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Inhibition of α7-containing nicotinic ACh receptors by muscarinic M_1 ACh receptors in rat hippocampal CA1 interneurones in slices

Jian-xin Shen, Bin Tu and Jerrel L. Yakel

Laboratory of Neurobiology, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, PO Box 12233, Research Triangle Park, NC 27709, USA

Cys-loop ligand-gated nicotinic ACh receptors (nAChRs) and G protein-coupled muscarinic ACh receptors (mAChRs) are expressed on rat hippocampal interneurones where they can regulate excitability, synaptic communication and cognitive function. Even though both nAChRs and mAChRs appear to co-localize to the same interneurones, it is not clear whether there is crosstalk between them. We utilized patch-clamp techniques to investigate this issue in rat hippocampal CA1 interneurones in slices under conditions where synaptic transmission was blocked. The α7 nAChR-mediated currents were activated by choline, and when the activation of this receptor was preceded by the activation of the M_1 mAChR subtype, the amplitude of α7 responses was significantly reduced in a rapidly reversible and voltage-independent manner, without any change in the kinetics of responses. This M_1 mAChR-mediated inhibition of α7 nAChRs was through a PLC-, calcium- and PKC-dependent signal transduction cascade. These data show that M_1 mAChRs and α7 nAChRs are functionally co-localized on individual rat hippocampal interneurones where the activation of these particular mAChRs inhibits α7 nAChR function. This information will help to understand how these cholinergic receptor systems might be regulating neuronal excitability in the hippocampus in a manner that has relevance for synaptic plasticity and cognition.

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Corresponding author J. L. Yakel: NIEHS, F2-08, PO Box 12233, 111 T.W. Alexander Drive, Research Triangle Park, NC 27709, USA. Email: yakel@niehs.nih.gov

Acetylcholine (ACh) acting through cholinergic receptors in the hippocampus, via the G protein-coupled muscarinic ACh receptors (mAChRs) and the Cys-loop ligand-gated nicotinic ACh receptors (nAChRs), can regulate neuronal excitability, synaptic communication and cognitive function (Cobb & Davies, 2005; Lawrence *et al.* 2006*a*). Furthermore, deficits in cholinergic signalling, in particular in the hippocampus, produce an array of disorders in learning and memory, and have been linked with a variety of neurological disorders and diseases, including Alzheimer's disease, schizophrenia and epilepsy (Terry & Buccafusco, 2003; Dani & Bertrand, 2007).

The primary cholinergic input to the hippocampus arises from the medial septum and diagonal band of Broca (MSDB); however, intrinsic cholinergic interneurones have also been described within the hippocampus (Frotscher & Léránth, 1985; Frotscher *et al.* 2000). The cholinergic projections from the MSDB innervate both principal glutamatergic cells and inhibitory GABAergic interneurones, the activation of which can initiate and sustain network oscillations (e.g. hippocampal theta rhythm) *in vivo* and *in vitro* (Frotscher & Léránth, 1985; Buzsáki, 2002; Cobb & Davies, 2005; Lawrence *et al.* 2006*a*,*b*). Hippocampal GABAergic interneurones coordinate the activity of large numbers of principal cells and are believed to be responsible for regulating hippocampal oscillations (Fisahn *et al.* 1998, 2002; Jones *et al.* 1999; Cobb & Davies, 2005). Besides cholinergic input from the MSDB, there is also a significant GABAergic input from the MSDB as well. It is the phasic GABAergic inputs from the MSDB, probably in concert with the tonic cholinergic excitation of interneurones, that entrain hippocampal interneurones, thereby inducing rhythmic inhibition of pyramidal cells (Freund & Antal, 1988; Buzsáki, 2002).

A variety of different subtypes of G protein-coupled mAChRs (M_{1-4}) have been shown to be expressed and regulate a variety of ionic conductances (both depolarizing and hyperpolarizing responses) and signal transduction

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cascades in both hippocampal pyramidal cells and interneurones (McQuiston & Madison, 1999*b*; Cobb & Davies, 2005; Lawrence *et al.* 2006*a*,*b*; Widmer *et al.* 2006). For nAChRs, the α7-containing receptors are the predominant subtype expressed on these interneurones, the activation of which will elicit local changes in cytoplasmic calcium ($[Ca^{2+}]_{in}$) levels due to its high Ca^{2+} permeability (Khiroug *et al.* 2003; Fayuk & Yakel, 2005, 2007). Even though hippocampal interneurones express both functional mAChRs and nAChRs and ACh is the endogenous neurotransmitter for both of these receptors, it is not yet known whether there is some interaction between these different cholinergic receptors, and what implications this may have concerning the modulation of the excitability of interneurones.

In the current study, we have investigated whether the activation of mAChRs had any effect on the properties of the α7-containing nAChRs in rat hippocampal interneurones in slices. We found that the activation of the M_1 mAChR, through a PLC-, calcium- and PKC-dependent signal transduction cascade, significantly reduced the amplitude of the α7 responses. This is the first demonstration of any crosstalk between the nAChR and mAChR systems in the hippocampus, and may help to understand how these two cholinergic receptor systems might be regulating neuronal excitability in the hippocampus in a way that has relevance for synaptic plasticity and cognition.

Methods

Slice preparation

All experiments were carried out in accordance with guidelines approved by the NIEHS Animal Care and Use Committee, which includes minimizing the number of animals used and their suffering. Standard techniques were used to prepare 310 μm thick acute hippocampal slices from 14- to 21-day-old rats (Fayuk & Yakel, 2005). Briefly, rats were anaesthetized with halothane (Sigma) and decapitated. Brains were quickly removed and placed into an ice-cold oxygenated, artificial cerebral spinal fluid (ACSF) containing (in mM): 126 NaCl, 3.5 KCl, 1.3 $MgCl_2$, 2 $CaCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$ and 11 glucose. Slices were placed on to nylon mesh immersed in oxygenated ACSF at room temperature (23–25°C) and then used for recordings after at least 1 h of recovery period and within about 6 h.

Electrophysiology

Whole-cell patch-clamp recordings were performed on hippocampal CA1 interneurones from the stratum radiatum in slices. Patch pipettes (Garner 8250 glass, with resistances of 3–5 MΩ) were filled with an intracellular solution (ICS) that contained (in mM): 120 potassium (or caesium where indicated) gluconate, 2 NaCl, 2 MgATP, 0.3 Na_2GTP, 1 EGTA and 10 Hepes; pH was adjusted to 7.2–7.3 with either KOH or CsOH, and osmolarity was adjusted to ∼285 mosmol l^{-1} with either KCl or CsCl. Slices were superfused at room temperature with oxygenated ACSF. Cells were clamped using an Axopatch 200B amplifier (Axon Instruments, Union City, CA, USA) at a holding potential of −70 mV (corrected for a 10 mV junction potential) using pClamp 10 software (Axon Instruments). Action potentials were recorded from under current-clamp conditions, and current was injected to depolarize neurones to induce spiking; neurones were defined as regular spiking if the rate of action potential firing evoked by minimal depolarization was < 40 Hz (Khiroug *et al.* 2003). After identifying the firing pattern, TTX (1 μM) was added to the perfusing ACSF to block action potential firing. The α7 nAChR-mediated responses were induced by a brief pressure application (50 ms at 35–140 kPa pressure) of choline (10 mM), which was delivered via a glass pipette placed 5–10 μm from the cell body using a Picospritzer II (General Valve Co., Fairfield, NJ, USA). The interval between choline applications was 80 s. The pressure application of control ACSF was given to every cell and it never produced any electrical response. To activate mAChRs, a double-barrelled glass pipette (∼20 MΩ) was used; one side was filled with either oxotremorine-M (100 μM, in ACSF with TTX) or ACh (50 μM, in ACSF with TTX and 10 μM dihydro-β-erythroidine (DHβE)) and the other with ACSF and TTX, or with ACSF, TTX and DHβE (when ACh was used in the other side), and the tip was positioned between 30 and 60 μm from the soma. Oxotremorine-M, ACh or ACSF was delivered by pressure application (duration of 2 s at 70–140 kPa pressure, followed by a delay of 5 s before choline application); the per cent change in the amplitude of the α7 nAChR-mediated responses was usually the average of two to three applications of either oxotremorine-M or ACh. Bath-applied drugs were diluted at final concentrations in ACSF and were delivered to the cell through a gravity-fed perfusion system. Drugs applied intracellularly were diluted at final concentrations in ICS and dialysed through the tip of the patch pipette for ∼20 min after obtaining the whole-cell recording configuration. Cells were discarded if the initial response was below 300 pA; this occurred in 24% (54 cells) of recordings.

Data analysis

Averaged data are presented as mean ± S.E.M., and statistical significance was tested using a *t* test or ANOVA when appropriate; P values of < 0.05 were considered significant.

Results

Muscarinic M_1 receptor activation decreases the amplitude of α7 nAChR-mediated responses

Whole-cell patch-clamp recordings were obtained from rat CA1 stratum radiatum hippocampal interneurones in acute slices. Similar to our previous report (Fayuk & Yakel, 2005), the α7 nAChRs were selectively activated by choline and completely blocked by the α7-selective antagonist methyllycaconitine (MLA). In the present study, the pressure application of choline (10 mM for 50 ms; see Methods) elicited rapidly activating and decaying inward currents (Fig. 1) that were completely blocked by MLA (10 nM; 12 cells). The average amplitude, rise-time (10–90%) and half-time of decay for whole-cell inward currents induced by the pressure application of choline were 790 ± 100 pA, 14 ± 3 ms and 81 ± 20 ms, respectively (16 cells). As previously observed (Khiroug *et al.* 2003), all of the interneurones analysed for firing properties (6 cells) had a regular-spiking action potential firing profile (the average firing rate was 22 ± 1 Hz (Fig. 1*A*, inset)).

When muscarinic ACh receptors (mAChR) were activated by oxotremorine-M (100 μM for 2 s; see Methods), there was a significant decrease in the peak amplitude of the α7 nAChR-mediated responses by $25 \pm 3\%$ (16 cells; range of 6–56%); Fig. 1*A* and *C*), without any change in the kinetics of responses (Fig. 1*D*). This inhibition by oxotremorine-M (under these conditions) was rapidly reversible after washout (within 2 min in most cases; Fig. 2*A*) and was due to the activation of mAChRs since the bath application of atropine (10 μM), the broad spectrum mAChR antagonist, completely blocked the inhibition of the α7 responses by oxotremorine-M (10 cells; Fig. 1*B* and *C*). The application of oxotremorine-M under these conditions had little to no affect (i.e. < 10 pA) on the baseline holding current in 81% of neurones, and only induced a small inward current response (i.e. < 25 pA) in 19% of neurones (16 cells; Supplemental Fig. 1). Therefore the inhibition of α7 responses by oxotremorine-M was independent of whether there were any changes in the baseline holding current induced by oxotremorine-M. To further confirm this, experiments were done where potassium in the intracellular solution was replaced by caesium. Under these conditions (8 cells), oxotremorine-M decreased the amplitude of the choline responses by $37 \pm 4\%$ (which was significantly larger than control, $P < 0.05$), an effect

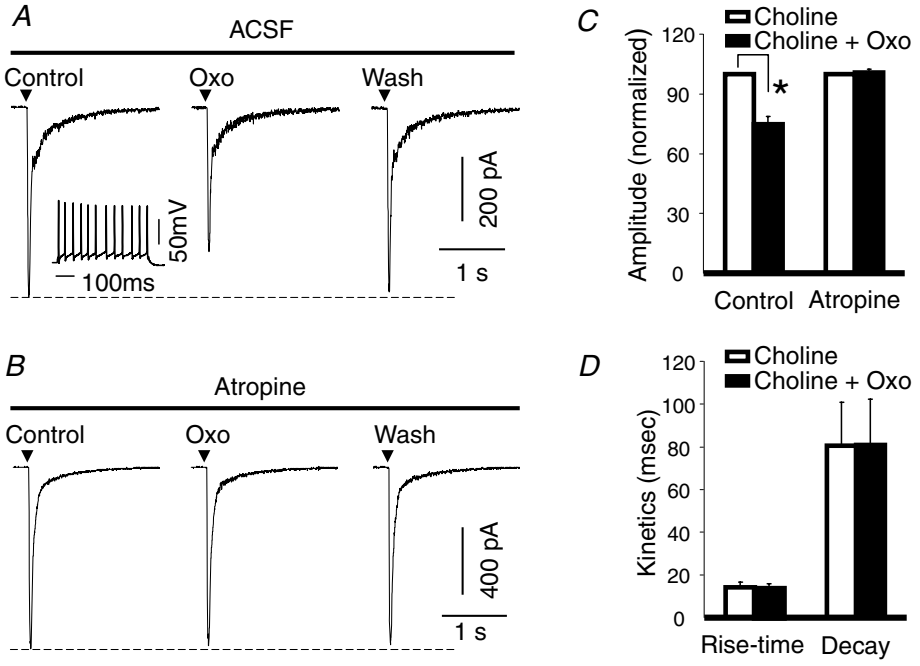

Figure 1. Muscarinic receptor activation decreases amplitude of α7 nAChR-mediated responses
A, representative rapidly activating and decaying inward current responses due to the pressure application of choline (10 mM for 50 ms; indicated by the arrowhead). The inset represents typical spike firing properties of these interneurones. An application of oxotremorine-M (Oxo; 100 μM for 2 s) preceding (by 7 s) the application of choline reduced the amplitude of responses (middle trace); the inhibition by oxotremorine-M was rapidly reversible (right trace; recovery was within 2 min). *B*, representative traces showing that during the bath application of atropine (10 μM), the inhibition of α7 responses by oxotremorine-M was completely blocked. *C*, bar graph of averaged data showing the relative change in amplitude by oxotremorine-M in control (ACSF; $n = 16$) and with atropine ($n = 10$) (*$P < 0.05$). *D*, bar graph of averaged data showing the rise-time and half-time of decay values.

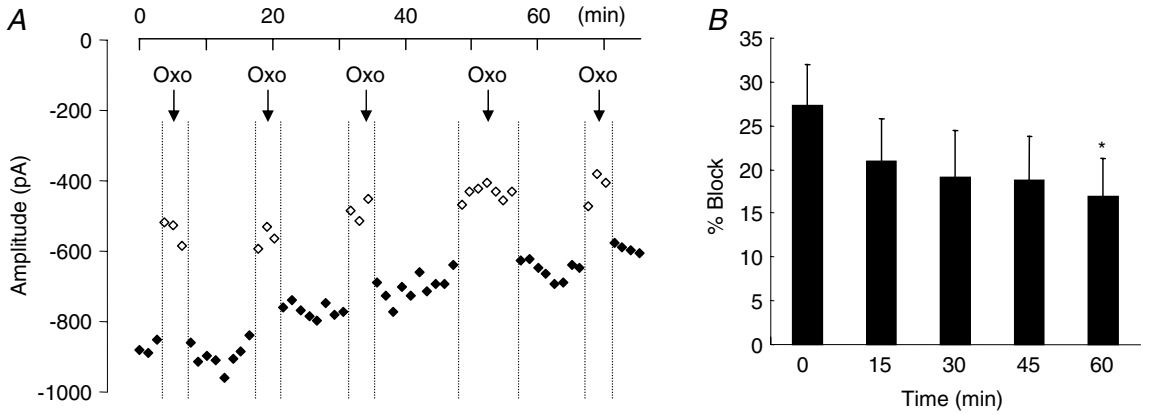

Figure 2. Inhibition of α7 nAChR-mediated responses on repeated activation of mAChRs
A, representative example showing the decrease in amplitude of α7 nAChR-mediated response in an interneurone due to repeated applications of oxotremorine-M. *B*, bar graph of averaged data showing the per cent reduction in amplitude by oxotremorine-M on repeated applications ($n = 6$) (*$P < 0.05$); 0 min is for the first application of oxotremorine-M, and repeated applications were at 15 min intervals.

completely blocked by atropine (10 μM), without any change in the baseline holding current in any of these cells. The fact that this inhibition was larger in recordings using caesium may be due to decreased space-clamp errors with higher input resistances under these conditions (Fayuk & Yakel, 2007), or rather a different intracellular milieu may be having an affect.

Repeated applications of oxotremorine-M (up to 5, at 15 min intervals) continued to decrease the amplitude of the α7 nAChR-mediated responses (Fig. 2). Even after more than 1 h of recording, there was still significant inhibition. These data indicate that there is only modest rundown of the oxotremorine-M-induced inhibition of α7 responses with time.

To determine which subtype of mAChR is involved in the inhibition of α7 nAChR-mediated responses, we utilized the M_1-selective antagonist, pirenzepine (3 μM), and the M_2-selective antagonist, AFDX-116 (1 μM). The bath application of pirenzepine completely blocked the oxotremorine-M-induced inhibition of α7 responses (8 cells; Fig. 3), whereas the bath application of AFDX-116 was not significantly different than control

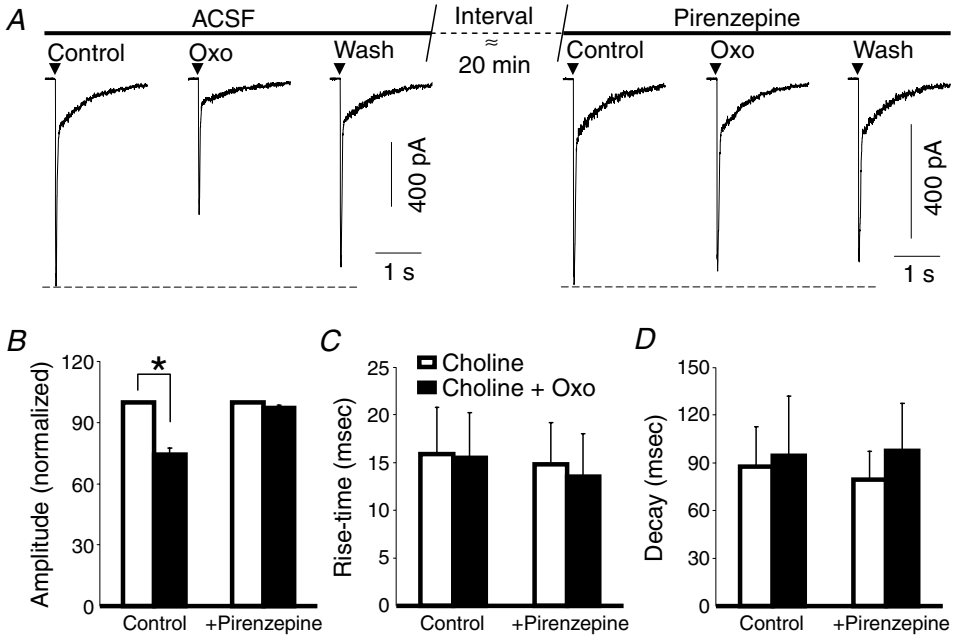

Figure 3. The M_1 subtype of mAChR involved in the inhibition of α7 nAChR-mediated responses
A, representative traces showing that the oxotremorine-M-induced inhibition of α7 responses (left traces) was completely blocked by the bath application of pirenzepine (3 μM; right traces). *B–D*, bar graph of averaged data showing the relative change in amplitude (*B*), rise-time (*C*) and half-time of decay (*D*) values by oxotremorine-M in control and with pirenzepine ($n = 8$) (*$P < 0.05$).

(the inhibition by oxotremorine-M was $16 \pm 6\%$, 7 cells, $P > 0.05$ compared to control; Fig. 4); this indicates that oxotremorine-M is acting through the M_1 mAChR. Lastly, this inhibition of the α7 nAChRs was not voltage dependent since the inhibition was the same at a holding potential of -110 mV *versus* -70 mV in the same cells ($18 \pm 4\%$ in both cases, 4 cells).

To explore whether the activation of the mAChRs may occur under more physiological conditions, we utilized the endogenous neurotransmitter ACh. When we replaced oxotremorine-M with a low dose of ACh (50 μM, which was previously found not to induce desensitization; see Methods) and added the non-α7 nAChR antagonist DHβE (10 μM; to prevent the activation of these receptors by ACh), the amplitude of the choline-induced α7 responses was decreased by $17 \pm 2\%$ (6 cells; Fig. 5), an effect that was completely blocked by atropine. These data indicate that ACh (50 μM) is exerting its action via the activation of mAChRs, and not through inducing desensitization.

α7 nAChRs on presynaptic terminals can enhance the release of a number of neurotransmitters (e.g. glutamatergic and GABAergic terminals; Wonnacott, 1997; Wonnacott *et al.* 2006). Therefore, there is the possibility that the effects of oxotremorine-M on the amplitude of the α7 nAChRs might be through an indirect action on presynaptic M_1 mAChRs. To rule this out, we collectively bath-applied CNQX (10 μM), AP5 (50 μM), N-Phenyl-7-(hydroxyimino)cyclopropa[b]chromen-1a-carbox amide (30 μM), gabazine (10 μM) and CGP 54626 (1 μM), which would block, respectively, AMPA and kainate receptors, NMDA receptors, metabotropic glutamate receptors, and ionotropic and metabotropic GABA receptors. Under these conditions, there was no significant change in the inhibition of the α7 responses by oxotremorine-M (7 cells; the inhibition was $16 \pm 3\%$ prior to and $13 \pm 2\%$ after the application of this cocktail of inhibitors; $P > 0.05$).

G protein and calcium-dependent cascade involved in M_1 mAChR inhibition of α7 nAChRs

The mAChRs couple to intracellular signal transduction cascades via heterotrimeric G proteins. In the hippocampus, the M_1 receptor G protein pathway can utilize the $G_{q/11}$ G protein family, which involves the activation of phospholipase C-β (PLC-β), inositol phospholipid breakdown, protein kinase C (PKC) activation, and intracellular calcium mobilization (Nash *et al.* 2004). To test for the involvement of G proteins, cells were dialysed with an intracellular solution containing GDPβS (300 μM), a non-hydrolysable analogue of GDP and competitive inhibitor of GTP binding to Gα subunits that is known to inhibit G protein-mediated responses. Under these conditions, the ability of oxotremorine-M to inhibit α7 responses was dramatically reduced to $4.0 \pm 1\%$ (Fig. 6*A* and *D*; 5 cells; $P < 0.05$, *versus* 25% for control).

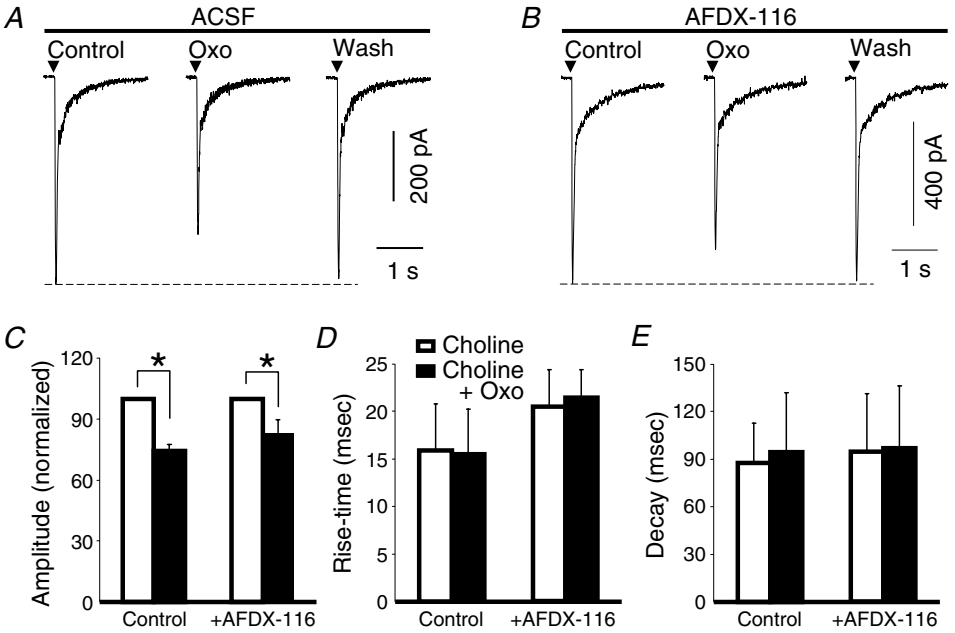

Figure 4. Lack of involvement of the M_2 subtype of mAChR in the inhibition of α7 nAChR-mediated responses

A and *B*, representative traces showing that the oxotremorine-M-induced inhibition of α7 responses (*A*) was not significantly affected by the bath application of AFDX-116 (1 μM; *B*). *C–E*, bar graphs of averaged data showing the relative change in amplitude (*C*), rise-time (*D*) and half-time of decay (*E*) values by oxotremorine-M in control and with AFDX-116 ($n = 7$) (*$P < 0.05$).

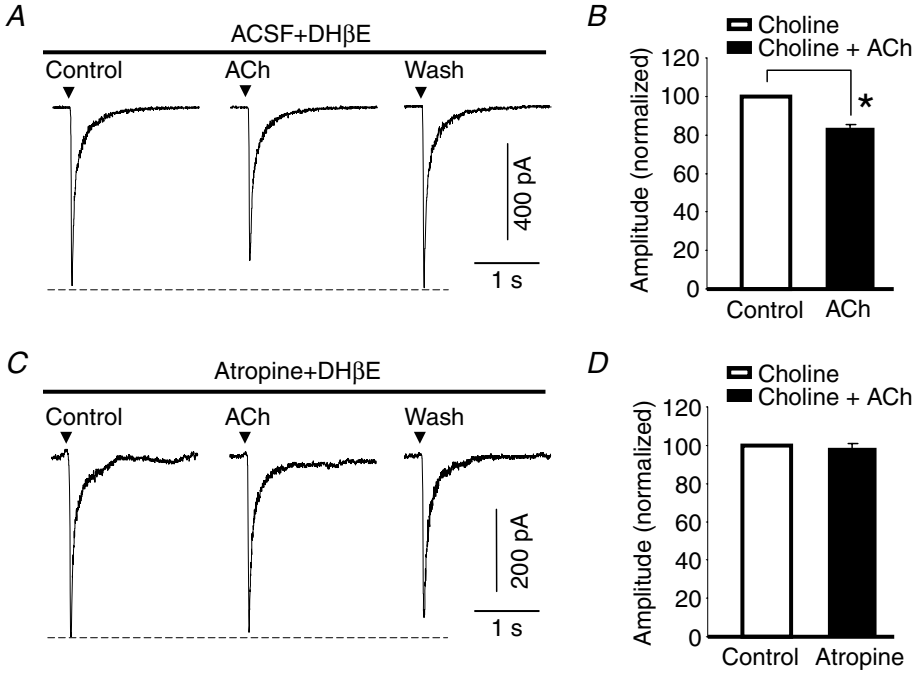

Figure 5. ACh acting through mAChRs inhibits α7 nAChR-mediated responses
A, representative traces showing that ACh (50 μM for 2 s; rather than oxotremorine-M) reduced the amplitude of choline-induced α7 responses (middle trace) in a rapidly reversible manner (right trace). All recordings have DHβE (10 μM) added to the ACSF. *B*, bar graph of averaged data showing the relative change in amplitude by ACh ($n = 6$) (*$P < 0.05$). *C*, representative traces showing that during the bath application of atropine (10 μM), the inhibition of α7 responses by ACh was completely blocked. *D*, bar graph of averaged data showing the relative change in amplitude by ACh with atropine ($n = 4$).

M_1 mAChR-mediated intracellular signalling events are known to be calcium dependent since chelating cytoplasmic calcium levels with BAPTA, both in central (Gulledge & Stuart, 2005) and peripheral (Selyanko & Brown, 1996; Zhu & Yakel, 1997) neurones, blocks M_1 mAChR-mediated responses. When neurones were dialysed with BAPTA (10 mM), the oxotremorine-M-induced inhibition of α7 responses was reduced to $6.9 \pm 2\%$ (Fig. 6*B* and *D*; 8 cells; $P < 0.05$, *versus* 25% for control). These data suggest that the inhibitory action of oxotremorine-M on α7 responses is coupled to a calcium-dependent G protein signal transduction cascade.

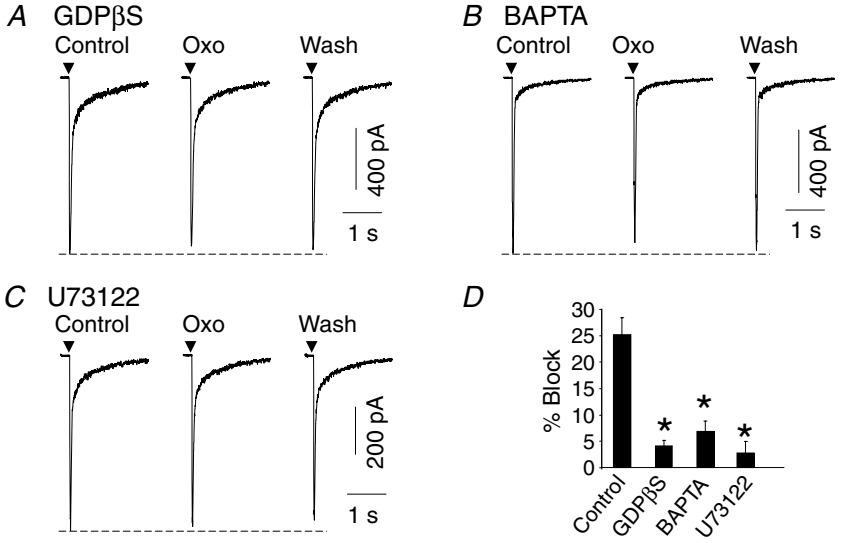

Figure 6. G protein, calcium- and PLC-dependent cascade involved in M_1 mAChR inhibition of α7 nAChRs
A, *B* and *C*, representative traces showing that the oxotremorine-M-induced inhibition of α7 responses was blocked or dramatically reduced by intracellular dialysis with either GDPβS (300 μM) (*A*), BAPTA (10 mM) (*B*) or U73122 (20 μM) (*C*). *D*, bar graph of averaged data showing the per cent reduction in amplitude by oxotremorine-M in control and with GDPβS ($n = 5$), BAPTA ($n = 8$) and U73122 ($n = 7$) (*$P < 0.05$ compared to control).

M_1 mAChR inhibition of α7 nAChRs requires PLC activity

To test whether the inhibition of the α7 nAChR-mediated response by oxotremorine-M works through phospholipase C (PLC), we dialysed neurones with the broad spectrum PLC inhibitor U73122 (20 μM). Under these conditions, there was no inhibitory effect of oxotremorine-M on the inhibition of α7 responses (Fig. 6*C* and *D*; 7 cells; $P < 0.05$, *versus* 25% for control), indicating that oxotremorine-M inhibits α7 nAChR-mediated responses via a pathway involving PLC.

Involvement of PKC in M_1 mAChR-mediated inhibition of α7 nAChRs

Lastly, we investigated the possible role played by PKC activation in the M_1 mAChR-mediated inhibition of α7 receptors. We dialysed neurones with the PKC inhibitor peptide (PKC-I_{19-31}; 200 μM). Under these conditions, the inhibition of α7 responses by oxotremorine-M was significantly reduced to $12 \pm 3\%$ (Fig. 7*A* and *D*; 7 cells; $P < 0.05$, *versus* 25% for control). Gö6976 is an inhibitor of the conventional, calcium-dependent PKC isoforms; when dialysed into cells (200 nM), this also significantly reduced the inhibition of α7 responses by oxotremorine-M to $8.8 \pm 2\%$ (Fig. 7*B* and *D*; 5 cells; $P < 0.05$, *versus* 25% for control), a value not significantly different from PKC-I_{19-31}-dialysed neurones. In contrast, when we dialysed neurones with a protein kinase A (PKA) inhibitory peptide (PKA-I_{5-24}; 200 μM), this had no effect on the inhibition of α7 responses since oxotremorine-M still decreased the peak amplitude of the α7 nAChR-mediated responses by $28 \pm 6\%$ (Fig. 7*C* and *D;* 8 cells), which was not significantly different than control ($P > 0.05$). Finally, of all the compounds that we added to the intracellular pipette solution, none had any significant effect on the amplitude or the kinetic properties of the α7 currents.

Discussion

We have shown that the activation of the M_1 mAChR, either by oxotremorine-M or the endogenous neurotransmitter ACh, on rat hippocampal CA1 interneurones in slices activates a PLC-, calcium- and PKC-dependent signal transduction cascade (Supplemental Fig. 2) that inhibits the amplitude of the α7 nAChR-mediated currents in these neurones, the first such demonstration of functional co-localization and crosstalk between these two cholinergic receptor systems in individual hippocampal neurones. Furthermore, the amplitude of α7 responses was significantly reduced in a rapidly reversible and voltage-independent manner, without any change in the kinetics of responses.

Both mAChRs and nAChRs are expressed on hippocampal interneurones, where they are thought to be responsible for regulating excitability, synaptic communication and cognitive function (Levey *et al.* 1995; Cobb & Davies, 2005; Lawrence *et al.* 2006*a*,*b*), and both are activated by the endogenous neurotransmitter ACh. The primary cholinergic input to the hippocampus arises from the medial septum and diagonal band of Broca (MSDB), and innervates both principal glutamatergic cells and inhibitory GABAergic interneurones, the activation of which can initiate and sustain network oscillations

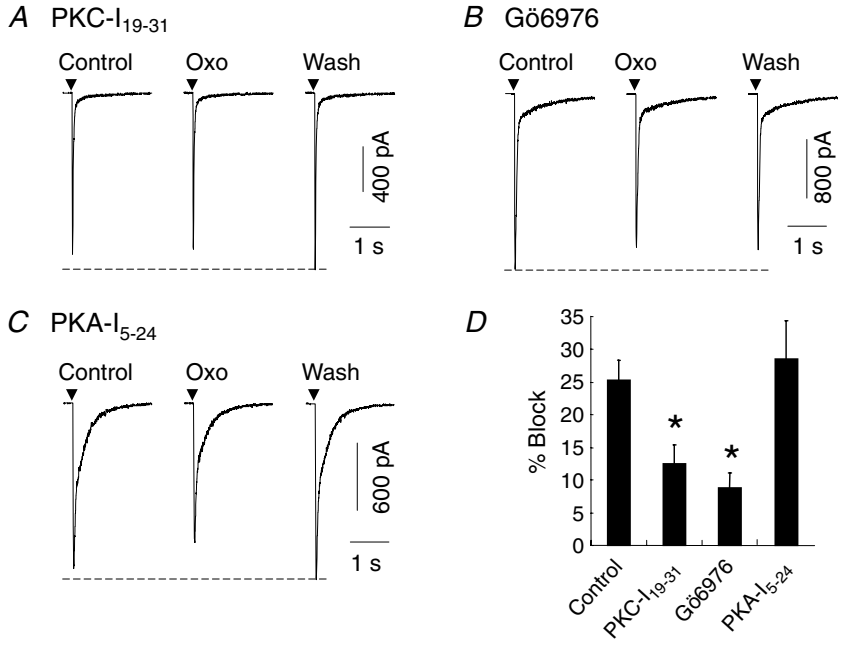

Figure 7. Involvement of PKC in M_1 mAChR-mediated inhibition of α7 nAChRs
A–C, representative traces showing that the oxotremorine-M-induced inhibition of α7 responses was reduced by intracellular dialysis with either PKC inhibitor peptide (PKC-I_{19-31}; 200 μM) (*A*) or Gö6976 (200 nM) (*B*), but not by PKA-I_{5-24} (200 μM) (*C*). *D*, bar graph of averaged data showing the per cent reduction in amplitude by oxotremorine-M in control and with PKC-I_{19-31} ($n = 7$), Gö6976 ($n = 5$) and PKA-I_{5-24} ($n = 8$) (*$P < 0.05$ compared to control).

(Frotscher & Léránth, 1985; Fisahn *et al.* 1998, 2002; Cobb & Davies, 2005; Lawrence *et al.* 2006*a*,*b*).

The stimulation of cholinergic inputs to hippocampal interneurones can regulate their excitability through a variety of different receptor subtypes and ionic conductances; mAChRs can both depolarize and hyperpolarize interneurones through multiple ionic conductances, whereas nAChR activation (principally through the α7 nAChR subtype) can directly excite these neurones (Jones *et al.* 1999; Cobb & Davies, 2005; Lawrence *et al.* 2006*a*,*b*; Widmer *et al.* 2006). Since hippocampal interneurones coordinate the activity of large numbers of principal neurones, they have a powerful and complex role in the regulation of hippocampal output via the organization of synchronous activity (Jones *et al.* 1999; Goldberg & Yuste, 2005). Pyramidal cells also express mAChRs and are innervated by cholinergic inputs from the MSDB, the activation of which will excite pyramidal cells through multiple ionic conductances as well (Cobb & Davies, 2005). Although nAChRs are preferentially expressed on interneurones in the hippocampus (Jones & Yakel, 1997; Frazier *et al.* 1998; McQuiston & Madison, 1999*a*), there have also been reports of functional nAChRs on pyramidal neurones (Hefft *et al.* 1999; Ji *et al.* 2001).

Multiple forms of synaptic plasticity can be regulated by both mAChR and nAChR activation. For example, the activation of presynaptic or postsynaptic mAChRs can either enhance or reduce LTP in the hippocampus (Seeger *et al.* 2004; Cobb & Davies, 2005). The activation of nAChRs can also affect synaptic plasticity, depending on various factors including location of nAChRs and timing of their regulation (Fujii & Sumikawa, 2001; Ji *et al.* 2001; McGehee, 2002). For example, the activation of α7 nAChR on interneurones can block concurrent short- and long-term potentiation in the pyramidal cells, whereas nAChRs on presynaptic terminals can enhance the release of glutamate and therefore increase the probability of producing LTP (Ji *et al.* 2001).

The coordinated signalling between the nAChR and mAChR systems would be expected to be frequency or activity dependent, which might help to explain the role these cholinergic receptors are having in regulating plasticity. For example, short-term cholinergic fibre stimulation would be expected to favour direct excitation of interneurones (e.g. through activation of α7 nAChRs); however, longer-term stimulation may recruit mAChR-mediated synaptic responses in interneurones and pyramidal neurones (Cobb & Davies, 2005). This delay in the activation of mAChRs might serve as a negative feedback system for the regulation of α7 receptors. In addition, the excessive stimulation of α7 receptors can lead to calcium overload (due to their high calcium permeability) and excitotoxicity (Fucile *et al.* 2006).

The influx of calcium through α7 nAChRs has implications for regulating various signal transduction cascades and synaptic plasticity (Dajas-Bailador & Wonnacott, 2004; Fucile, 2004). In chick ciliary ganglion (CG) neurones or cultured rat hippocampal neurones, this increase in $[Ca^{2+}]_{in}$ levels activated calcium-dependent Ca^{2+}–calmodulin-dependent protein kinase (CaMK) and mitogen-activated protein kinase (MAPK) pathways, leading to activation of the transcription factor CREB and gene expression (Hu *et al.* 2002). The transition from GABA-induced excitation to inhibition during development in chick CG neurones is in part due to the activation of the α7-containing nAChRs, demonstrating that nAChR activation is important for development (Liu *et al.* 2006). Furthermore, in both rodent hippocampal interneurones and chick CG neurones, Ca^{2+} influx through the α7-containing nAChRs down-regulates $GABA_A$ receptor-induced currents via calcium-dependent cascades (Wanaverbecq *et al.* 2007; Zhang & Berg, 2007). The precise spatiotemporal dynamics of the $[Ca^{2+}]_{in}$ signals are critical in determining what effect this will have on ion channel regulation, various forms of synaptic plasticity, gene expression, as well as excitability. Perhaps this increase in $[Ca^{2+}]_{in}$ levels through the α7 receptors might also regulate G protein pathways, through which the M_1 mAChRs could be modulated. Under our control recording conditions (i.e. with EGTA), the influx of calcium through the α7 nAChRs did not appear to interfere with the mAChR-mediated signalling (which is calcium dependent) to these α7 receptors, which may have been due to the buffering of calcium by EGTA.

In myenteric neurones of the enteric nervous system of guinea pigs, the activation of M_1 mAChRs alters the desensitization kinetics of the nAChRs by increasing the rate and extent of desensitization (Brown & Galligan, 2003). This crosstalk between the nAChRs and M_1 mAChRs in these myenteric neurones is different to what we observed in rat hippocampal interneurones since we found no changes in the kinetics of the α7 nAChRs in interneurones; this is probably due to the fact that the nAChRs in myenteric neurones are different and likely to contain α3, α5 and β2 subunits (Zhou *et al.* 2002). In rat intrinsic cardiac ganglion (ICG) neurones, nAChRs are probably composed of α3, α5, α7, β2 and β4 subunits (Poth *et al.* 1997; Bibevski *et al.* 2000). In both whole-cell and excised patches, nAChRs in these ICG neurones were potentiated by G_o protein subunits through a direct interaction between $G_o\alpha$ and $G\beta\gamma$ and the α3, α5 and β2 nAChR subunits, but neither α7 nor β4 subunits (Fischer *et al.* 2005). Therefore these data indicate that non-α7 nAChRs in the periphery are modulated by $G_q/_{11}$ and G_o signal transduction cascades in a manner different to what we have observed for α7-containing nAChRs in rat hippocampal interneurones. Lastly, in neurosecretory cells from the cockroach, the activation of M_1 mAChRs, via two different PKC isozymes, both up- and down-regulated nAChR function via a pathway very similar to that

presented here in rat hippocampal interneurones (Courjaret *et al.* 2003).

In conclusion, we have shown that in rat hippocampal interneurones in slices, the activation of the M_1 mAChR subtype significantly reduced the amplitude of α7 nAChR-mediated responses. Although the signal transduction cascade was a PLC- and calcium-dependent pathway and regulated by PKC, it still remains to be determined precisely how the α7 receptor channels are inhibited. Furthermore, it remains to be determined if α7 receptor activation likewise regulates G protein cascades.

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Supplemental material

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