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ORIGINAL INVESTIGATION

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Activation of the mesocorticolimbic dopaminergic system by stimulation of muscarinic cholinergic receptors in the ventral tegmental area

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Abstract *Objective:* To investigate the function of muscarinic receptors in the ventral tegmental area in vivo, the release of endogenous monoamines was simultaneously measured in the somatodendritic (ventral tegmental area) and terminal (frontal cortex and nucleus accumbens) regions of the mesocorticolimbic dopaminergic system in rats, using dual probe microdialysis. *Methods:* Rats were implanted with dual microdialysis probes ipsilaterally into the ventral tegmental area (VTA) and nucleus accumbens (NAC) or frontal cortex (FC). *Results:* Intrategmental infusion of the muscarinic agonist oxotremorine M (OXO M, 0.1 and 1 mM) increased extracellular levels of dopamine and serotonin, but not noradrenaline, in the VTA to a maximum of 200% over baseline in both urethane-anaesthetized and unanaesthetized rats. In freely moving animals, this effect was accompanied by strong motor agitation. Both VTA dopamine and serotonin levels dropped to 60% or less of baseline when the perfusion medium was replaced by a calcium-free medium containing OXO M. In the NAC and FC, a similar increase in extracellular dopamine, but not serotonin and noradrenaline, was observed during OXO M infusion in the VTA. The removal of calcium during OXO M infusion in the VTA did not cause a decrease in NAC dopamine levels. Activation of serotonin and dopamine release by OXO M in the VTA and FC was dramatically reduced or prevented by the coinfusion of the muscarinic antagonist *N*-methylscopolamine (0.1 mM). *Conclusion:* These data demonstrate that VTA dopamine cells possess functional muscarinic receptors whose activation stimulates the release of dopamine in the VTA, NAC and FC. These results also suggest that muscarinic receptors may modulate the synaptic release of serotonin in the VTA.

Key words A10 region · Muscarinic receptor · Microdialysis · Mesocorticolimbic dopaminergic system

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Introduction

The mesocorticolimbic dopaminergic system plays an important role in the mediation of motor, cognitive and affective functions, and is thought to be intimately involved in the aetiology of a variety of neuropsychiatric disorders, including schizophrenia and addictive disorders. This system originates in the ventral tegmental area (VTA or A10 region), a midbrain site where dopamine cell bodies are densely distributed, and sends projections to cortical and limbic regions. It receives, in turn, a variety of inputs that can modulate the activity of the tegmental dopamine cells and therefore alter the release of dopamine in terminal regions. Characterization of the physiological and pharmacological roles played by neurotransmitters in regulating the activity of dopamine cells is fundamental for establishing new strategies for the treatment of neuropsychiatric disorders.

Morphological studies suggest the existence of a cholinergic projection from the mesopontine cholinergic nuclei (particularly the laterodorsal tegmentantal nucleus) to the VTA (Henderson and Sheriff 1991; Oakman et al. 1995). In addition, different cholinergic biochemical markers, indicative of the presence of cholinergic terminals, are present in the midbrain dopamine nuclei (Butcher 1977; Butcher and Wolfe 1982; Bernard et al. 1995). Interestingly, chemical stimulation of the pediculopontine tegmentum nucleus, an area adjacent to the LTDG, produced an increase in dopamine turnover in the nucleus accumbens and striatum, indicative of a functional interaction between mesopontine cholinergic and midbrain dopamine neurons (Niijima and Yoshida 1988). However, despite this neuroanatomical relationship, the function of cholinergic receptors (muscarinic or nicotin-

ic) in the VTA is not well understood. The presence of nicotinic receptors, which have been studied in greater detail than muscarinic receptors, on the dopamine cell bodies has been clearly demonstrated by lesion studies (Clarke and Pert 1985; Clarke et al. 1985). Nicotinic receptor activation in the VTA is known to enhance the burst firing of dopamine neurons and dopamine release in the nucleus accumbens (Grenhoff et al. 1986; Mereu et al. 1987; Nisell et al. 1994a, 1994b, 1996; Blaha et al. 1996). This neuronal activation seems to involve, at least partially, presynaptic nicotinic receptors located on glutamatergic terminals (Schilstrom et al. 1998). The presence of muscarinic receptors in the VTA is less clear since they are barely detectable (Mash and Potter 1986). However, expression of mRNA for the m5 receptor subtype has been demonstrated in midbrain dopamine neurons (Villaro et al. 1990; Weiner et al. 1990). Moreover, previous in vitro (Lacey et al. 1990) and in vivo electrophysiological studies (Gronier and Rasmussen 1998) have demonstrated that microiontophoretic or bath application of selective muscarinic agonists can potently increase the firing rate of A10 dopamine cells. We have also recently demonstrated that the muscarinic receptor involved in this neuronal activation belongs to the M_1 receptor subfamily (i.e., m_1, m_3 and m_5; Gronier and Rasmussen 1999). Additional support for the muscarinic modulation of dopamine neuronal activity has come from recent investigations showing that the peripheral administration of muscarinic agents can modulate dopamine release in mesocorticolimbic areas (Perry et al. 1997) and alter dopamine-related behaviours (Yeomans et al. 1993; Yeomans 1995; Bymaster et al. 1998; Fink-Jensen et al. 1998).

On the basis of these findings, it becomes relevant to determine whether tegmental muscarinic receptors modulate the release of dopamine in terminal regions. However, dopamine is released not only from dopamine terminals but also from cell bodies and dendrites, where dopamine is synthesized, stored, and metabolized (Cheramy et al. 1981; Nissbrandt et al. 1989). Dopamine released in the VTA plays a role in the self-regulation of dopaminergic cell activity, and in the control of the release of neurotransmitters from cortical and limbic afferent fibres. However, whether the characteristics of dopamine release in dendrites and cell bodies differ from that in terminals is still controversial (Cheramy et al. 1981; Nissbrandt et al. 1989; Westerink 1992).

In the present study, using dual probe microdialysis, we investigated whether the activation of tegmental muscarinic receptors alters dopamine release in the somatodendritic region (VTA) and in the main dopamine terminal areas (ipsilateral nucleus accumbens and frontal cortex) of the mesocorticolimbic dopaminergic system. In addition, we also measured the effect of tegmental muscarinic activation on serotonin and noradrenaline levels in these different areas.

Materials and methods

Subjects

Male Sprague Dawley rats weighing 280–350 g at the time of surgery were housed in groups of four under standard laboratory conditions, and maintained under a 12-h light and dark cycle with unlimited access to food and water.

Surgery

For microdialysis studies in the awake animals, two guide cannulae (BAS, Lafayette, Ind., USA) were implanted into the brain of the rat under chloropent anaesthesia (0.33 ml/100 g, IP). One cannula was inserted into the VTA (AP –7.2 mm, L 1 mm, V 7 mm, set at a 15° angle), and a second was placed into the ipsilateral nucleus accumbens (AP 2.5 mm, L 1.5 mm, V 6 mm), or the ipsilateral frontal cortex (AP 3.3 mm, L 1 mm, V 2 mm). Body temperature was maintained at 37°C during the surgery. Rats were allowed to recover for 36–48 h. Twelve hours before the experiments, the probes (BAS, 2 mm for studies in the nucleus accumbens and VTA, and 4 mm for studies in the frontal cortex) were inserted. Animals that were studied under anaesthesia were anaesthetized with urethane (1.25 g/kg, IP). The probes were implanted directly at the same co-ordinates as for unanaesthetized rats. Body temperature was maintained at 37°C.

Microdialysis and chromatographic analysis

The levels of dopamine, serotonin, noradrenaline, and their metabolites were recorded from both the VTA and ipsilateral frontal cortex or nucleus accumbens in the same animal. Probes were perfused with a Ringer solution at a flow rate of 1.5 μl/min (BAS infusion pump) and fractions were collected every 20 min. The composition of the Ringer solution was: NaCl, 150 mM, KCl, 3 mM, $CaCl_2$ 1.7 mM, $MgCl_2$, 0.9 mM, with the pH adjusted to 6.9. Samples (30 μl for 20 min) were collected in an acidic and antioxidant-containing medium (EDTA 0.22 mM, L-cysteine 0.33 mM, ascorbic acid 0.05 mM, acetic acid 0.1 M, 10 μl) and stored frozen at –50°C. The content of dopamine, serotonin, noradrenaline and their main metabolites were determined by electrochemical detection. On the day of analysis, the samples were transferred into a temperature-controlled (2°C) sample tray on an HPLC sample injector (Gilson Model 231). The dialysate samples (20 μl) were injected by using a ten-port Valco valve with electric actuator through a cleanup column (BDS-Hypersil 3 m C18, 2× 10 mm), and then onto the analytical column (BDS-Hypersil 3 m C18, 2×150 mm from Keystone Scientific, Bellefonte, Pa., USA). The mobile phase (75 mM sodium phosphate monobasic, 350 mg/l 1-octane sulphonic acid sodium salt, 0.5 mM EDTA, 0.8% tetrahydrofuran (HPLC grade, inhibitor free) and 8% acetonitrile at pH 3, adjusted with phosphoric acid) and the flow rate (0.2 ml/min) for both columns was the same. Monoamines were detected with an electrochemical detector (EG & G PARC, Princeton, N.J., USA) with dual glassy carbon electrodes (E1=600 mV, E2=–10 mV, range=0.5 nA on both electrodes). Serotonin was detected at E1 and dopamine and noradrenaline were detected at E2. Data from both channels were collected by a Compaq computer running a chromatography data system (Ezchrom Scientific Software, San Ramon, Calif., USA) which determines peak heights and calculates sample concentrations. Sensitivity for dopamine, serotonin and noradrenaline was 0.1 pmol/ml dialysate (which was 3× noise level) or 2 fmol/sample (20 μl). This HPLC method also allows detection of the main metabolites of DA and 5-HT: 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), at a 100-fold lower sensitivity than for monoamines. At the end of the experiment, the animal was killed by administration of an overdose of chloral hydrate. Fast green FCF (0.3%) was then infused through the probes during 2–3 min and

Table 1 Mean (±SEM) basal extracellular levels (nM) of dopamine, serotonin, noradrenaline, 5-HIAA, DOPAC and HVA from the ventral tegmental area (*VTA*), frontal cortex, and nucleus accumbens, in anaesthetized and conscious animals

		Dopamine	Serotonin	Noradrenaline	DOPAC	HVA	5-HIAA
VTA							
	Freely moving	0.24±0.09 (9)	0.36±0.14 (9)	1.62±0.47 (8)	63±21 (8)	43±10 (8)	306±60 (8)
	Anaesthetized	0.33±0.03 (34)	0.29±0.04 (34)	0.45±0.22 (13) *	63±5 (34)	47±4 (34)	351±28 (34)
Frontal cortex							
	Freely moving	0.10±0.03 (7)	0.30±0.10 (7)	2.27±0.55 (7)	74±22 (7)	99±30 (7)	231±56 (7)
	Anaesthetized	0.34±0.06 (9)**	0.42±0.15 (8)	1.66±0.90 (7)	130±32 (9)	151±35 (9)	310±52 (9)
Accumbens							
	Anaesthetized	1.6±0.18 (24)	0.25±0.04 (23)	0.17±0.16 (18)	857±78 (23)	445±37 (23)	257±21 (23)

Values between brackets represent the number of animals studied
*P<0.02; **P<0.01, Student's t-test

the brain was collected and frozen for histological verification according to the atlas of Paxinos and Watson (1982).

The two drugs used during our experiments which were infused through the probe into the VTA, oxotremorine M and *N*-methylscopolamine, were purchased from RBI (Natick, Mass., USA).

Statistics

All values given are expressed as percentage of controls. The average concentration of the last three stable samples before the drug treatment were considered as a control baseline. Within-group analyses were conducted using the Kruskal-Wallis test for non-parametric analysis of variance, followed, when appropriate, by the Mann-Whitney *U*-test (with sequentially rejective Bonferroni's test, Hochberg variation, for *P* values readjustment) for multiple comparisons. Comparisons of time courses between groups were carried out by the Mann-Whitney *U*-test, based on calculation of area values (i.e. total of responses at each time point). Differences between the average dialysate concentrations in the anaesthetized and unanaesthetized preparations were performed using Student's *t*-test. The level of significance was set at P<0.05.

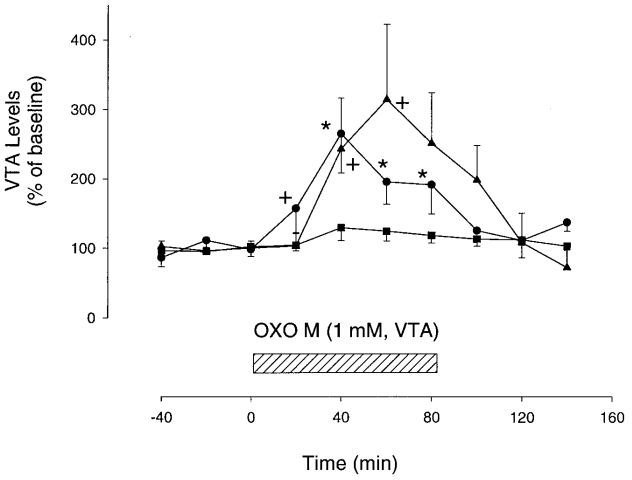

Fig. 1 Effects of oxotremorine M (*OXO M*; 1 mM), infused for 80 min into the ventral tegmental area (*hatched bar*), on tegmental extracellular levels of serotonin, noradrenaline, and dopamine in freely moving animals. Data are presented as mean (±SEM) percent change of baseline. *P<0.01, +P<0.03; compared to the corresponding baseline values (Mann-Whitney *U*-test with adjustment). ● Dopamine (n=8), ▲ serotonin (n=8), ■ noradrenaline (n=6)

Results

Basal extracellular levels of dopamine, noradrenaline, and serotonin in the different brain areas are shown in Table 1. They are in the same range in the anaesthetized and unanaesthetized rats. However, slight differences were observed between the two types of preparations. Basal extracellular levels of dopamine in the frontal cortex are significantly higher in the anaesthetized rats, and basal extracellular levels of noradrenaline are significantly higher in the conscious animals. In the anaesthetized preparation, monoamine values remain stable with normal artificial CSF administration over the timecourse of the experiment (Table 1, Fig. 2, Fig. 3, Fig. 4). During the removal of calcium from the CSF medium in the VTA, levels of serotonin and dopamine decrease by more than 40% in the VTA, while accumbens dopamine levels increased by more than 60% (Fig. 2, Fig. 3, Fig. 4). Experiments in the anaesthetized animals were carried out within the first 5 h following the onset of the anaesthesia because the basal levels of dopamine and metabolites tended to increase progressively when the anaesthesia was prolonged by more than 6 h, probably due to a progressive loss of tissue oxygenation (not shown). Results from the rare experiments where rats were not breathing normally under urethane anaesthesia were not considered.

Effect of tegmental infusion of oxotremorine M on the dialysate content of monoamines in the VTA

The selective muscarinic agonist oxotremorine M (OXO M; 0.1 or 1 mM) was infused in the VTA after collecting baseline levels for 90–140 min. In the conscious animals, tegmental infusion of OXO M caused a strong behavioural activation, including hyperlocomotion, turning behaviour, rearing, and grooming. This agitated state usually started 10–20 min after the onset of OXO M infusion and lasted 30–40 min, after which the rats stayed immobilized until the end of the perfusion. After the cessation of OXO M infusion, the rats tended to return to a

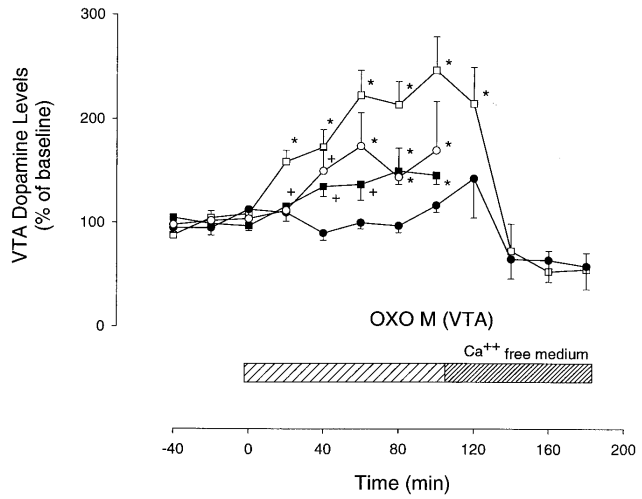

Fig. 2 Effect of two concentrations (0.1 mM and 1 mM) of oxotremorine M (*OXO M*), infused into the ventral tegmental area, alone or in combination with the muscarinic antagonist *N*-methylscopolamine (0.1 mM), and in the presence (*light-hatched bar*) or absence (*dark-hatched bar*) of calcium in the perfusion medium, on the tegmental extracellular levels of dopamine, in urethane-anaesthetized rats. Data are presented as mean (±SEM) percent change of baseline. **P*<0.01, +*P*<0.04, compared to the corresponding baseline levels (Mann-Whitney *U*-test with adjustment). □ OXO M 1 mM (*n*=10), ■ OXO M 1 mM+0.1 mM methylscopolamine (*n*=4), ○ OXO M 0.1 mM (*n*=5), ● OXO M 0 mM (*n*=7–10)

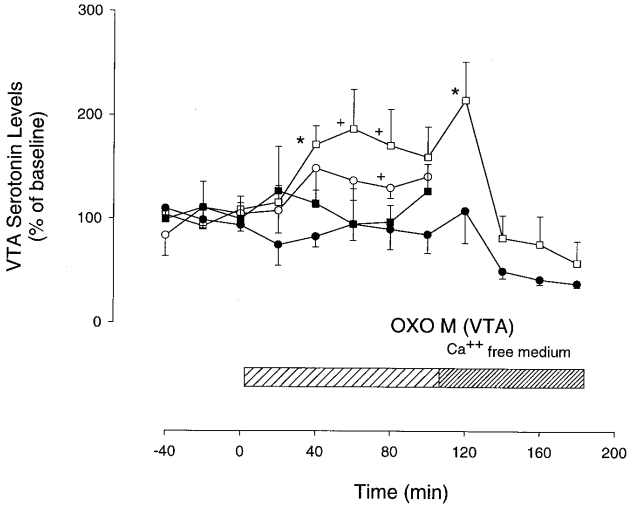

Fig. 3 Effect of two concentrations (0.1 mM and 1 mM) of oxotremorine M (*OXO M*), infused into the ventral tegmental area, alone or in combination with the muscarinic antagonist *N*-methylscopolamine (0.1 mM), and in the presence (*light-hatched bar*) or absence (*dark-hatched bar*) of calcium in the perfusion medium, on the tegmental extracellular levels of serotonin in urethane-anaesthetized rats. Data are presented as mean (±SEM) percent change of baseline. **P*<0.01, +*P*<0.05 compared to the corresponding baseline levels (Mann-Whitney *U*-test with adjustment). □ OXO M 1 mM (*n*=5–10), ○ OXO M 0.1 mM (*n*=5), ■ OXO M 1 mM+0.1 mM methylscopolamine (*n*=4), ● OXO M 0 mM (*n*=7–11)

normal behavioural state. Figure 1 shows that intrategmental infusion of the selective muscarinic agonist OXO M (1 mM) in the awake animal induced a robust increase in dopamine (χ^2_7=37.3, *P*=0.0001) and serotonin levels (χ^2_7=21.5, *P*=0.0031), but not noradrenaline levels (χ^2_7=10.7, *P*=0.15), in the VTA. When the artificial CSF medium was reintroduced, serotonin and dopamine levels gradually decreased and tended to stabilize at initial baseline levels. In order to rule out any indirect effect of stress or locomotor agitation, and to prevent high animal discomfort, rats were also tested under deep anaesthesia (urethane, 1.35 g/kg, IP). A significant augmentation of tegmental extracellular serotonin (χ^2_9=31.6, *P*=0.0002) and dopamine levels (χ^2_9=67, *P*=0.0001) during the intrategmental infusion of OXO M (1 mM) was also seen in the anaesthetized animals (Fig. 2, Fig. 3). However, the maximal increase was greater in the unanaesthetized animal (250% versus 300% for dopamine, and 180% versus 320% for serotonin, in the anaesthetized and conscious animals, respectively). Levels of serotonin and dopamine fell below the baseline level when calcium was removed from the artificial CSF medium, still containing the muscarinic agonist (1 mM). As indicated previously, in the absence of OXO M (control conditions) levels of serotonin and dopamine decreased substantially (40–60% of baseline) when calcium was removed from the artificial buffer (Fig. 2, Fig. 3). The selective muscarinic antagonist *N*-methyl-scopolamine (0.1 mM), infused 40 min before the onset of the infusion of the agonist (1 mM), significantly reduced the muscarinic activation of dopamine release (*P*<0.023, Mann-Whitney *U*-test, based on calculation of area values, Fig. 2). Serotonin levels remained generally unchanged during the concomitant infusion of *N*-methylscopolamine and OXO M (χ^2_5=13, *P*=0.93, Fig. 3). In each experiment involving *N*-methylscopolamine, it was verified that this drug by itself had no effect on the basal levels of dopamine and serotonin (not shown). Infusion of a lower concentration of OXO M (0.1 mM) also increased significantly the tegmental levels of dopamine (χ^2_5=20.12, *P*=0.0012) and of serotonin (χ^2_5=12.8, *P*=0.025, Fig. 2, Fig. 3). In both the anaesthetized and unanaesthetized preparations, the activation of tegmental dopamine release was accompanied by a slight, but significant, increase in dopamine metabolites (HVA, χ^2_7=26.4, *P*=0.0004 and χ^2_5=28.4, *P*=0.0008; DOPAC, χ^2_7=37.3, *P*=0.0001 and χ^2_5=39.5, *P*=0.0001; in the freely moving and anaesthetized animals, respectively, Fig. 7, Fig. 8). On the other hand, there was no change in the levels of the serotonin metabolite 5-HIAA (χ^2_7=5.5, *P*=0.59 and χ^2_5=5.3, *P*=0.39 in the freely moving and anaesthetized animals, respectively, Fig. 7).

Effect of tegmental infusion of OXO M on the dialysate content of monoamines in the ipsilateral nucleus accumbens

In the nucleus accumbens of urethane-anaesthetized rats, the tegmental infusion of OXO M (1 mM) results in a

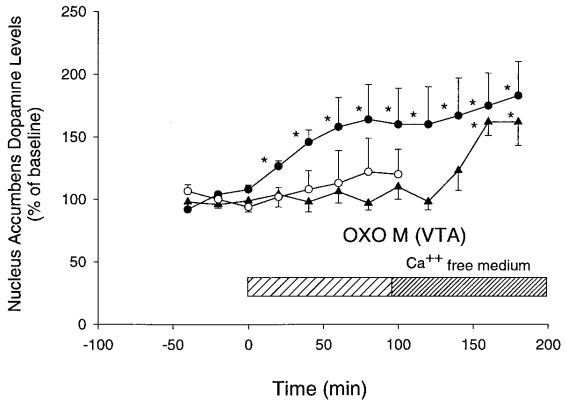

Fig. 4 Effect of two concentrations (0.1 mM and 1 mM) of oxotremorine M (*OXO M*), infused into the ventral tegmental area in the presence (*light-hatched bar*) or absence (*dark-hatched bar*) of calcium in the perfusion medium, on the extracellular levels of dopamine in the nucleus accumbens of urethane-anaesthetized rats. Data are presented as mean (±SEM) percent change of baseline. **P*<0.01, compared to corresponding baseline values (Mann-Whitney *U*-test with adjustment). ▲ OXO M 0 mM (*n*=6–9), ○ OXO M 0.1 mM (*n*=5), ● OXO M 1 mM (*n*=5)

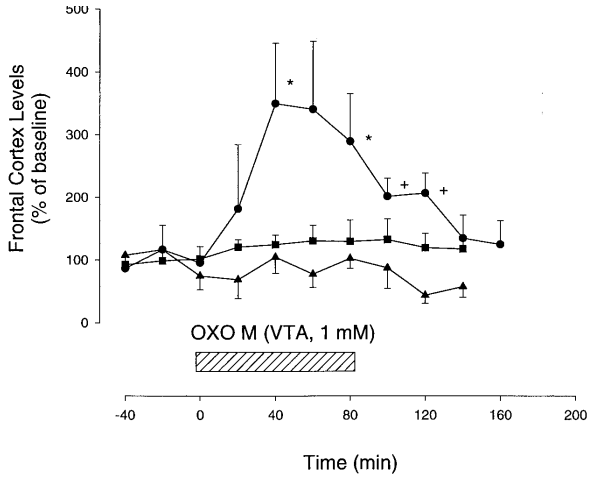

Fig. 5 Effect of oxotremorine M (*OXO M*; 1 mM), infused for 80 min into the ventral tegmental area (*hatched bar*) on the extracellular levels of dopamine, serotonin, and noradrenaline in the frontal cortex of freely moving rats. Data are presented as mean (±SEM) percent change of baseline. **P*<0.02, +*P*<0.034, compared to the corresponding baseline values (Mann-Whitney *U*-test with adjustment). ● Dopamine (*n*=6), ▲ serotonin (n=5), ■ noradrenaline (*n*=5)

gradual increase in the extracellular levels of dopamine (χ^2_9=32.9, *P*=0.0001, maximal peak 166%, at t=80 min, Fig. 4), but not in those of serotonin and noradrenaline (not shown). The removal of calcium from the infusion medium in the VTA only slightly increased accumbens extracellular dopamine levels. On the other hand, as indicated previously, in control condition, extracellular levels of accumbens dopamine increased substantially during the removal of calcium from the CSF infusion medium in the VTA (Fig. 4). Levels of HVA and

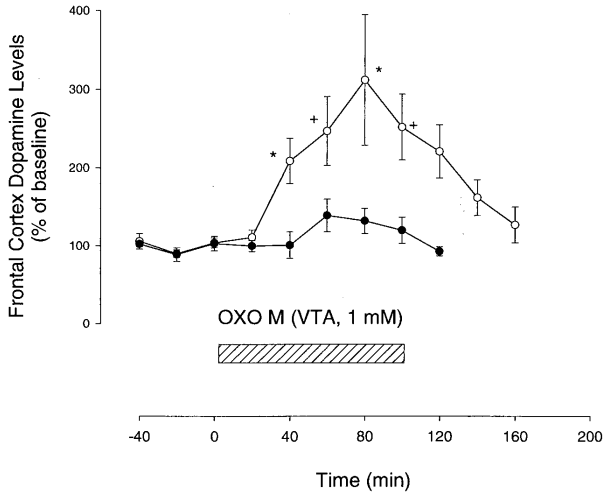

Fig. 6 Effect of oxotremorine M (*OXO M*; 1 mM), infused into the ventral tegmental area (*hatched bar*), alone or in combination with the muscarinic antagonist *N*-methylscopolamine (0.1 mM) in the perfusion medium, on the extracellular levels of dopamine in the frontal cortex of urethane-anaesthetized animals. Data are presented as mean (±SEM) percent change of baseline. **P*<0.01, +*P*<0.03, compared to the corresponding baseline values, (Mann-Whitney *U*-test with adjustment). ○ OXO M 1 mM (*n*=5), ● OXO M 1 mM+scopolamine 0.1 mM (*n*=4)

DOPAC in the nucleus accumbens were slightly but significantly increased during the tegmental infusion of 1 mM OXO M (DOPAC, χ^2_9=19, *P*=0.023; HVA, χ^2_9=23, *P*=0.0062, Fig. 8). On the other hand, levels of dopamine in the nucleus accumbens were not significantly increased during the tegmental infusion of OXO M at lower concentration (0.1 mM, χ^2_5=1.22, *P*=0.94, Fig. 4). Other results from three preparations indicate that levels of extracellular dopamine only slightly decreased within 1–2 h after OXO M was removed from the infusion medium which still contains calcium (not shown).

Effect of tegmental infusion of OXO M on the dialysate content of monoamines in the ipsilateral frontal cortex

During the intrategmental infusion of OXO M (1 mM), both in awake and anaesthetized animals, the extracellular levels of dopamine, but not those of serotonin or noradrenaline, increased dramatically 40–60 min after the onset of the infusion (maximum increase: 320% and 350% in the anaesthetized and freely moving animals) in the ipsilateral frontal cortex (χ^2_7=20.3, *P*=0.0049 in freely moving animals and χ^2_8=24.5, *P*=0.0023 in anaesthetized animals, Fig. 5, Fig. 6). Compared with the nucleus accumbens, the cortex displayed a higher dopamine peak effect, and a more rapid return to baseline. When the selective muscarinic antagonist *N*-methyl-scopolamine was co-infused with OXO M (1 mM) at a lower concentration (0.1 mM) in the VTA of anaesthetized animals the levels of dopamine release did not vary significantly (χ^2_6=10.6, *P*=0.1). In both freely moving and anaesthe-

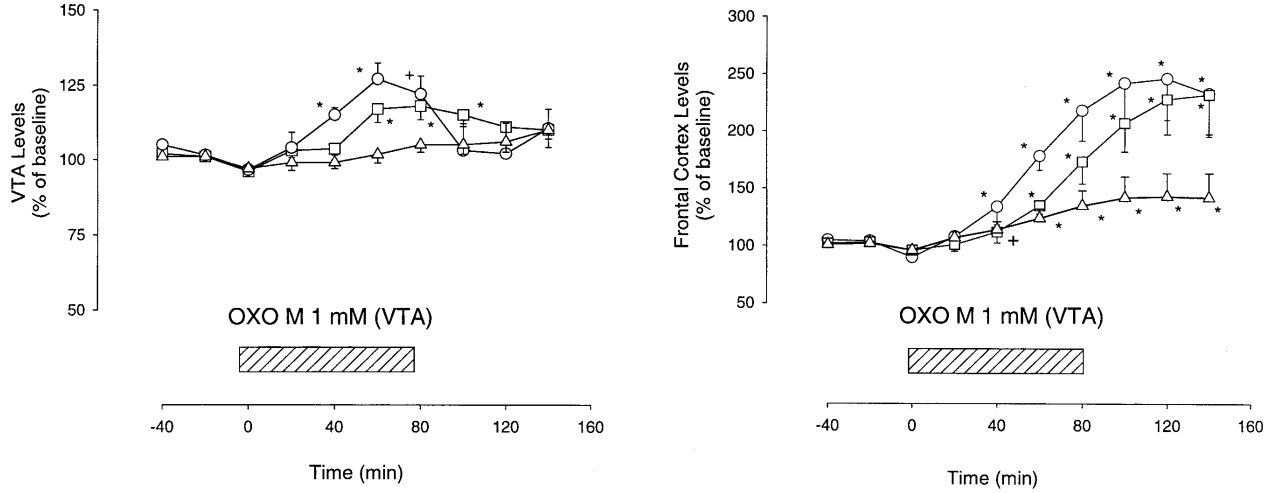

Fig. 7 Effect of oxotremorine M (*OXO M*; 1 mM), infused for 80 min into the ventral tegmental area (*hatched bars*) on the extracellular levels of dopamine (DOPAC and HVA) and serotonin (5-HIAA) metabolites in the ventral tegmental area (*left*), and frontal cortex (*right*) of freely-moving rats. Data are presented as mean (±SEM) percent change of baseline. **P*<0.01, +*P*<0.05, compared to the corresponding baseline values (Mann-Whitney *U*-test with adjustment). □ HVA, ○ DOPAC, Δ 5HIAA

tized animals, the increase in extracellular dopamine was followed by increases in dopamine metabolites in the ipsilateral frontal cortex (HVA, χ^2_7=42, *P*=0.0001 and χ^2_8=41, *P*=0.0001; DOPAC, χ^2_7=41.7, *P*=0.0001 and χ^2_8=42.5, *P*=0.0001; in the freely moving and anaesthetized animals, respectively). The augmentation in HVA levels tends to be more potent in the unanaesthetized animals (Fig. 7, Fig. 8). These increases in metabolites persisted even after the cessation of OXO M infusion and were greater than those observed in the nucleus accumbens and in the VTA (Fig. 7, Fig. 8). In addition, a slight (peak 25% at t=120 and 140 min) but significant increase in the frontal cortex extracellular levels of the serotonin metabolite 5-HIAA (χ^2_8=26, *P*=0.0005) was also observed during the tegmental infusion of OXO M in the freely moving animals but not in the anaesthetized animal (Fig. 7).

Discussion

In this study, a dual probe approach was used to examine the relationship between somatodendritic and terminal dopamine release following pharmacological manipula-

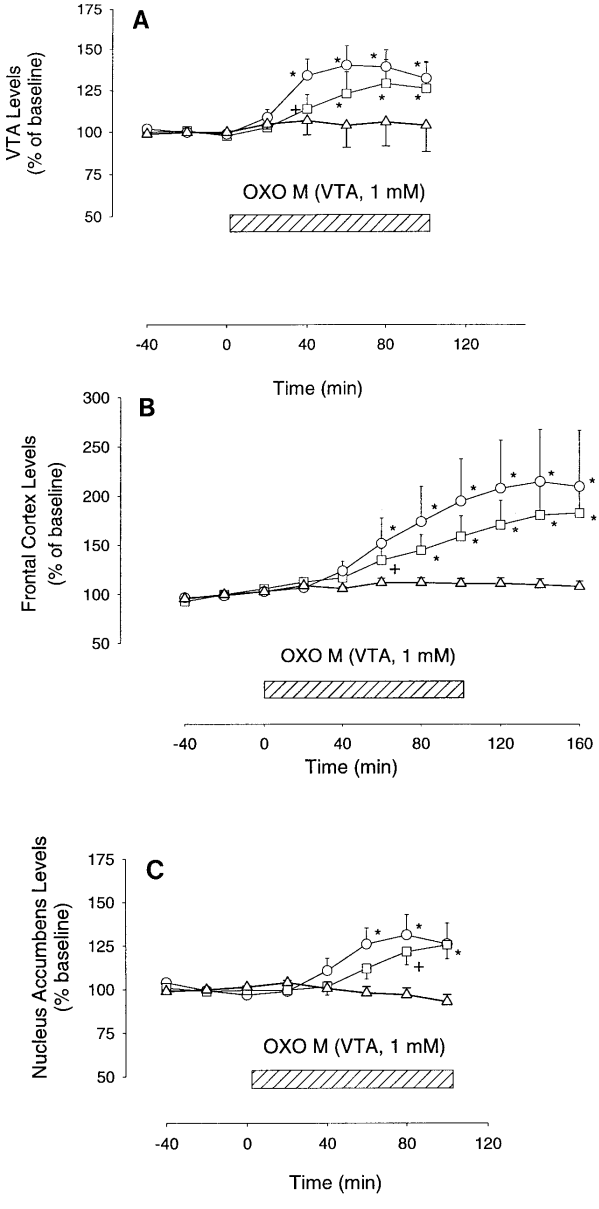

Fig. 8A–C Effect of oxotremorine M (*OXO M*), infused during 100 min into the ventral tegmental area (*hatched bars*) on the extracellular levels of dopamine (DOPAC and HVA) and serotonin (5-HIAA) metabolites in the ventral tegmental area (**A**), frontal cortex (**B**) and nucleus accumbens (**C**) of urethane-anaesthetized rats. Data are presented as mean (±SEM) percent change of baseline and were analysed with one-way ANOVA with repeated measures. **P*<0.01, +*P*<0.05 compared to the corresponding baseline values (Mann-Whitney *U*-test with adjustment). □ HVA, ○ DOPAC, Δ 5HIAA

tion of muscarinic receptors in the VTA. The dual probe approach has been successfully used previously to demonstrate the presence in vivo of functional glutamatergic, GABAergic and dopaminergic receptors in the VTA, activation of which alters dopamine release in terminal areas (Westerink et al. 1996, 1998; Ikemoto et al. 1997; Kohl et al. 1998). The cholinergic agent carbachol or the cholinesterase inhibitor neostigmine, infused into the VTA, have also been found to increase dopamine levels in the accumbens or frontal cortex (Blaha et al. 1996; Westerink et al. 1996, 1998). Interestingly, the effect of neostigmine was prevented by a chemical lesion of the LDTG (Blaha et al. 1996). However, carbachol is a non-selective muscarinic/nicotinic agonist and neostigmine may promote stimulation of both nicotinic and muscarinic receptors. Therefore, no conclusion could be made concerning the selectivity of these effects. Our study clearly demonstrates that stimulation of muscarinic receptors in the VTA activates dopamine release in both the terminal and somatodendritic area of the mesocorticolimbic dopaminergic system.

Local infusion of the selective muscarinic agonist oxotremorine M (OXO M) within the VTA potently increases the extracellular levels of dopamine in this cell body region, confirming our previous electrophysiological observation that functional muscarinic receptors are present within the VTA (Gronier and Rasmussen 1998). This large increase in extracellular dopamine in the VTA is calcium-dependent, since dopamine levels dropped when the buffer was replaced by a calcium-free buffer containing OXO M. Therefore, the increase in dopamine levels is not due to a non-specific toxic effect of the muscarinic agonist and reflects changes in neuronal outflow of dopamine (which likely originates from synaptic vesicles released via an impulse-dependent mechanism). These data also confirm that the somatodendritic release of dopamine can be modulated by non-dopaminergic receptors.

The muscarinic activation of somatodendritic dopamine release likely reflects alterations in the activity of tegmental dopamine neurons since the infusion of the selective muscarinic agonist OXO M in the VTA also strongly increases extracellular dopamine in the nucleus accumbens and frontal cortex, the main dopamine projection areas of the mesocorticolimbic dopaminergic systems. These results are in keeping with our previous electrophysiological observations that selective muscarinic agonists, locally applied onto midbrain dopamine neurons, can potently increase the firing rate and amount of burst activity of A9 and A10 dopamine neurons (Gronier and Rasmussen 1998). Therefore, these changes in firing rate were indicative of an activity-dependent release of dopamine in terminal fields. The fact that somatodendritic and terminal dopamine release in accumbens and frontal cortex respond in a concordant way to the activation of tegmental muscarinic receptors agrees with previous studies which have characterized similar alterations of dopamine release following glutamate, GABA and dopamine tegmental receptor manipulation (Westerink et al. 1992; Kohl et al. 1998).

It is noteworthy that the changes in tegmental and terminal dopamine release are on the same order of magnitude but not strictly correlated. For example, it was necessary to infuse the higher concentration of OXO M (1 mM) in order to observe a clear effect in the accumbens, while an effect was already detected in the VTA with the lower concentration (0.1 mM). This may be due, in part, to the diffusion characteristics of the drug that may need to diffuse through the whole somatodendritic area before a pharmacological effect is detectable in the nucleus accumbens. This concentration dependence may also be due to a difference in sensitivity of limbic dopamine neurons to the muscarinic agonist. Indeed, the increase in dopamine release was greater in the cortex (350% of baseline) than in the nucleus accumbens (180%). In parallel, the augmentation in the outflow of dopamine metabolites is larger in the cortical region than in the limbic region. These results agree with a recent electron microscopic immunohistological study, showing that the majority of cholinergic terminals in the VTA target dendrites containing low levels of dopamine transporters (Garzon et al. 1999). Such dendrites are thought to belong to mesocortical dopamine neurons, and also GABA neurons (Freed and Revay 1995). These results are also in keeping with our electrophysiological observations that fast firing and highly bursting VTA dopamine neurons, which are thought to be more likely mesocortical neurons (White and Wang 1985), are more sensitive to muscarinic agonists (Gronier and Rasmussen, unpublished observation). These data seem also concordant with the recent observation of Perry et al. (1998) that peripheral administration of the preferential muscarinic M_1 agonist xanomeline activates dopamine release in the frontal cortex. Thus, our data suggest that the cholinergic fibres originating from the laterodorsal tegmental nucleus (LDTG) may play an important role in the control of cortical dopaminergic function.

Dysfunction of prefrontal cortex neurotransmission has been proposed to contribute to the pathophysiology of schizophrenia (Knable and Weinberger 1997), particularly in the expression of negative symptoms. An increase in the number of LDTG cholinergic cells has been observed in schizophrenic patients (Garcia-Rill 1995). To what extent this apparent cholinergic hyperactivity could influence cortical neurotransmission remains to be determined. In this regard, it is interesting to note that antimuscarinic drugs have been shown to have some beneficial effects on the negative symptoms of schizophrenic patients, while they tend to worsen positive symptoms (Tandon et al. 1990, 1991; Zemishlany et al. 1996). In addition, behavioral studies also implicate cholinergic neurotransmission in schizophrenia (Yeomans 1995). Further, a recent multidisciplinary investigation has shown that the muscarinic agent 6-(3-propyl-1,2,5-thiadiazol-4-yl)-1-azabicyclo-(3.2.1.)octane (PTAC) displays antipsychotic-like activity in behavioral, neurochemical and electrophysiological paradigms (Bymaster et al. 1998; Fink-Jensen et al. 1998). Thus, the role of the cholinergic system in schizophrenia and the utility of

muscarinic agents in the treatment of schizophrenia warrant additional investigations.

The removal of calcium from the VTA perfusion medium induced different effects on accumbens dopamine release in the presence or absence of OXO M. In control conditions, accumbens dopamine release increased dramatically following the removal of calcium in the VTA. This may reflect an increase in the firing rate of tegmental mesolimbic dopamine neurons. Indeed, in the absence of calcium, the VTA dopamine levels dropped, reflecting a strong reduction of somatodendritic dopamine release which would lead to a reduced dopamine autoreceptor stimulation and subsequent loss of negative feedback on neuronal activity. On the other hand, in the presence of OXO M, accumbens dopamine release only slightly increased during the removal of calcium in the VTA. The fact that the effects of calcium removal and of muscarinic receptor stimulation are not additive may indicate that mesolimbic dopamine neurons have already reached their maximal level of activation during intense muscarinic receptor stimulation. In other words, the subsequent loss of VTA dopamine autoreceptor stimulation by calcium removal could no longer activate neurons that have already been strongly stimulated by the muscarinic agonist. Alternatively, one could also suggest that a proportion of mesolimbic dopamine neurons have been inactivated by depolarization block during calcium removal in the VTA (because of overactivation), while other neurons have become even more activated by calcium removal, the combination of these two opposite effects leading to only a slight increase in accumbens dopamine release.

Of additional interest is the activation of tegmental serotonin release observed during the infusion of the muscarinic agonist, which raises the possibility that muscarinic receptors may mediate serotonin release on serotonin terminals. The VTA receives a substantial serotonin innervation from the dorsal and medial raphe nuclei (Herve et al. 1987; Oades and Halliday 1987), and is known to contain appreciable levels of serotonin (Guan and MacBride 1989; also this study). This activation of serotonin release is neuron-mediated, since it is calcium dependent, and is altered during the co-infusion of the muscarinic antagonist *N*-methylscopolamine. It is not possible to determine whether this activation of serotonin release involves muscarinic receptors located on serotonin nerve terminals or occurs as a consequence of the increase of the release of another neurotransmitter. Further studies are necessary to investigate the mechanism of a possible muscarinic modulation of serotonin release in different serotonin rich areas, including the VTA. To our knowledge, the presence of muscarinic receptors modulating the release of serotonin from serotonin nerve endings has never been described in the literature, although some studies have demonstrated that nicotinic receptor activation may promote the release of serotonin in frontal cortex and striatum (Toth et al. 1992; Takahashi et al. 1998).

In conclusion, our results provide evidence for a direct muscarinic influence on the activity of the mesocorticolimbic dopaminergic system. Therefore, cholinergic afferents to the VTA, via the activation of muscarinic receptors, are a significant modulator of dopamine neurotransmission in mesocorticolimbic structures.

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