor is commonly found as a postsynaptic muscarinic receptor on striatal output neurons (Hersch et al., 1994). Thus, it is possible that striatal muscarinic M_4 receptors are involved in the induction of jaw movements by pilocarpine. Development of more potent and selective muscarinic M_4 receptor antagonists and agonists will allow further study of the role of striatal M_4 receptors in the production of pilocarpine-induced tremulous jaw movements. In view of the hypothesized relation between cholinomimetic-induced jaw movements and parkinsonism, the present data suggest that a centrally-acting muscarinic M_4 receptor antagonist could be useful for the treatment of parkinsonian tremor (Salamone, 1997).

Acknowledgements

This work was supported by a grant from the NIH (NINDS).

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