Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 76 (2003) 53–61
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Role of cholinergic receptors in locomotion induced by scopolamine and oxotremorine-M

Araba Chintoh, James Fulton, Nicole Koziel, Mariam Aziz, Manu Sud, John S. Yeomans*

Department of Psychology, University of Toronto, 100 St. George Street, Toronto, Ontario, Canada M5S 3G3

Received 27 June 2002; received in revised form 17 June 2003; accepted 24 June 2003

Abstract

Mesopontine cholinergic neurons activate dopamine neurons important for reward-seeking and locomotor activity. The present studies tested whether cholinergic receptor blockade in the ventral tegmental area (VTA) altered locomotion induced by scopolamine (3 mg/kg ip) or by oxotremorine-M (0.1 μg bilaterally in the VTA). It was predicted that cholinergic blockers in the VTA would attenuate these cholinergic-induced locomotor increases. Locomotor activity was increased by scopolamine and oxotremorine-M administration in all treatments. When dihydro-β-erythroidine (DHBE), a nicotinic receptor antagonist, was applied in VTA prior to oxotremorine-M, locomotion was reduced to slightly above saline baseline levels, but atropine, a muscarinic antagonist, had no effect. This suggests that the locomotor effect of oxotremorine-M at this dose was mediated mainly via nicotinic, not muscarinic, receptors. Intra-VTA injections of DHBE, however, did not attenuate scopolamine-induced locomotion indicating that scopolamine-induced locomotion is not mediated mainly via VTA cholinergic receptors. In mutant mice with a deletion in the M5 muscarinic receptor gene, scopolamine-induced locomotion was increased versus wild type mice after scopolamine injection. This suggests that the M5 receptor has an inhibitory effect on scopolamine-induced locomotion.

Keywords: Dihydro-β-erythroidine; Locomotion; Muscarinic; Nicotinic; Reward; M5

1. Introduction

Locomotor activity in rats can be increased by systemic administration of nicotinic (e.g., nicotine 0.1–0.4 mg/kg sc in nicotine tolerant rats) or muscarinic antagonists (e.g., scopolamine 0.5–10 mg/kg ip) (Abood and Biel, 1962; Clarke and Kumar, 1983). It has been proposed that dopamine neurons of the substantia nigra and ventral tegmental area (VTA), which are directly activated by mesopontine cholinergic neurons via nicotinic and muscarinic receptors, are important for these effects (Clarke et al., 1988; Mathur et al., 1997; Yeomans, 1995). For example, nicotinic agonists infused directly into the VTA increase locomotor activity (Reavill and Stolerman, 1990; Museo and Wise, 1990a,b; Panagis et al., 1996). Nicotine injections in VTA also increase dopamine release in the nucleus accumbens (Blaha et al., 1996) and facilitate brain stimulation reward (Bauco and Wise, 1994). The facilitating effects of systemic nicotine on locomotor activity, dopamine release, and nicotine self-administration are blocked by nicotinic blockers in VTA or by 6-hydroxydopamine lesions of mesolimbic dopamine neurons (Clarke et al., 1988; Corrigall et al., 1992, 1994; Louis and Clarke, 1998; Nissell et al., 1994).

The facilitating effects of systemic muscarinic antagonists are associated with mesopontine cholinergic neurons. Increases in locomotor activity and striatal dopamine release result following infusions of scopolamine (10–150 μg) into the pedunculopontine tegmental nucleus (Chapman et al., 1997; Mathur et al., 1997). Lesions of the laterodorsal tegmental nucleus or carbachol infusions in the pedunculopontine tegmental nucleus reduce the locomotor-activating effects of systemic scopolamine (LaViolette et al., 2000; Mathur et al., 1997). Therefore, disinhibition of mesopontine cholinergic neurons via muscarinic autoreceptors is one mechanism by which scopolamine could increase locomotion.

Whether the locomotor-activating effects of scopolamine are due to activation of dopamine neurons, however, is less clear. Systemic scopolamine (1–10 mg/kg ip) increases dopamine release in the striatum (Chapman et al., 1997) at the same doses that increase locomotor activity (Joyce and Koob, 1981; Mathur et al., 1997). Although locomotion induced by antimuscarinics was attenuated in mice by

* Corresponding author. Tel.: +1-416-978-3402; fax: +1-416-978-4811.
E-mail address: yeomans@psych.utoronto.ca (J.S. Yeomans).

0091-3057/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0091-3057(03)00196-5

systemic administration of α-methyltyrosine, a catecholamine synthesis inhibitor (Thornberg and Moore, 1990), 6-hydroxydopamine lesions of the nucleus accumbens did not block scopolamine-induced locomotion in rats (Joyce and Koob, 1981).

The present studies tested whether nicotinic or muscarinic receptors in the VTA are important for the locomotor-activating effects of scopolamine or oxotremorine-M. Oxotremorine-M is a potent cholinergic agonist that, when infused into the VTA, increases the firing rate of dopamine neurons in a dose-dependent way mainly via muscarinic receptors (Gronier and Rasmussen, 1998). Oxotremorine-M in VTA also increases dopamine levels in the nucleus accumbens and frontal cortex (Gronier et al., 2000). In the first study, we measured the locomotor-activating effect of oxotremorine-M in VTA of rats and tested whether this effect is blocked by VTA pretreatment of the nicotinic antagonist, dihydro-β-erythroidine (DHBE), or the muscarinic antagonist, atropine, at doses found to inhibit brain stimulation reward (Yeomans and Baptista, 1997). In the second study, we tested whether DHBE similarly attenuates the locomotor-activating effect of systemic scopolamine.

Systemically administered scopolamine can increase locomotor activity in mice (Shannon and Peters, 1990). Mutant mice with altered cholinergic receptor genes have been used to identify genetically defined receptors needed for the locomotor effects of stimulants (e.g., Picciotto et al., 1998; Gomeza et al., 1999). M5 muscarinic receptor mRNA is associated with dopamine neurons of the VTA and substantia nigra, pars compacta (Vilaro et al., 1990; Weiner et al., 1990). Six-hydroxydopamine lesions of these dopamine neurons remove M5 mRNA and reduce M5-selective receptor binding (Vilaro et al., 1990; Reever et al., 1997; Yeomans et al., 2001). Infusions of M5 antisense oligonucleotides into the VTA inhibited brain stimulation reward in rats (Yeomans et al., 2000), suggesting that M5 receptors in VTA mediate activation of dopamine neurons.

Mutant mice with truncated M5 receptor genes showed a complete loss in sustained dopamine release in the nucleus accumbens core in response to electrical stimulation of the laterodorsal tegmental nucleus (Forster et al., 2001; Takeuchi et al., 2002; Yeomans et al., 2001). This sustained dopamine release was also blocked by the muscarinic receptor blocker scopolamine in the VTA of rats (Forster and Blaha, 2000). In the third study, therefore, we tested whether M5 mutant mice show reduced locomotion in response to systemic scopolamine.

2. Methods

2.1. Subjects

2.1.1. Rats

Male Wistar rats (Charles River, Quebec) weighing 300–400 g at the time of surgery were individually housed in plastic cages with food pellets and water available ad libitum. The cages were kept in temperature controlled quarters (21 ± 1 °C) on a 12:12-h light–dark cycle (lights on at 7:00 a.m.).

2.1.2. Mice

Mutant mice were bred in our colony from 129SvJ X CD-1 mice described previously (Takeuchi et al., 2002). The deletion in the M5 muscarinic receptor gene included most of the third intracellular loop. This change is expected to block expression of the second half of the M5 receptor protein from AA251-531.

Twenty-four male mice were housed on a 12:12-h light–dark cycle (lights turned on at 10 a.m.) at an ambient temperature of 21 °C. Twelve mice were homozygous (−/−) for the M5 mutation and 12 were wild type (+/+). The two groups were matched for age (3–4 months old) and generation and were kept in separate cages with food and water in the mouse colony for at least 3 days prior to the beginning of the first locomotor activity tests.

2.2. Surgery

The rats were pretreated with atropine (0.4 mg/kg ip) (Sigma, St. Louis, MO) and anaesthetized with sodium pentobarbital (60 mg/kg ip) (MTC Pharmaceuticals, Cambridge, ON, Canada). Supplements of sodium pentobarbital were administered as necessary. The rats were placed in a stereotaxic instrument using blunt ear bars. Bilateral stainless steel guide cannulae (23 gauge) were aimed at the VTA based on the coordinates of Paxinos and Watson (1997) (anterior/posterior −5.0 mm, medial/lateral −0.9 mm, dorsal/ventral −7.0 mm, relative to bregma). The rats were allowed at least 1 week to recover from surgery before any experimental manipulations.

2.3. Apparatus

Locomotor activity was measured in three automated, open-field locomotion boxes (43 × 43 × 30 cm) (MED Associates, St. Alban's VT). Infrared beams across the base of the box, and 15 cm above the floor, recorded horizontal movements made by each animal. Activity was recorded in 5-min bins. Each test session lasted 60 min and locomotion was measured in terms of total distance traveled in each 5-min period.

2.4. Systemic injections

All systemic injections of scopolamine hydrobromide (Sigma) were given immediately after VTA injections (Mathur et al., 1997). Scopolamine (3.0 mg/kg ip) was injected in a volume of 0.6 ml sterile physiological saline.

2.5. Intracranial microinjections

All VTA microinjections were made bilaterally via a Hamilton microsyringe. The 0.5-μl volume on each side was slowly infused over a 120-s period, and the injector was left in place for 30 s after injection before slow removal. DHBE (Research Biochemicals, Natick, MA) was administered at a concentration of 60 mg/ml. Oxotremorine-M (Research Biochemicals) was administered at a concentration of 0.2 mg/ml. Atropine was administered at a concentration of 60 mg/ml.

2.6. General procedure for rats

Rats were given 3 h of habituation (1 h/day for 3 days) in the locomotion boxes prior to drug testing. In addition, rats were placed in the activity boxes for 30 min before each test session as pretest habituation. All testing occurred between 9 a.m. and 1 p.m. The experimental protocol used in these experiments was approved by the University of Toronto Animal Care Committee, following the rules of the Canadian Council on Animal Care.

2.7. Experiment 1: Oxotremorine-M

Eleven rats were infused intracranially on four different days separated by at least 48 h between infusions. On the first day, all animals were administered with an infusion of saline followed 20 min later by saline to establish baseline locomotor activity. On the next three infusion days, the animals were given counterbalanced infusions of (1) DHBE (30 μg on each side) followed 20 min later by infusion of oxotremorine-M (0.1 μg on each side); (2) saline followed 20 min later by oxotremorine-M; and (3) DHBE followed 20 min later by saline. Conditions were counterbalanced within each group of three rats (except for the last group of two rats) by assigning one rat on each infusion day to a given drug. Activity was assessed for 60 min in the locomotion boxes in a darkened atmosphere following the final injection on each day.

In 4 of the 11 previously tested rats, a fifth day of drug testing was added: atropine (30 μg on each side), followed 20 min later by oxotremorine-M.

2.8. Experiment 2: Scopolamine

Eight rats were given three intracranial infusions (each in a volume of 0.5 μl saline) on 3 days, with infusions separated by 48 h. (1) Rats were infused intracranially with DHBE (30 μg on each side of the VTA) prior to a systemic injection of scopolamine (3.0 mg/kg ip). (2) Saline (0.5 μl) was administered prior to a systemic scopolamine injection. (3) Saline (0.5 μl) was administered prior to a systemic saline injection as a control condition and to test whether any carry-over effects from Experiment 1 were evident in the six rats that were tested in both experiments. Rats were counterbalanced into drug conditions as in Experiment 1.

2.9. Histology

Upon completion of behavioral studies, rats were acutely anaesthetized with sodium pentobarbital (60 mg/kg), then perfused intracardially with 100 ml of sterile physiological saline, followed by 100 ml of 10% formalin. The rats were decapitated and the brains were removed and stored for at least 1 day in vials containing 10% formalin and 30% sucrose solution. The brains were sliced in a cryostat (Cryocut 1800, Reichert Jung) at -20 °C. The 40-μm thick sections were mounted onto slides and stained with cresyl violet for histological localization of the cannula placements.

2.10. Statistical analysis

The total distance traveled during the entire 60-min period from each animal in each condition was analyzed using a two-way repeated measures ANOVA (Statistica), with drug condition and time as the variables. In the first experiment, the results from the four drug conditions (saline/saline, DHBE/saline, DHBE/oxotremorine, atropine/oxotremorine, and saline/oxotremorine) were compared statistically. The results using atropine were tested separately. In the second experiment, the results from the three drug conditions (saline/saline, DHBE/scopolamine, and saline/scopolamine) were compared in a two-way ANOVA with time and drug condition as the two variables. Subsequently, Fisher's least significant difference (LSD) test was applied to assess individual differences between the drug conditions.

2.11. Experiment 3: Scopolamine in M5 mutant mice

Procedure. The 24 mice were divided into four sets of mice (mutants injected with saline, mutants injected with scopolamine, wild types injected with saline, and wild types injected with scopolamine), with six mice in each group. Each experiment was run for 5 days on a set of six mice, three from each genotype. The mice were tested for locomotor activity between 1 and 5 p.m. each day. The open-field locomotor boxes (described above) were cleaned with 10% ethanol at the end of each test day. Mice were tested in different boxes each day in a counterbalanced order to minimize any effect of the location of the box or of the previous exposure.

The first 3 days of testing for each set of mice involved 90 min of habituation in an open-field box for each mouse. On Day 4, each mouse was placed in the box for 30 min to habituate, then was removed and given an injection of saline (0.7 ml ip) or scopolamine (3 mg/kg ip) followed by 60 min of recording locomotor activity. On Day 5, after 30 min of habituation to the apparatus, each mouse was given an

injection of scopolamine (if given saline previously) or saline (if given scopolamine previously). This same procedure was followed for five subsequent days for each of the other three sets of mice. Statistical analysis of locomotor activity was performed using a three-way repeated measures ANOVA (Statistica) with genotype, drug, and time as the three variables.

3. Results

3.1. Histology

Locations of cannula tips in rats are shown in Fig. 1. All sites were within 1 mm of the boundaries of the VTA, with a range in anterior/posterior coordinates from −4.2 to −5.2 mm, medial/lateral coordinates from 0.3 to 1.2 mm, and dorsal/ventral coordinates from 6.8 to 8.8 mm. One rat used in Experiment 1 was deleted from further analysis because of extensive cell damage and gliosis observed near both VTA cannulae postmortem. All other subjects were included in the statistical analysis. No differences were found between the distribution of VTA sites that were used in Experiment 1 only ($N=4$, shown as squares in Fig. 1), or used in both Experiments 1 and 2 ($N=6$, shown as circles), or used only in Experiment 2 ($N=2$, shown as stars in Fig. 1).

3.2. Experiment 1: Oxotremorine-M

Bilateral injections of oxotremorine-M (0.1 μg) in 11 VTA sites significantly increased locomotor activity (Fig. 2). A

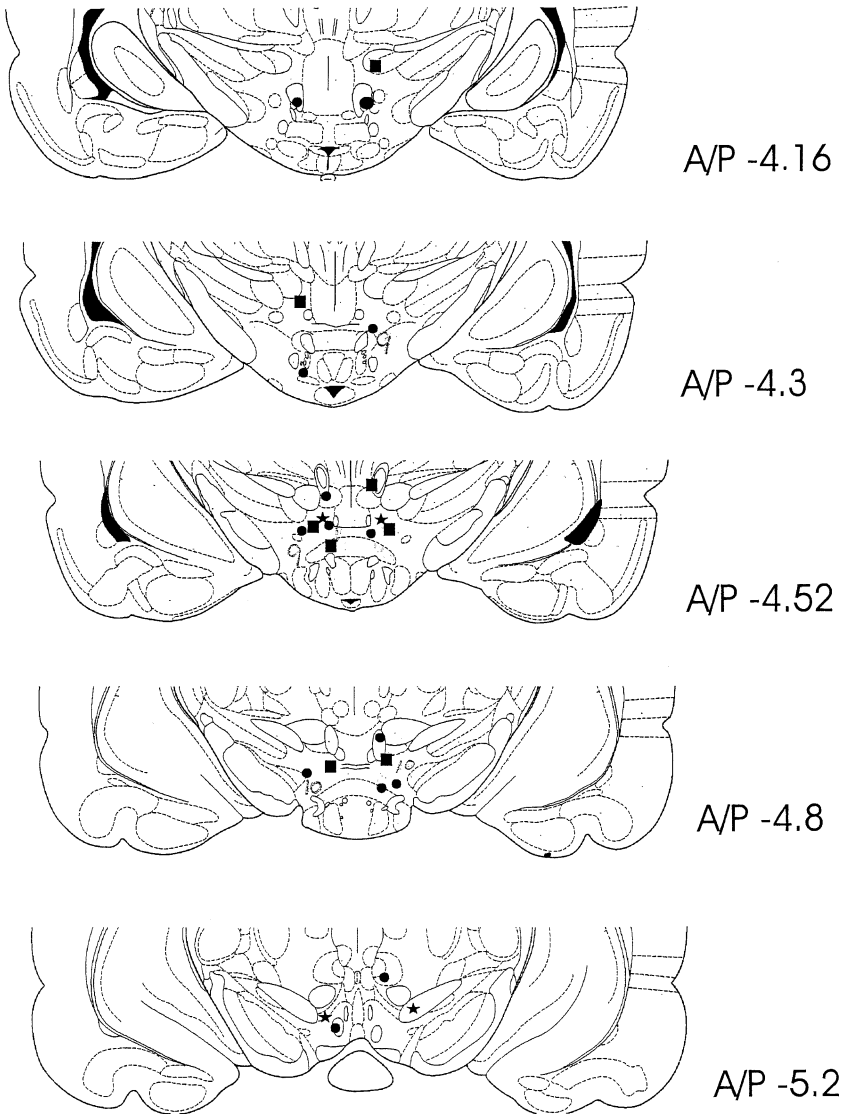

Fig. 1. Histologically determined injection sites displayed on coronal sections from the Paxinos and Watson (1997) atlas. Numbers to the right of each section indicate the distance in millimeters posterior to bregma. Sites identified by squares were tested in Experiment 1 only; sites identified by circles were tested in both Experiments 1 and 2. Sites identified by stars were tested in Experiment 2 only.

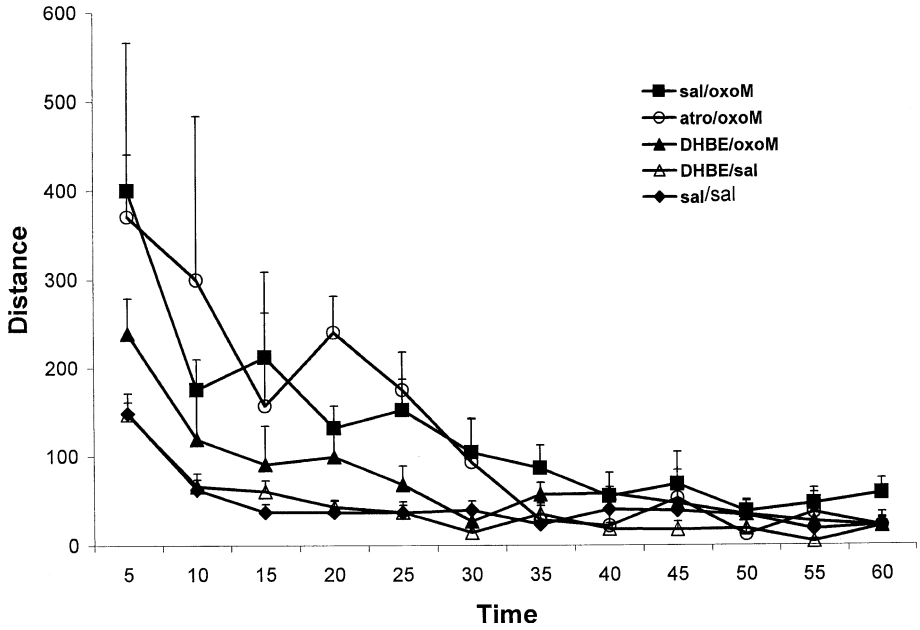

Fig. 2. Locomotion induced by bilateral VTA injections of oxotremorine-M (0.1 μg) following bilateral VTA injections of 30 μg dihydro-β-erythroidine ("DHBE/oxoM"), 30 μg atropine ("atro/oxoM"), or saline ("sal/oxoM") in VTA of rats. Control conditions consisted of either two bilateral saline injections ("sal/sal") or bilateral injections of dihydro-β-erythroidine followed by bilateral injections of saline ("DHBE/sal"). Distance in inches is shown on the vertical axis and time in minutes after oxotremorine injection is shown on the horizontal axis. The number of rats tested was 11 in all conditions except 4 in the "atro/oxoM" condition. Error bars in all figures indicate the standard error of the mean.

two-way repeated measures ANOVA found a significant difference between the means of the drug conditions [$F(3,27) = 12.2$, $P < .0001$], a significant effect of time [$F(11,99) = 33.3$ $P < .0001$], and a significant interaction between drug and time [$F(33,297) = 3.09$, $P < .0001$]. Post hoc analysis with Fisher's LSD revealed no significant difference between the means of the saline/saline condition and the DHBE/saline condition (Fisher's LSD: P=.805), indicating no reliable effect of DHBE. These means differed from both the oxotremorine-M conditions (saline/saline vs. DHBE/oxotremorine-M, P=.07; DHBE/saline vs. DHBE/oxotremorine-M, P=.032; saline/saline vs. saline/oxotremorine-M, $P < .0001$; DHBE/saline vs. saline/oxotremorine-M, $P < .0001$), indicating a reliable effect of oxotremorine-M without DHBE and a reliable effect of oxotremorine-M with DHBE. In addition, a significant difference was found between the means of the DHBE/oxotremorine-M and saline/oxotremorine-M conditions (Fisher's LSD: P=.0021), indicating a reliable reduction in the oxotremorine-M effect on locomotion by DHBE.

The mean total distance traveled in the oxotremorine-M condition was almost three times that for the saline control condition. In particular, the locomotor-activating effect of oxotremorine-M was greatest in the first 15 min postinjection but remained above control conditions for 35 min.

A subanalysis was conducted for the four rats tested with atropine (30 μg) in VTA. The mean distances traveled for these four rats were saline/saline = 544 in.; atropine/oxotremorine-M = 1250 in.; and saline/oxotremorine-M = 1619 in. The mean locomotor activity in the saline condition differed significantly from the saline/oxotremorine-M group (P=.028) but not the atropine/oxotremorine-M condition (P=.108). The two oxotremorine-M conditions were not significantly different (P=.363), indicating no reliable inhibition of locomotion by atropine.

3.3. Experiment 2: Scopolamine

Systemic injections of scopolamine (3 mg/kg) in both experimental conditions resulted in a significant increase in locomotor activity (Fig. 3). The main effect of drug condition was found to be significant [$F(2,14) = 14.75$, $P < .0004$]. The main effect of time was significant [$F(11,77) = 8.55$, $P < .0001$]. The interaction between drug and time was also significant [$F(22,154) = 2.18$, $P < .0031$]. The mean distance traveled for the saline/scopolamine group was over six times that in the saline/saline group. These groups differed significantly as assessed by post hoc tests, Fisher's LSD: $P < .0001$. The mean locomotor activity for the rats treated with bilateral DHBE in VTA and systemic scopolamine also differed significantly from saline controls (Fisher's LSD: $P < .0001$) but did not differ significantly from the saline/scopolamine group (Fisher's LSD: P=.379). Therefore, scopolamine increased activity, as expected, but DHBE in VTA did not reduce scopolamine-induced activity.

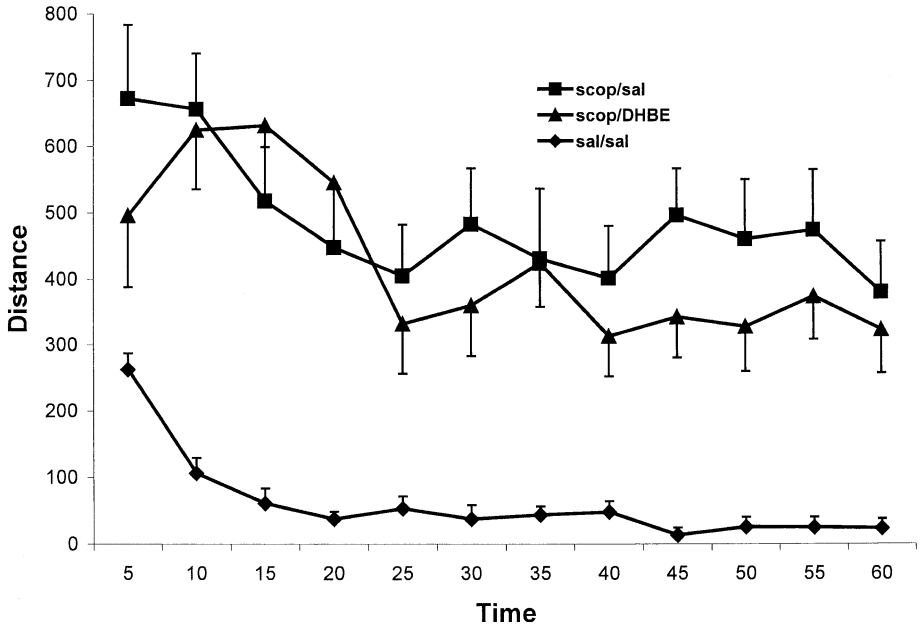

Fig. 3. Locomotion induced by scopolamine (3 mg/kg ip) in rats. Preinjection of DHBE 30 μg bilaterally in VTA ("scop/DHBE") was no more effective than saline ("scop/sal") in reducing the effect of scopolamine. Scales and error bars the same as Fig. 2. Saline control tests consisted of a systemic injection of physiological saline immediately preceded by bilateral VTA injections of saline ("sal/sal"). Eight rats were tested in all conditions.

3.4. Experiment 3: Scopolamine in M5 mutant mice

Both groups of mice were much more active than rats in all conditions tested. Scopolamine (3 mg/kg ip) significantly increased locomotor activity in both mutant and wild type mice as compared to saline controls (Fig. 4). Similar locomotor activity was observed in the two groups of mice in saline control conditions at all times postinjection, with little decline in activity as a function of time. After scopolamine injections, wild type mice increased locomotor distance traveled by about twice over saline levels, while mutant mice increased distance traveled by almost three times over saline controls. In both groups, activity reached a peak 10 min after scopolamine injection and then declined.

Statistically, the main effect of drug was significant [$F(22) = 9.594$, $P < .0052$], as was the main effect of time [$F(242) = 5.324$, $P < .0001$]. The main effect of genotype was not significant [$F(22) = 1.42$, $P = .246$], but the interaction between genotype and time was highly significant [$F(242) = 2.971$, $P = .0010$], indicating that the inhibitory effect of the M5 receptor increased as a function of time

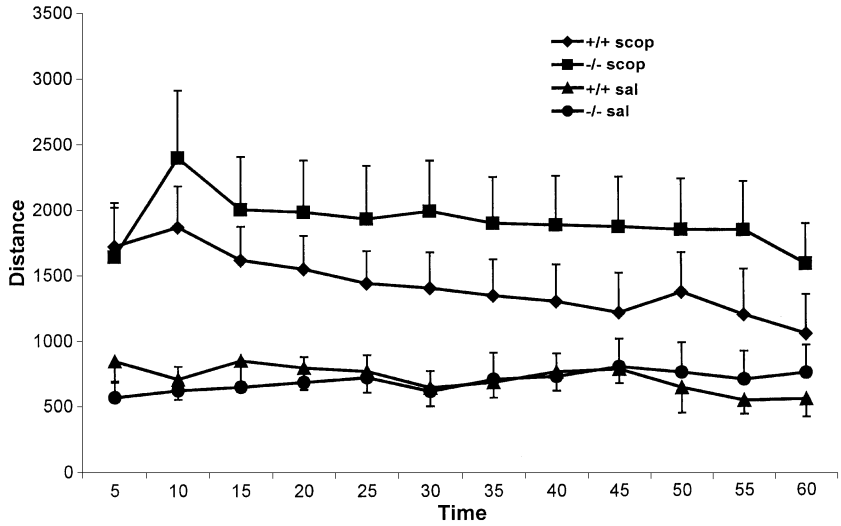

Fig. 4. Locomotion induced by scopolamine (3 mg/kg ip) in M5 mutant mice. Scopolamine ("scop") increased mean total distance traveled for both mutant (−/−) and wild type (+/+) mice. Spontaneous locomotion, observed after saline ("sal") injections, was similar in both groups of mice. Six mice were tested in each group.

after injection. In addition, the interaction between drug and time was highly significant [$F(242) = 5.58$, $P < .0001$], indicating that the effect of scopolamine decreased over time.

4. General discussion

The strong locomotor-activating effects of oxotremorine-M (0.1 μg bilaterally) in VTA, found here in rats, depended mainly on nicotinic receptors, and not muscarinic receptors, near the VTA. By contrast, the locomotor-activating effects of systemic scopolamine in rats were not dependent on nicotinic receptors in the VTA. In addition, the activating effects of scopolamine were not dependent on M5 muscarinic receptors in M5 mutant mice, but, in fact, the M5 receptor had an inhibitory effect on scopolamine-induced locomotion.

4.1. Oxotremorine-M-induced locomotion in rats

Oxotremorine-M is a potent, muscarinic agonist that can also activate nicotinic receptors (Akk and Auerbach, 1999; Reitstetter et al., 1994). VTA dopamine neurons are activated by oxotremorine-M mainly via muscarinic receptors (Gronier and Rasmussen, 1998; Gronier et al., 2000). In Experiment 1, oxotremorine-M (0.1 μg bilaterally) increased locomotor activity by three times as compared with saline. This effect was not significantly reduced by pretreatment with the muscarinic blocker, atropine, but was strongly inhibited by the nicotinic blocker, DHBE. The dose of atropine used (30 μg in VTA) is the same dose that was effective in inhibiting brain stimulation reward (Yeomans and Baptista, 1997) but which did not inhibit nicotine self-administration (Corrigall et al., 1994) in rats. The dose of DHBE used (30 μg in VTA) is the same dose used to inhibit nicotine self-administration (Corrigall et al., 1994) and brain stimulation reward in rats (Yeomans and Baptista, 1997). These results indicate that the locomotor-activating effect of oxotremorine-M at this dose is mediated mainly through nicotinic receptors near VTA.

This conclusion, while unexpected, is consistent with other results. First, although atropine methyl nitrate injections in VTA blocked cocaine self-administration, they increased locomotor activity (Munn and Wise, 1999). Previously, we have found that locomotor activity is increased by VTA infusions of the nonselective cholinergic agonist carbachol (1–4 μg) (Kofman, unpublished dissertation), but not by the muscarinic agonist pilocarpine (1–4 μg). Finally, oxotremorine-M can activate α-4,β-2 nicotinic receptors, the most abundant of many nicotinic receptor subtypes in the VTA region (Akk and Auerbach, 1999; Klink et al., 2001). Together, these results suggest that nicotinic receptors in VTA mediate locomotor stimulating effects (as previously found by Reavill and Stolerman, 1990, and Museo and Wise, 1990a,b) and further indicate that muscarinic receptors in VTA do not mediate the increase of locomotor activity but rather have a weak inhibitory effect. Although atropine in the present study had no effect on oxotremorine-M-induced locomotion, it is possible that this lack of effect includes both facilitating effects of atropine alone (not tested) and inhibiting effects of atropine on oxotremorine-M-induced locomotion.

Muscarinic receptors in VTA are important for the slow activation of many mesolimbic VTA dopamine neurons (Lacey et al., 1990; Gronier and Rasmussen, 1998; Gronier et al., 2000; Forster and Blaha, 2000; Forster et al., 2001). VTA muscarinic receptors are also important for the rewarding effects of hypothalamic brain stimulation (Yeomans et al., 1985, 2001; Kofman et al., 1990; Yeomans and Baptista, 1997) of cocaine (Munn and Wise, 1999) of carbachol (Ikemoto and Wise, 2002) and possibly of feeding (Rada et al., 2000). It is not yet clear why muscarinic receptors have no role in locomotor-activating functions. One possibility is that locomotor activation occurs via a select population of nicotinic-activated, but not muscarinic-activated, neurons near VTA or that a select population of muscarinic-activated neurons inhibit locomotor activity. Given the diversity of nicotinic and muscarinic receptor subtypes on dopaminergic and GABAergic neurons in the VTA, many selective mechanisms are possible (Klink et al., 2001; Levey, 1993).

4.2. Scopolamine-induced locomotion in rats

Scopolamine (3 mg/kg ip) increased locomotion for over 60 min, as has been shown many times previously. Intra-VTA infusion of the nicotinic blocker DHBE, however, did not significantly attenuate scopolamine-induced locomotion. DHBE infusions in VTA at the same dose in these rats inhibited oxotremorine-M-induced locomotion. The difference in these results indicates that nicotinic receptors near VTA are not important for the locomotion induced by systemic scopolamine at this dose. This suggests that scopolamine induces locomotion via systems other than direct mesopontine cholinergic activation of dopamine neurons.

This conclusion is consistent with previous work showing that nucleus accumbens dopamine terminals are not critical for scopolamine-induced locomotion (Joyce and Koob, 1981). Mesopontine neurons are important for scopolamine-induced locomotion, however, since lesions of the laterodorsal tegmental nucleus inhibit scopolamine-induced locomotion (LaViolette et al., 2000). Also, scopolamine in the pedunculopontine tegmental nucleus increases locomotion by blocking muscarinic receptors (Mathur et al., 1997). Therefore, scopolamine-induced locomotion is likely mediated by another nondopaminergic projection of mesopontine cholinergic neurons (LaViolette et al., 2000) or by blockade of other muscarinic receptors such as those associated with basal forebrain neurons and their projections (Mattson et al., 2002).

4.3. Scopolamine-induced locomotion in M5 mutant mice

M5 receptors in VTA are important for hypothalamic brain stimulation reward in rats (Yeomans et al., 2000) and critical for sustained dopamine release in the nucleus accumbens activated by stimulation of the laterodorsal tegmental nucleus in M5 mutant mice (Forster et al., 2001). In the M5 mutant mice here, scopolamine-induced locomotion was slightly increased, an unexpected finding, while spontaneous locomotor activity (observed after saline injections) was unaffected. These results further indicate that M5 receptors, while important for reward and sustained activation of dopamine neurons projecting to the nucleus accumbens core, are not critical for locomotion evoked by systemic scopolamine. The small increase in locomotor activity in M5 mutant mice may be related to the increase in locomotor activity following atropine methyl nitrate in VTA of rats found by Munn and Wise (1999). In the present study, however, it cannot be determined where in the mouse brain, the M5 mutation facilitates scopolamine-induced locomotion.

In fact, lesion of nucleus accumbens dopamine terminals with 6-hydroxydopamine block amphetamine-induced locomotion, but not scopolamine-induced locomotion (Joyce and Koob, 1981). M5 receptors facilitate dopamine release in the nucleus accumbens following terminal activation by potassium (Zhang et al., 2002) or stimulation of laterodorsal tegmental nucleus cholinergic neurons (Forster et al., 2001). Consistent with this, amphetamine-induced locomotion is reduced in M5 knockout mice (Yeomans et al., 2003). Therefore, scopolamine-induced locomotion is likely independent of mesolimbic dopamine neuron activation.

Acknowledgements

We thank Nadeem Ansari and Gautam Shah for help in collecting pilot data in the initial stages of the experiments.

References

Abood L, Biel JH. Anticholinergic psychotomimetic agents. Int Rev Neurobiol 1962;4:217–73.

Akk G, Auerbach A. Activation of muscle nicotinic acetylcholine receptor channels by nicotinic and muscarinic agonists. Br J Pharmacol 1999; 128:1467–76.

Bauco P, Wise RA. Potentiation of lateral hypothalamic and midline mesencephalic brain stimulation reinforcement by nicotine: examination of repeated treatment. J Pharmacol Exp Ther 1994;272:294–301.

Blaha CD, Allen L, Das S, Inglis WL, Latimer M, Vincent SR, et al. Modulation of dopamine efflux in the nucleus accumbens after cholinergic stimulation of the ventral tegmental area in intact and pedunculopontine nucleus-lesioned, and laterodorsal tegmental nucleus-lesioned rats. J Neurosci 1996;16:714–22.

Chapman CA, Yeomans JS, Blaha CD, Blackburn JR. Increased striatal dopamine efflux follows scopolamine administered systemically or to the tegmental pedunculopontine nucleus. Neuroscience 1997;76: 177–86.

Clarke PBS, Kumar R. Effects of nicotine and amphetamine on intracranial self-stimulation in a shuttle box in rats. Psychopharmacology 1983;84: 109–14.

Clarke PBS, Fu DS, Jakubovic A, Fibiger HC. Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant actions of nicotine in rats. J Pharmacol Exp Ther 1988;246:701–8.

Corrigall WA, Franklin KBJ, Coen KM, Clarke PBS. The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. Psychopharmacology 1992;107:285–9.

Corrigall WA, Coen KM, Adamson KL. Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. Brain Res 1994;653:278–84.

Forster GL, Blaha CD. Laterodorsal tegmental stimulation elicits dopamine efflux in the rat nucleus accumbens by activation of acetylcholine and glutamate receptors in the ventral tegmental area. Eur J Neurosci 2000; 12:3596–604.

Forster G, Yeomans JS, Takeuchi J, Blaha CD. M5 muscarinic receptors are required for prolonged accumbal dopamine release after electrical stimulation of the pons in mice. J Neurosci 2001;21 [RC190(1-6)].

Gomeza J, Zhang L, Kostenis E, Felder C, Bymaster F, Brodkin J, et al. Enhancement of D1 dopamine mediated locomotor stimulation in M4 muscarinic acetylcholine receptor knockout mice. Proc Natl Acad Sci U S A 1999;96:10483–8.

Gronier B, Rasmussen K. Activation of midbrain presumed dopaminergic neurones by muscarinic cholinergic receptors: an in vivo electrophysiological study in the rat. Br J Pharmacol 1998;124:455–64.

Gronier B, Perry KW, Rasmussen K. Activation of the mesocorticolimbic dopaminergic system by stimulation of muscarinic cholinergic receptors in the ventral tegmental area. Psychopharmacology 2000;147: 347–55.

Ikemoto S, Wise RA. Rewarding effects of the cholinergic agents carbachol and neostigmine in the posterior ventral tegmental area. J Neurosci 2002;22:9895–904.

Joyce EM, Koob GF. Amphetamine, scopolamine and caffeine-induced locomotor activity following 6-hydroxydopamine lesions of the mesolimbic dopamine system. Psychopharmacology (Berl) 1981;73:311–3.

Klink R, d'Exaerde AD, Zoli M, Changeux J. Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. J Neurosci 2001;21:1452–63.

Kofman O, McGlynn S, Olmstead MC, Yeomans JS. Differential effects of atropine, procaine and dopamine in the rat ventral tegmentum on lateral hypothalamic rewarding brain stimulation. Behav Brain Res 1990;38: 55–68.

Lacey MG, Calabresi P, North RA. Muscarine depolarizes rat substantia nigra zona compacta and ventral tegmental neurons in vitro through M1-like receptors. J Pharmacol Exp Ther 1990;253:395–400.

LaViolette SR, Priebe RP, Yeomans JS. Role of the laterodorsal tegmental nucleus in scopolamine- and amphetamine-induced locomotion and stereotypy. Pharmacol Biochem Behav 2000;65:163–74.

Levey AI. Immunological localization of m1–m5 muscarinic acetylcholine receptors in peripheral tissues and brain. Life Sci 1993;52:441–8.

Louis M, Clarke PB. Effect of ventral tegmental 6-hydroxydopamine lesions on the locomotor stimulant action of nicotine in rats. Neuropharmacology 1998;37:1503–13.

Mathur A, Shandarin A, LaViolette SR, Parker J, Yeomans JS. Locomotion and stereotypy induced by scopolamine: contributions of muscarinic receptors near the pedunculopontine tegmental nucleus. Brain Res 1997;775:144–55.

Mattson A, Ogren SO, Ogren L. Facilitation of dopamine-mediated locomotor activity in rats following cholinergic denervation. Exp Neurol 2002;174:96–108.

Munn EM, Wise R. Atropine injections into the ventral tegmental area (VTA) reduce reward but increase locomotor activity and dopamine (DA) levels in the accumbens (Acc). Soc Neurosci Abstr 1999;25:305.

Museo E, Wise RA. Locomotion induced by ventral tegmental microinjections of a nicotinic agonist. Pharmacol Biochem Behav 1990a;35:735–7.

Museo E, Wise RA. Microinjections of a nicotinic agonist into dopamine

terminal fields: effects on locomotion. Pharmacol Biochem Behav 1990b;37:113–6.
Nissell M, Nomikos GG, Svensson TH. Systemic nicotine induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. Synapse 1994;16:36–44.
Panagis G, Nisell M, Nomikos GG, Chergui K, Svensson TH. Nicotine injections into the ventral tegmental area increase locomotion and Fos-like immunoreactivity in the nucleus accumbens of the rat. Brain Res 1996;730:133–42.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates, compact. 3rd ed. New York: Academic Press; 1997.
Picciotto M, Zoli M, Rimondi R, Lena C, Marubio LM, Pich EM, et al. Acetylcholine receptors containing the β2 subunit are involved in the reinforcing properties of nicotine. Nature 1998;391:173–7.
Rada P, Mark GP, Yeomans JS, Hoebel BG. Acetylcholine release in ventral tegmental area by hypothalamic self-stimulation, eating and drinking. Pharmacol Biochem Behav 2000;55:131–41.
Reavill C, Stolerman IP. Locomotor activity in rats after administration of nicotinic agonists intracerebrally. Br J Pharmacol 1990;99:273–8.
Reever CM, Ferrari-DiLeo G, Flynn DD. The M5 (m5) receptor subtype: fact or fiction? Life Sci 1997;60:1105–12.
Reitstetter R, He DS, Gruener R. Oxotremorine-M activates single nicotinic acetylcholine receptor channels in cultured Xenopus myocytes. Eur J Pharmacol 1994;264:27–32.
Shannon HE, Peters SC. A comparison of the effects of cholinergic and dopaminergic agents on scopolamine-induced hyperactivity in mice. J Pharmacol Exp Ther 1990;255:549–53.
Takeuchi J, Fulton J, Jia Z, Abramov-Newerly W, Jamot L, Sud M, et al. Increased drinking in mutant mice with truncated M5 muscarinic receptor genes. Pharmacol Biochem Behav 2002;72:117–23.
Thornberg JE, Moore KE. Inhibition of anticholinergic drug-induced locomotor stimulation in mice by α-methyltyrosine. Neuropharmacology 1990;12:1179–85.
Vilaro MT, Palacios JM, Mengod G. Localization of m5 muscarinic mRNA in rat brain examined by in situ hybridization histochemistry. Neurosci Lett 1990;43:31–6.
Weiner DM, Levey AI, Brann MR. Expression of muscarinic acetylcholine receptors and dopamine receptor mRNAs in basal ganglia. Proc Natl Acad Sci U S A 1990;87:7050–4.
Yeomans JS. Role of tegmental cholinergic neurons in dopaminergic activation, antimuscarinic psychosis and schizophrenia. Neuropsychopharmacology 1995;12:3–16.
Yeomans JS, Baptista M. Both nicotinic and muscarinic receptors in ventral tegmental area contribute to brain stimulation reward. Pharmacol Biochem Behav 1997;57:915–21.
Yeomans JS, Kofman O, McFarlane V. Cholinergic involvement in lateral hypothalamic self-stimulation. Brain Res 1985;329:19–26.
Yeomans JS, Takeuchi J, Baptista M, Flynn DD, Lepik K, Nobrega J, et al. Brain-stimulation reward thresholds raised by an antisense oligonucleotide for the M5 receptor infused near dopamine cells. J Neurosci 2000;20:8861–7.
Yeomans J, Forster G, Blaha C. M5 muscarinic receptors are needed for slow activation of dopamine neurons and for rewarding brain stimulation. Life Sci 2001;68:2449–56.
Yeomans JS, Ng K, Hayes D, Rothenberg L, Forster G, Blaha CD, et al. Improvement in latent inhibition learning, and decrease in amphetamine-induced locomotion in M5 muscarinic receptor knockout mice. Soc. Neurosci Abstr 2003 [in preparation].
Zhang W, Yamada M, Gomeza J, Basile AS, Wess J. Multiple muscarinic acetylcholine receptor subtypes modulate striatal dopamine release, as studied with M1–M5 muscarinic receptor knockout mice. J Neurosci 2002;22:6347–52.