Muscarinic Receptors Regulate Extracellular Glutamate Levels in the Rat Striatum: An In Vivo Microdialysis Study

SCOTT M. RAWLS and JACQUELINE F. MCGINTY
Department of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, North Carolina

ABSTRACT

Regulation of extracellular glutamate levels by muscarinic receptors in the striatum of unanesthetized rats was investigated by microdialysis. Extracellular glutamate levels were elevated by intrastriatal perfusion of L-trans-pyrrolidine-2,4-dicarboxylic acid (L-trans-PDC), a competitive substrate of plasma membrane excitatory amino acid transporters. The nonselective muscarinic agonist, oxotremorine (0.5–54 μM) significantly decreased L-trans-PDC-evoked glutamate levels in a concentration-dependent manner. Scopolamine (0.1–10 μM), a nonselective muscarinic receptor antagonist, reversed the effect of oxotremorine, which confirms that muscarinic receptor activation mediated the reduction of L-trans-PDC-evoked glutamate levels. In addition, scopolamine (10 μM) significantly elevated basal extracellular glutamate levels, an effect prevented by oxotremorine, which suggests that acetylcholine tonically regulates glutamatergic transmission in the striatum. Previous data from this laboratory have shown that L-trans-PDC-evoked glutamate levels are partially calcium-dependent. The present study demonstrated that attenuation of L-trans-PDC-evoked glutamate levels by reduced calcium was not altered by oxotremorine. Therefore, it is likely that muscarinic receptors regulate calcium-dependent glutamate release evoked by L-trans-PDC.

Acetylcholine interacts with dopamine and glutamate to regulate striatal output. Disturbance of the balance among these neurotransmitters has been linked to basal ganglia movement disorders and psychostimulant effects. Systemic scopolamine administration elevates extracellular striatal dopamine levels (Chapman et al., 1997; Dewey et al., 1993), which suggests that muscarinic transmission exerts a braking action on dopaminergic transmission. However, not all muscarinic effects are mediated intrastriatally (Chapman et al., 1996) and those that are depend on the muscarinic subtype stimulated (Xu et al., 1989).

Histochemical and autoradiographic studies have shown that the striatum contains high levels of acetylcholine, muscarinic receptors and high-affinity choline uptake sites, as well as high activities of choline acetyltransferase and acetylcholinesterase activity (Kasa, 1986). Striatal acetylcholine arises from interneurons, which account for only 3% of the striatal neuronal population (Bolam et al., 1984). Nonetheless, their highly branched axons innervate all striatal projection neurons (Semba et al., 1987). These medium spiny neurons integrate excitatory glutamatergic input from the cortex and thalamus with dopaminergic input from the midbrain (Parent and Hazrati, 1995).

Cholinergic transmission is mediated by muscarinic and nicotinic receptors. However, nicotinic receptors apparently do not affect striatal glutamate levels (Toth et al., 1993). Muscarinic receptors are encoded by five genes (m1–m5) that have been cloned and characterized pharmacologically (Kubo et al., 1988; Bonner et al., 1988). Clusters of cells expressing m1, m2 and m4 mRNA are detected in regions of the striatum (Bernard et al., 1992) where muscarinic receptor immunoreactivity is abundant (Levey et al., 1991). Because m1, m2 and m4 receptor immunoreactivity has been detected in presynaptic terminals making asymmetric contacts (Hersch and Levey, 1995), it is likely that muscarinic receptors are located on glutamate terminals and presynaptically regulate extracellular glutamate levels.

Previous in vitro investigations have reported that muscarinic agonists inhibit EAA release. For example, in hippocampal synaptosomes, muscarinic receptor activation by oxotremorine or carbachol, another nonselective muscarinic agonist, decreased glutamate release (Marchi and Raiteri, 1989). In addition, presynaptic muscarinic receptor activation decreases EAA transmission in striatal slices (Hernandez et al., 1996) without affecting the postsynaptic membrane potential.

Regulation of in vivo extracellular glutamate levels in the striatum by muscarinic receptors has not been investigated extensively. Godhukin and colleagues (1984) demonstrated

ABBREVIATIONS: L-trans-PDC, L-trans-pyrrolidine-2,4-dicarboxylic acid; EAA, excitatory amino acid; NDA, naphthalene-2,3-dicarboxaldehyde; ACSF, artificial cerebrospinal fluid; HPLC, high-performance liquid chromatography; AUC, area under the curve.
by push-pull perfusion that local application of carbachol decreased basal and potassium-evoked \([\text{H}]\text{glutamate release. In addition, carbachol decreased glutamate efflux evoked by cortical stimulation, which suggests that action potential-dependent glutamate release from corticostriatal terminals is regulated presynaptically by muscarinic receptors (Godhukin et al., 1984). Recently, intrastral perfusion of 10 mM, but not 500 \(\mu\text{M}\), of the partial muscarinic agonist, pilocarpine, decreased basal extracellular glutamate levels (Smolders et al., 1997). Furthermore, the m2/m4 selective antagonist, methoctramine, increased basal striatal glutamate levels, which suggests that presynaptic heteroreceptors may have mediated the effect (Smolders et al., 1997).

To our knowledge, in vivo microdialysis studies examining muscarinic regulation of evoked calcium-dependent glutamate levels have not been conducted. Because of rapid re-uptake of glutamate by EAA transporters, perturbations in extracellular glutamate levels can be variable and difficult to detect even in response to elevated KCl (Rawls SM and McGinty JF, unpublished observations). In contrast, extracellular glutamate levels are elevated reliably by EAA uptake inhibitors, such as L-\text{trans}-PDC. Although in vitro studies have shown that L-\text{trans}-PDC is a substrate for EAA transporters (Waldmeier et al., 1993; Griffiths et al., 1994), our laboratory demonstrated that L-\text{trans}-PDC-evoked glutamate levels in vivo contain a significant calcium- and action potential-dependent component (Rawls and McGinty, 1997a). Furthermore, L-\text{trans}-PDC increased extracellular glutamate levels without producing neurohistological damage (Rawls and McGinty, 1997a, b; Massieu et al., 1995).

The major aim of the present study was to investigate the effects of oxotremorine and scopolamine on L-\text{trans}-PDC-evoked extracellular glutamate levels in the rat striatum by in vivo microdialysis coupled with reverse-phase HPLC. We also investigated the effects of scopolamine and/or oxotremorine on basal glutamate levels to determine whether acetylcholine tonically regulates striatal glutamate efflux. Finally, we examined whether oxotremorine affected the calcium-sensitive component of L-\text{trans}-PDC-evoked glutamate levels.

Materials and Methods

Animals and surgery. All animal use procedures were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee. Male Wistar rats weighing between 250 and 350 g (Charles River, Raleigh, NC) were housed individually with food and water on a 12-hr light/dark cycle.

Fig. 1. The concentration-dependent effects of oxotremorine (OXO) on glutamate levels evoked by L-\text{trans}-PDC (PDC) in the striatum of freely moving rats. Horizontal bars indicate the duration of treatment perfusion. PDC perfusion was started at 0 min. The perfusion of oxotremorine was initiated at -15 min. Data are expressed as the percentage of the mean base-line concentrations (±S.E.M.) of glutamate. (Inset) The histogram represents AUC values from 0 to 30 min of PDC perfusion. *P < .05, as compared with glutamate levels evoked by PDC perfusion alone.
After rats were anesthetized with chloral hydrate (400 mg/kg i.p.), a 21-gauge guide cannula (Plastics One Inc., Roanoke, VA) with a stainless steel tip protruding 2 mm below the dura was implanted into each rat as described (Rawls and McGinty, 1997a). A plastic stylet (Plastics One Inc., Roanoke, VA), which also extended 2 mm below the dural surface, was screwed into the guide cannula after solidification of the dental acrylic. Rats were given a postoperative anesthetic (Toradol, 3 mg/kg i.p.) and allowed to recover at least 48 hr before insertion of the microdialysis probe.

**Microdialysis probe construction.** The concentric microdialysis probes were constructed according to the method of Church and Justice (1987) as described previously (Rawls and McGinty, 1997a, b). One fused silica line was connected to a CMA 100 microinjection pump (CMA/Microdialysis, Acton, MA) and served as the inlet tube. The second fused silica line was placed into a microcentrifuge tube that collected dialysate. Each microdialysis probe was flushed with double-deionized water at a perfusion rate of 0.30 ml/min for at least 12 hr before experimental use, assuring probe fidelity.

**In vivo microdialysis perfusion.** The microdialysis perfusion flow was 1.0 μl/min and was controlled by the CMA 100 microinjection pump. The perfusion medium was ACSF containing NaCl (149.4 mM), CaCl$_2$ (1.1 mM), KCl (3.2 mM), MgCl$_2$ (1.2 mM), and glucose (6.1 mM) adjusted to a pH of 7.4 with NaOH. The ACSF was filtered by a 0.2-micron vacuum filtration and degassed with helium for at least 30 min. After unilateral probe implantation into the striatum of unanesthetized rats, basal neurotransmitter levels were allowed to stabilize during a 3-hr washout period. Dialysate samples were collected into microcentrifuge tubes at 15-min intervals and stored at −70°C until HPLC analysis was conducted. All treatments were administered locally through the microdialysis probe into the striatum. Stock solutions of L-trans-PDC, oxotremorine, and scopolamine were prepared in double-deionized water and diluted to final concentrations in ACSF. Calculations were based on the free-base forms of oxotremorine and scopolamine. Perfusate media were exchanged manually, with each switch taking 10 to 20 sec. Dead volume lag time for each probe was approximately 30 sec.

**L-trans-PDC administration.** We previously demonstrated that 1 mM L-trans-PDC reliably increases extracellular glutamate levels in vivo (Rawls and McGinty, 1997a, b). These glutamate levels contained significant calcium- and action potential-dependent components. Therefore, 1 mM L-trans-PDC was used to evoke extracellular glutamate levels in the present study.

In the L-trans-PDC-treated groups, normal ACSF was perfused through the probe during a 60-min base-line period, followed by 1

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**Fig. 2.** The effect of scopolamine (0.1–10 μM) on the reduction of L-trans-PDC (PDC)-evoked glutamate levels by 5 μM oxotremorine in the striatum of freely moving rats. Horizontal bars indicate the duration of treatment perfusion. PDC perfusion was started at 0 min. The perfusion of oxotremorine (OXO), alone or in combination with scopolamine (SCO), was initiated at −15 min. Data are expressed as the percentage of the mean base-line concentrations (±S.E.M.) of glutamate. (Inset) The histogram represents AUC values from 0 to 30 min of PDC perfusion. *P < .05, as compared with glutamate levels evoked by PDC perfusion alone. +P < .05, as compared with glutamate levels evoked by coperfusion of oxotremorine and PDC.
mM L-trans-PDC for 60 min. After removing L-trans-PDC from the perfusate, normal ACSF was perfused for 60 min.

Experiment 1: Effect of intrastratial perfusion of oxotremorine on L-trans-PDC-evoked extracellular glutamate levels. Because multiple subtypes of muscarinic receptors are expressed in the striatum and selective muscarinic agonists are unavailable, the nonselective muscarinic agonist, oxotremorine, was chosen for this study. Oxotremorine binds with higher overall affinity to muscarinic receptor subtypes than other classical agonists (Messer et al., 1989). Results from previous studies were used as guidelines to determine an effective concentration of oxotremorine perfused by reverse microdialysis (Xu et al., 1989).

After a 45-min base-line period, normal ACSF was replaced by ACSF containing either 54 μM (n = 5), 5 μM (n = 5) or 0.5 μM (n = 6) oxotremorine sesquifumarate (oxotremorine) for 45 min. Fifteen minutes after the initiation of oxotremorine perfusion, L-trans-PDC was perfused for 60 min, followed by normal ACSF for 60 min.

Experiment 2: Effect of intrastratial perfusion of oxotremorine and scopolamine on L-trans-PDC-evoked extracellular glutamate levels. Microdialysis studies investigating the effect of the nonselective muscarinic antagonist, scopolamine, on extracellular glutamate levels have not been conducted. However, scopolamine (1, 10 μM) reduced vinconate-evoked extracellular dopamine levels in the striatum of awake rats (Iino et al., 1995). Based on these data and our pilot studies, a concentration range of 0.1 to 10 μM was chosen.

After a 45-min base-line period, 5 μM oxotremorine was perfused with either 0.1 μM (n = 5), 1 μM (n = 6) or 10 μM (n = 5) scopolamine hydrobromide (scopolamine) in ACSF for 45 min. Fifteen minutes after beginning coperfusion of oxotremorine and scopolamine, L-trans-PDC was administered for 60 min, followed by normal ACSF for 60 min.

Experiment 3: Effect of intrastratial scopolamine and/or oxotremorine perfusion on basal extracellular glutamate levels. The sensitivity of basal extracellular glutamate levels to scopolamine, oxotremorine, or a combination of the two drugs was examined. After a 45-min base-line period, 10 μM scopolamine (n = 5) was perfused for 45 min, followed by normal ACSF for 90 min. In a second group, 5 μM oxotremorine (n = 4) was perfused for 60 min after a 30 min base-line period. In a third group (n = 5), 5 μM oxotremorine perfusion was initiated 15 min before and during 10 μM scopolamine perfusion. In a fourth group, normal ACSF (n = 5) was perfused for 180 min.

Experiment 4: Effect of reduced calcium ACSF on the oxotremorine-induced reduction of L-trans-PDC-evoked extracellular glutamate levels. We previously reported that lowering the calcium concentration in the perfusate reduces L-trans-PDC-evoked extracellular glutamate levels (Rawls and McGinty, 1997a).

Fig. 3. The effect of scopolamine (0.1–10 μM) on basal glutamate levels in the striatum of freely moving rats. Horizontal bars indicate the duration of treatment perfusion. The perfusion of scopolamine (SCO) was initiated at 0 min. Data are expressed as the percentage of the mean base-line concentrations (± S.E.M.) of glutamate. (Inset) Histogram represents AUC values from 0 to 45 min. *P < .05, as compared with glutamate levels evoked by ACSF perfusion.
In addition, we demonstrated that U-69593, a selective kappa opioid receptor agonist, did not alter the calcium-independent component of l-trans-PDC-evoked glutamate levels (Rawls and McGinty, 1997b). In the present study, we examined the effect of oxotremorine on the calcium-independent component of L-trans-PDC-evoked glutamate levels.

After a 45-min baseline period, normal ACSF was replaced with ACSF containing 0.1 mM calcium (n = 4) for 45 min. Fifteen minutes after the initiation of 0.1 mM calcium perfusion, 1 mM L-trans-PDC perfusion was administered for 60 min, followed by normal ACSF for 60 min. In separate groups, after a 45-min baseline period, 5 µM oxotremorine was perfused in the presence (n = 5) or absence (n = 4) of 0.1 mM calcium for 45 min. Fifteen minutes after the initiation of oxotremorine plus or minus 0.1 mM calcium perfusion, 1 mM L-trans-PDC was administered for 60 min, followed by normal ACSF for 60 min. In still another group, after a 60-min baseline period, 1 mM L-trans-PDC alone (n = 4) was perfused for 60 min, followed by normal ACSF for 60 min.

Amino acid analysis. For glutamate derivatization, 5 µl of dialysate or amino acid standard was mixed with 5 µl of sodium borate (8 mM, pH 9.5), followed by the addition of 5 µl of potassium cyanide (12 mM). The resulting solution was mixed with 2 µl of NDA solution and allowed to derivatize for 5 min, as described previously (Rawls and McGinty, 1997b, a). Reacting primary amines (i.e., glutamate) with NDA in the presence of cyanide ion in borate buffer yields a stable, electrochemically and UV-detectable 1-cyano-(f)-isoindole derivative (de Montigny et al., 1987; O’Shea et al., 1992).

After derivatization, 17 µl of the derivatization mixture was injected into a 5-micron C18 reversed phase column (150 x 4.6 mm) (Phenomenex, Inc., Torrance, CA) with a LKB 2150 HPLC pump (Pharmacia Biotech, Uppsala, Sweden) and eluted with one of the following mobile phases: 1) 5 mM sodium citrate buffer (pH = 7.5) used in a linear gradient elution with 30% methanol, or 2) 5 mM sodium acetate buffer (pH = 7.5) containing 40% methanol under isocratic conditions. Varying the buffer and gradient conditions affected glutamate retention times but did not affect quantitative analysis. Flow rates were varied between 0.5 and 0.75 ml/min.

Glutamate was detected with a model HP 1050 diode array detector (Hewlett-Packard Company, Atlanta, GA). Glutamate was identified by overlaying absorption spectra at excitation wavelengths of 250, 420 and 440 nm. Glutamate was quantified at 420 nm by HP CHEM STATION software (Hewlett-Packard Company, Atlanta, GA). Glutamate was identified by overlaying absorption spectra at excitation wavelengths of 250, 420 and 440 nm. Glutamate was quantified at 420 nm by HP CHEM STATION software (Hewlett-Packard Company, Atlanta, GA). Glutamate was identified by overlaying absorption spectra at excitation wavelengths of 250, 420 and 440 nm. Glutamate was quantified at 420 nm by HP CHEM STATION software (Hewlett-Packard Company, Atlanta, GA).

Results

In vitro probe recoveries, basal glutamate levels and histology. In vitro probe recoveries averaged 30 ± 10%. Basal extracellular glutamate levels, uncorrected for recovery, ranged from 1 to 5 µM. Probe sites were viewed microscopically to ensure correct placement in the dorsal striatum and minimal damage caused by drug perfusion and probe insertion. Similar to those shown previously, probe sites were located primarily in the medial-central striatum (Rawls and McGinty, 1997a).

Effect of oxotremorine on l-trans-PDC-evoked extracellular glutamate levels. Perfusion of 1 mM l-trans-PDC into the striatum elevated basal glutamate levels approximately 4-fold, with the peak increase occurring during the first 15 min of perfusion (fig. 1). During the first 15 min of oxotremorine perfusion (0.5 or 54 µM), basal glutamate levels were not affected (fig. 1). However, oxotremorine decreased the magnitude and duration of the l-trans-PDC effect in a concentration-dependent manner. Five micromolar, but not 0.5 µM, oxotremorine significantly decreased l-trans-PDC-evoked glutamate levels as determined by AUC analysis during the first 30 min of l-trans-PDC perfusion (fig. 1, inset). A higher concentration of oxotremorine, 54 µM, significantly reduced l-trans-PDC-evoked glutamate levels but also produced neurohistological damage (data not shown).

Effect of oxotremorine and scopolamine on l-trans-PDC-evoked extracellular glutamate levels. Scopolamine concentration-dependently reversed the oxotremorine-induced reduction of l-trans-PDC-evoked glutamate levels (fig. 2). However, only the highest concentration of scopolamine, 10 µM, was effective, as determined by AUC analysis during the first 30 min of l-trans-PDC perfusion (fig. 2, inset).

Effect of scopolamine and/or oxotremorine on basal extracellular glutamate levels. Ten and 1 µM scopolamine significantly increased basal extracellular glutamate levels, as determined by AUC analysis during the full 45 min of scopolamine perfusion (fig. 3, inset). The augmentation was delayed, with the peak increase occurring 30 to 45 min after the initiation of scopolamine perfusion (fig. 3). Five micromolar oxotremorine reversed the scopolamine-induced increase in basal extracellular glutamate levels (fig. 4, inset). However, perfusion of oxotremorine alone did not affect basal glutamate levels (fig. 4).

Effect of oxotremorine in 0.1 mM calcium-containing ACSF on l-trans-PDC-evoked extracellular glutamate levels. The effects of 0.1 mM calcium, 5 µM oxotremorine and 0.1 mM calcium plus 5 µM oxotremorine on l-trans-PDC-evoked glutamate levels are illustrated in figure 4. Consistent with our previous results (Rawls and McGinty, 1997a, b), l-trans-PDC-evoked glutamate levels were reduced significantly when the calcium concentration in the ACSF was reduced from 1.1 to 0.1 mM (fig. 5, inset). Perfusion of 5 µM oxotremorine, in the presence of both physiological and 0.1 mM calcium, significantly reduced l-trans-PDC-evoked glu-
tamate levels during the first 30 min of L-trans-PDC perfusion (fig. 5, inset). When 5 mM oxotremorine was perfused in 0.1 mM calcium ACSF, the reduction of L-trans-PDC-evoked glutamate levels did not differ from that observed in the 0.1 mM calcium group (fig. 5, inset).

Discussion

Reduction by oxotremorine of L-trans-PDC-evoked extracellular glutamate levels. Local perfusion of oxotremorine by reverse microdialysis significantly reduced L-trans-PDC-evoked extracellular glutamate levels in the striatum of unanesthetized rats. Basal glutamate levels were not affected by perfusion of oxotremorine. Scopolamine reversed the effect of oxotremorine, confirming that oxotremorine reduced L-trans-PDC-evoked glutamate levels by activating muscarinic receptors. To our knowledge, in vivo evidence that selective activation of muscarinic receptors decreases striatal glutamate levels in unanesthetized rats has not been reported previously. Although Smolders and colleagues (1997) demonstrated that 10 mM pilocarpine immediately reduced basal extracellular glutamate levels in the striatum, they did not determine whether muscarinic receptors mediated the effect. High concentrations of pilocarpine are known to induce limbic seizures (Smolders et al., 1997, 1995) and neurotoxic damage similar to that produced by glutamate (Clifford et al., 1987). Because Smolders and colleagues neither reported histological data nor attempted to reverse the pilocarpine-induced effect with a muscarinic antagonist, the possibility exists that pilocarpine acted through mechanisms other than muscarinic receptor activation to reduce glutamate levels. Consistent with the possibility of pilocarpine acting nonselectively, another in vivo study reported that striatal acetylcholine levels were reduced by oxotremorine but increased by high doses of systemic pilocarpine (Murakami et al., 1996). In that study, however, only the effect of oxotremorine was reversed by scopolamine. Based on these data and the low $M_2$-binding affinity of pilocarpine (Hoss et al., 1990), it is unclear whether muscarinic receptors mediated the effect of pilocarpine in the Smolders' study.

Scopolamine increases basal extracellular glutamate levels. Blockade of muscarinic receptors by scopolamine elevated basal glutamate levels in a delayed and pro-
longed manner. Oxotremorine prevented the scopolamine-induced increase in basal glutamate levels. Therefore, it is possible that scopolamine increased basal glutamate levels by blocking muscarinic receptor binding sites on glutamatergic terminals and reducing tonic inhibition of basal glutamate efflux by acetylcholine. The possibility of an inhibitory cholinergic tone regulating basal extracellular glutamate levels is supported by the data of Smolders and colleagues (1997), who reported that methoctramine, a selective m2/m4 muscarinic antagonist, increased basal glutamate levels in a prolonged manner. However, a postsynaptic mechanism cannot be discounted at this point.

How does muscarinic receptor activation reduce L-trans-PDC-evoked extracellular glutamate levels?

Immunocytochemical studies indicate that m1, m2 and m4 receptors account for most muscarinic binding sites in the rat striatum (Levey et al., 1991). Medium spiny neurons contain m1 and m4 receptor immunoreactivity, whereas cholinergic interneurons contain m2 receptor immunoreactivity (Levey et al., 1991). In addition, robust m1, m2 and m4 receptor immunoreactivity was detected in presynaptic terminals, presumably glutamatergic, making asymmetric contacts within the striatum (Hersch and Levey, 1995). Because multiple subtypes of muscarinic receptors are expressed in the striatum and truly selective ligands are unavailable, it was not determined which receptor subtype(s) oxotremorine activated to reduce L-trans-PDC-evoked extracellular glutamate levels. However, it is likely that the m2/m4 class of receptors, which are negatively coupled to adenylate cyclase, are involved.

Muscarinic agonists inhibit the activation of voltage-dependent calcium channels (Howe and Surmeier, 1995). Therefore, oxotremorine may reduce L-trans-PDC-evoked extracellular glutamate levels by preventing calcium influx. In support of this hypothesis, oxotremorine did not affect the calcium-independent component of L-trans-PDC-evoked glutamate levels when the calcium concentration in the perfusate was lowered. These data strongly suggest that oxotremorine reduced calcium-dependent glutamate levels evoked by L-trans-PDC.
Although it is possible that oxotremorine decreases calcium influx at presynaptic glutamate terminals, other possibilities must be considered. For example, stimulation of m2 and m4 receptors decreases adenylate cyclase activity (Hulme et al., 1990). Because adenylate cyclase activation is positively coupled to glutamate release (Herrero and Sanchez-Prieto, 1996), oxotremorine may have reduced L-trans-PDC-evoked glutamate levels by suppressing cAMP-dependent phosphorylation events in glutamatergic terminals. In fact, in the hippocampus, activation of presynaptic muscarinic receptors decreased glutamatergic transmission by interfering with the neurotransmitter release process at some point subsequent to calcium influx (Scanziani et al., 1995). Muscarinic receptors are also present on glial cells (Ashkenazi et al., 1989; Norohna-Blob et al., 1987), providing another mechanism by which oxotremorine might reduce extracellular glutamate levels. However, because oxotremorine did not reduce the calcium-independent component of L-trans-PDC-evoked glutamate levels in our hands, it is unlikely that the effect of oxotremorine resulted from interactions with glial cells. In future studies with striatal synaptosomes, we will investigate the presynaptic versus postsynaptic mechanism by which muscarinic receptors regulate striatal glutamate.

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