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Short communication

Biphasic effects of oxotremorine-M on turning behavior induced by caffeine in 6-OHDA-lesioned rats

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Abstract

This work studied the interactions between cholinergic and adenosine systems in the denervated striatum. For that purpose, we evaluated the effects of an intrastriatal administration of the muscarincic receptor agonist, oxotremorine-M on turning behavior induced by systemic caffeine in unilaterally 6-hydroxydopamine-lesioned rats. Low doses of oxotremorine-M (0.1 ng/μl) enhanced, whereas high doses (100 ng/μl) attenuated contralateral turning induced by caffeine. These results support a functional link between muscarinic and adenosinergic systems in the denervated striatum and suggest opposite actions of muscarinic M_2 and M_1 receptors on caffeine-induced turning behavior.

Keywords: Caffeine; Oxotremorine-M; Turning behavior; Striatum; Adenosine/acetylcholine interaction

1. Introduction

In 6-hydroxydopamine-lesioned rats, caffeine as well as other methylxantine derivatives induce long-lasting contralateral rotational behavior (Casas et al., 1989; Herrera-Marschitz et al., 1988). Functional interactions with the dopaminergic system explaining the psychostimulant effects of caffeine have been postulated (Ferre et al., 1997).

On the other hand, there is a significant amount of data showing that caffeine-induced turning behavior can also be modulated by the cholinergic system. In this respect, we have previously shown that the muscarinic receptor antagonist, scopolamine, inhibited tolerance to caffeine-induced turning behavior (Casas et al., 1999). In addition, the intrastriatal administration of the muscarinic receptor antagonist, atropine, reduced the inhibitory effects of 2-*p*-(carboxyethyl)phenethylamino-5′ -*N*-ethylcarboxamidoadenosine hydrochloride (CGS 21680), an adenosine A_1 receptor agonist, on apomorphine-induced contralateral rotation in 6-hydroxydopamine-lesioned rats. Together, these data indicate that both adenosine and muscarinic systems interact in the striatum (Vellucci et al., 1993). Supporting the abovementioned studies, both anatomical and functional evidence show that adenosine receptors can modulate acetylcholine release (Preston et al., 2000). Striatal acetylcholine can act at five different subtypes (M_1–M_5) of muscarinic receptors (Yan et al., 2001), but it is still unknown through which subtype of receptor it modulates caffeine turning behavior. These different subtypes of receptors have singular anatomical localizations. For example, both the muscarinic M_1 and M_4 subtypes are preferentially localized postsynaptically on striatopallidal and

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striatonigral output spiny neurons, respectively (Santiago and Potter, 2001; Yan et al., 2001). Muscarinic M_2 receptors, on the other hand, are present on cholinergic interneurons (Rouse et al., 1997). In addition, functional dissimilarities between some of these receptors have been found. Indeed, antagonism of muscarininc M_2 receptors enhanced firing of spiny neurons, and this effect was blocked by the selective muscarinic M_1 receptor antagonist, pirenzepine, suggesting opposite actions of muscarinic M_1 and M_2 receptors in the neostriatal output (Galarraga et al., 1999).

Thus, the aim of this study was to further explore adenosine/acetylcholine interactions in the striatum and to evaluate how a differential pharmacological activation of presynaptic and postsynaptic striatal muscarinic receptors would affect caffeine-induced contralateral turning behavior in unilaterally 6-hydroxydopamine-lesioned rats.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 150±10 g were used in this study (Crifa Edo, France). The animals were housed four per cage with free access to rat chow and water. They were kept in a temperature-controlled environment (21+1 °C) on a 12-h light/dark cycle (lights on at 8 a.m.) when they were not in experimental sessions. This experiment was carried out in compliance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) for care and use of laboratory animals.

2.2. Drugs

Caffeine anhydrous was diluted in physiological saline and administered subcutaneously. Oxotremorine-M was diluted in an artificial cerebrospinal fluid (CSF) and administered intrastriatally.

2.3. General procedure

Unilateral 6-hydroxydopamine lesion of the medial forebrain bundle was performed as previously described elsewhere (Casas et al., 1999). Thirty days postlesion, all rats were challenged with apomorphine (0.05 mg/kg s.c.) four times, once a week. Rats showing fewer than 500 half-turns in 1 h during the last two tests were not included in the experiment. Guide cannula implantation surgery was conducted 1 month after the last apomorphine challenge. Guide cannulae (22 G) were implanted into the denervated striatum, aimed at the following coordinates: A, +0.2; L, −3.0; V, −2.0, according to the atlas of Paxinos and Watson (1986). Microinjection procedure was conducted 1 week later. For intrastriatal microinjections, rats were gently restrained, and an internal cannula (28 G) that extended 2.5 mm below the tip of the guide cannula was inserted. Solutions were infused through the internal cannula using a Hamilton syringe that was connected via PE-tubing and delivered in a volume of 1.0 μl at a flow rate of 0.2 μl/min with a microsyringe pump. The internal cannula was left in place for an additional minute prior to removal of the cannula. Twenty-four hours before the experiment, all rats received an intrastriatal microinjection of CSF in order to minimize confounding effects as a consequence of likely interactions between injection cannula-induced damage and oxotremorine-M neurochemical actions.

The day of testing, different groups of rats received an infusion of either CSF or oxotremorine-M (0.1, 1.0, 10, or 100 ng/μl). Subsequently, rats were individually placed into the plastic hemispherical bowls (40 cm in diameter) attached to a harness and connected to photoelectric detectors, and saline or caffeine (30 mg/kg) were administered 15 min later in order to exclude any contribution of apomorphine-conditioned turning. Both, contralateral and ipsilateral half-turns were recorded for 2 h following to systemic injections. Only rats with histologically correct probe placements were used for statistical analysis.

2.4. Statistics

Data were transformed to $\log_{10}$ in order to homogenize the variance. A repeated measures analysis of variance (ANOVA), with 30 min TIME-block as a within-subjects factor (four levels) and both, caffeine (two levels) and oxotremorine-M (five levels) as between-subjects factors was used to assess the effects of the treatments on contralateral turning. When significant interactions were observed, two- and one-way ANOVAs were performed. When appropriate, Student's *t*-test or Duncan's multiple range post hoc test were used for comparisons between groups. The accepted level of significance for all of tests was $P<0.05$.

3. Results

Caffeine induced a small amount of ipsilateral turning, while oxotremorine-M did not affect this behavior (data not shown).

With respect to contralateral turning, repeated measures ANOVA (TIME-block×oxotremorine-M×caffeine) revealed a significant main effect of TIME-block ($F_{(3,183)}=81.2$, $P<0.001$). Both TIME-block×oxotremorine-M ($F_{(12,183)}=7.0$, $P<0.001$) and TIME-block×oxotremorine-M×caffeine ($F_{(12,183)}=2.6$, $P<0.005$) significant interactions were also revealed. However, no significant TIME-block×caffeine interaction was revealed ($F_{(3,183)}=0.4$, $P=0.7$).

In caffeine-treated rats (Fig. 1A–D, scratched bars), repeated measures ANOVA (TIME-block×oxotremorine-M) revealed a significant main effect of TIME-block ($F_{(3,96)}=70.3$, $P<0.001$) and a significant TIME-block-

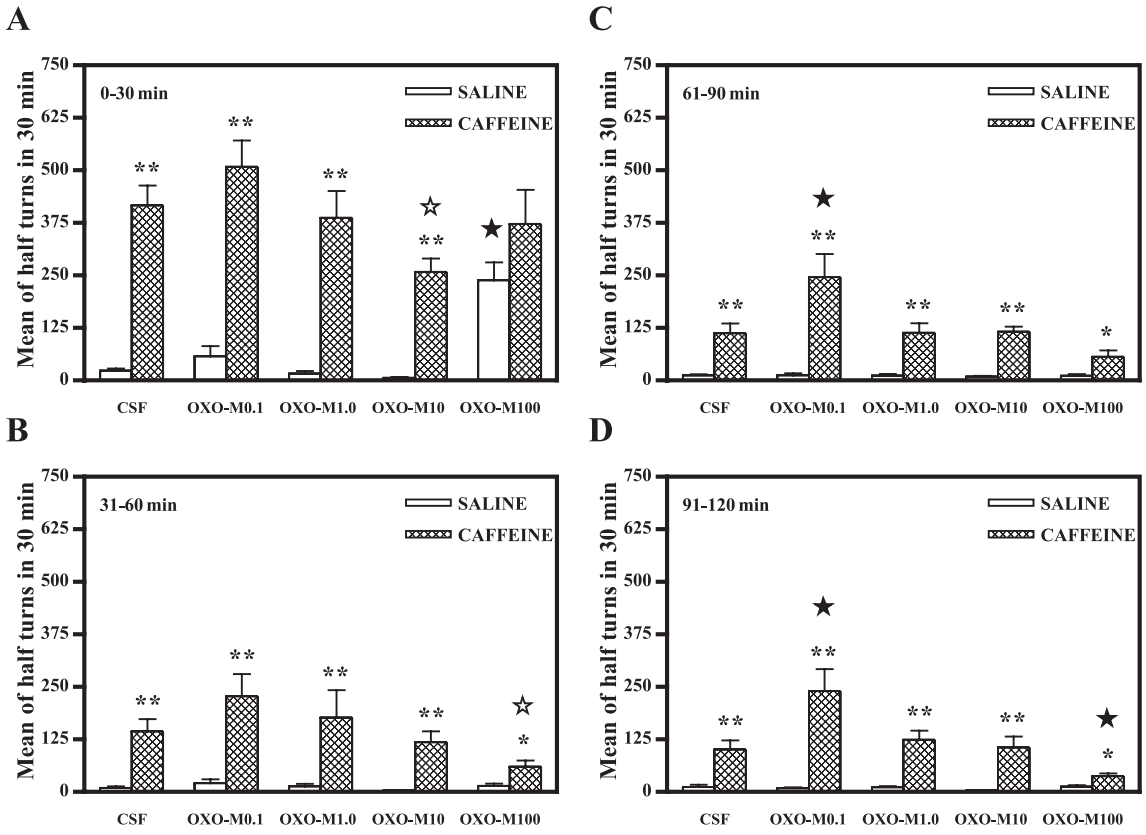

Fig. 1. Effects of intrastriatal administration of oxotremorine-M (0, 0.1, 1, 10, 100 ng/μl) in unilaterally 6-hydroxydopamine-lesioned rats on contralateral turning for 2 h shown in 30 min blocks: 0–30 (A), 31–60 (B), 61–90 (C) and 91–120 min (D). Saline-treated rats (empty bars, n=5–8 per group) and caffeine-treated rats (filled bars, n=5–10 per group); *P<0.05 and **P<0.001 vs. respective saline group (Student's t-test). ☆: P<0.05 vs. the respective CSF-treated group. ★: P<0.05 vs. all other respective groups (Duncan's test). Values are presented as means of half turns±S.E.M.

×oxotremorine-M interaction ($F_{(12,96)}$=2.1, P<0.05). One-way ANOVA revealed significant effects of oxotremorine-M in all time blocks (block 1: $F_{(4,36)}$=3.1, P<0.05; block 2: $F_{(4,36)}$=2.8, P<0.05; block 3: $F_{(4,36)}$=4.7, P<0.005 and block 4: $F_{(4,36)}$=7.2, P<0.001). Duncan's multiple-range post hoc tests showed that in the first 30 min block (Fig. 1A, scratched bars), rats infused with oxotremorine-M (10 ng/μl) rotated less than CSF-infused rats (P<0.05). In the second 30 min block (Fig. 1B, scratched bars), the highest concentration of oxotremorine-M (100 ng/μl) significantly attenuated caffeine-induced contralateral turning (P<0.05). In the two last 30 min blocks (Fig. 1C and D, scratched bars), the lowest concentration of oxotremorine-M (0.1 ng/μl) significantly enhanced the effects of caffeine plus CSF, and the highest concentration still significantly attenuated these effects in the last 30 min block (P<0.05).

In saline-treated rats (Fig. 1A–D, empty bars), repeated measures ANOVA (TIME-block×oxotremorine-M) revealed both a significant TIME-block main effect ($F_{(3,87)}$=27.5, P<0.001) and a TIME-block×oxotremorine-M interaction ($F_{(12,87)}$=6.2, P<0.001). One-way ANOVA revealed a significant effect of oxotremorine-M only in the first 30 min block ($F_{(4,33)}$=17.0, P<0.001). Post hoc analyses showed that in the first 30 min (Fig. 1A, empty bars), the highest concentration of oxotremorine-M (100 ng/μl) induced contralateral turning with respect to all other saline groups (P<0.05, Duncan's multiple range test).

Individual comparisons between saline- and caffeine-treated rats for each treatment during each time block showed that caffeine significantly increased contralateral turning with respect to saline treatment in all groups of rats, and in all time blocks, except in those rats treated with the highest dose of oxotremorine-M (100 ng/μl) during the first 30 min time block (P<0.05, Student's t-test; Fig. 1A–D).

4. Discussion

Our results show that intrastriatal infusion of the muscarininc receptor agonist oxotremorine-M into the 6-hydroxydopamine denervated striatum of rats modulated caffeine-induced contralateral turning behavior, supporting a functional link between cholinergic and adenosine systems. Dose-related biphasic effects of oxotremorine-M on contralateral turning were observed. At the lowest dose, oxotremorine-M potentiated, whereas at higher doses it attenuated caffeine-induced contralateral turning.

Both, M_1 and M_4 muscarinic receptors are mainly expressed in medium spiny neurons in the striatum (Santiago and Potter, 2001; Yan et al., 2001). These receptors are preferentially located in striatopallidal and striatonigral output pathways, respectively. The M_2 muscarinic receptor is expressed by cholinergic interneurons (Rouse et al., 1997) but not by medium spiny neurons (Yan et al., 2001), and an autoreceptor function has been attributed to this subtype of muscarinic receptor (Weiler, 1989).

A higher relative potency of oxotremorine-M relative to other muscarinic agonists in activating M_2- versus M_1-like muscarinic receptors has been reported (Richards, 1990). Thus, by comparing the EC_{50} values, it was found that oxotremorine-M was more potent at M_2 muscarinic autoreceptors than at postsynaptic IP-1-coupled muscarinic receptors (Richards, 1990). Therefore, it can be expected that oxotremorine-M at very low doses would be acting as a preferential M_2 muscarinic agonist, whereas at very high doses, this specificity would disappear and, consequently, M_4 and/or M_1 muscarinic receptors would be also activated.

On the other hand, it has been reported that adenosine A_{2A} receptor antagonism reduced acetylcholine release from rat striatal cholinergic interneurons (Preston et al., 2000). Given that oxotremorine-M (0.1 ng/μl) enhanced caffeine-induced contralateral turning in this study, presynaptic combined actions of oxotremorine-M and caffeine on acetylcholine release can be suggested. Thus, reduced cholinergic stimulation of postsynaptic M_1 and/or M_4 muscarinic receptors would favour caffeine behavioral effects as a consequence of both, postsynaptic striatonigral A_1 and striatopallidal A_2 adenosine receptors blockade (Ferre et al., 1997).

Unlike the lowest dose, higher doses of oxotremorine-M (10 and 100 ng/μl) attenuated caffeine-induced contralateral turning, suggesting that oxotremorine-M could be behaving as a nonspecific muscarinic agonist. Thus, postsynaptic agonistic actions of oxotremorine-M at M_4 and/or M_1 muscarinic receptors would be in opposition to those of caffeine at both A_1 and A_{2A} adenosine receptors located in striatal output pathways. In a similar manner, muscarinic receptor activation also attenuated contralateral turning induced by dopaminergic agonists (Kuruvilla and Uretsky, 1984). These results showing the biphasic effects of oxotremorine-M on caffeine-induced contralateral turning are in line with a recent study showing opposite functions of M_1 and M_2 muscarinic receptors on neostriatal output (Galarraga et al., 1999).

Paradoxically, oxotremorine-M at the highest dose (100 ng/μl) induced contralateral rotation in saline-treated rats, although this effect was transient taking place only during the first 30 min. Thus, it is possible that at this dose, oxotremorine-M may enhance apomorphine-conditioned turning. This issue is being currently addressed in our laboratory.

In summary, the present results suggest opposite actions of presynaptic M_2 versus postsynaptic M_1 and/or M_4 muscarinic receptors on caffeine-induced contralateral turning behavior. Moreover, they support the putative use of caffeine in combination with anticholinergic drugs in the treatment of Parkinson's disease.

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