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Muscarinic suppression in stratum radiatum of CA1 shows dependence on presynaptic M1 receptors and is not dependent on effects at $GABA_B$ receptors

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Abstract

Cholinergic modulation of synaptic transmission is vital to memory processes and may be responsible for setting network dynamics in the hippocampus appropriate for encoding of information. Sheridan and Sutor (1990) found evidence suggesting M1 receptors cause presynaptic inhibition of glutamatergic transmission, while Dutar and Nicoll (1988a) research supports a role of the M2 receptor. We examined muscarinic inhibition of fEPSPs in stratum radiatum of mice lacking m1 subtype receptors (KO) compared to wild type (WT) controls. WT mice exhibit greater suppression of transmission by muscarine as compared to KO in a dose dependent fashion. Oxotremorine shows no significant difference in suppression between WT and KO, while MCN-A-343, an M1 agonist, exhibits a significant difference between KO and WT, with KO showing no suppression. One hundred micromolar SGS-742, a selective $GABA_B$ antagonist, fails to affect either normal transmission or muscarinic suppression in either WT or KO suggesting that differences in suppression between the groups is not attributable to differences in $GABA_B$ receptor activation due to muscarinic activation of GABAergic interneurons. These findings support a role for presynaptic m1 mAChRs in modulation of synaptic transmission in CA1, but indicate that other muscarinic receptor subtypes, such as M2, are also involved in suppression of synaptic potentials.

Keywords: Acetylcholine; Hippocampus; Muscarinic; Encoding; Inhibition; Presynaptic

1. Introduction

Acetylcholine plays an important role in the encoding of new memories, as determined from behavioral data showing impairments of encoding caused by muscarinic receptor blockade (Hagan & Morris, 1988; Rasmusson, 2000). These behavioral effects could be due to the effects of acetylcholine (ACh) on a number of cellular neurophysiological properties. For example, activation of muscarinic receptors has been shown to enhance LTP in the CA1 region of the hippocampus (Auerbach & Segal, 1994; Blitzer, Gil, & Landau, 1990) in dentate gyrus (Natsume & Kometani, 1997), and in piriform cortex (Patil, Linster, Lubenov, & Hasselmo, 1998). In addition to this enhancement of LTP, other effects may also contribute to setting dynamics in the hippocampus appropriate for encoding information (Hasselmo & Schnell, 1994). In particular, a large body of research has demonstrated the involvement of mAChRs in the regulation of glutamatergic transmission, including suppression of transmission at the Schaffer collateral (SC) synapses in the hippocampus by a presynaptic mechanism

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(reviewed in Hasselmo, 1999b; Hounsgaard, 1978; Kimura and Baughman, 1997; Levey, 1996; Rasmusson, 2000; Valentino and Dingledine, 1981). Prior research from this laboratory has presented physiological data on ACh suppression (Hasselmo & Fehlau, 2001; Hasselmo & Schnell, 1994; Hasselmo, Schnell, & Barkai, 1995) as well as a neural model of how this suppression may be acting to regulate the dynamics of the hippocampus to enhance pattern separation and facilitate encoding (Hasselmo, 1999a, 1999b; Hasselmo, Bodelon, & Wyble, 2002; Hasselmo & McClelland, 1999; Hasselmo & Schnell, 1994; Hasselmo, Wyble, & Wallenstein, 1996; Wyble, Linster, & Hasselmo, 2000). However, this prior work has only used general agonists and has not looked at the potential contributions to suppression of glutamatergic transmission by mAChR subtypes.

One potential way that ACh may simultaneously cause many different physiological effects is by acting through different subtypes of receptors, and by differential expression ratios of these types in particular locations (Perry et al., 1993; Rouse, Edmunds, Yi, Gilmor, & Levey, 2000; Rouse, Gilmor, & Levey, 1998; Rouse, Marino, Potter, Conn, & Levey, 1999). The contributions and involvement of the subtypes of mAChRs has remained unclear, however, primarily from lack of highly specific agonists and antagonists (Caulfield & Birdsall, 1998; Levey, 1996; Wess, 2003; Wess et al., 2003).

Five separate subtypes of mAChRs have been identified by genetic analyses and pharmacological characterization (Caulfield & Birdsall, 1998; Wess, 2003; Wess et al., 2003). These have been commonly subdivided into the M1 family (m1, m3, and m5 mAChRs) and the M2 family (m2 and m4 mAChRs) based upon their second messenger linkage, with the M1 family acting through $G_{q/11}$ proteins and the M2 family through the $G_{i/o}$ proteins (Kostenis, Zeng, & Wess, 1999). The M1 and the M2 mAChR also appear to both modulate N- and L-type Ca^{2+} channels, but their physiological time courses of activity may be up to an order of magnitude different (Shapiro et al., 2001). This suggests not only a potentially different functional role, but also a differing contribution to control of the rhythmic activity evident in the hippocampus. This divergence supports the hypothesis that different families cause quite different actions in neural circuitry and that certain subtypes may play a more pivotal role in various aspects of behavior.

Utilizing carbachol (CCh), a non-specific cholinergic agonist, with antagonist challenge by the M1 selective antagonist pirenzepine, Sheridan and Sutor (1990) found results suggesting M1 receptors cause presynaptic inhibition of glutamatergic transmission in the hippocampus. In contrast, Dutar and Nicoll (1988a) using similar methods concluded that the M2 subtype was responsible for the presynaptic inhibition of the Schaffer collateral projection. More recently, Psarropoulou, Beaucher, and Harnois (1998) also found that depression of synaptic transmission in the hippocampus was primarily due to activation of M2 receptors causing presynaptic inhibition of glutamatergic transmission. Further, the m2 subtype has been suggested to be the predominant subtype in the hippocampus (Mrzljak, Levey, Belcher, & Goldman-Rakic, 1998). While the pharmacologically based research seemingly leads to mixed findings, this may be primarily from the lack of highly specific antagonists and the frequent use of the non-specific agonist CCh.

The genotyping of the specific subtypes of mAChRs has allowed more detailed information to be attained both from visual localization and from more specific agonists and antagonists and selective toxins. Research utilizing m1 selective toxins combined with electron microscopy and genetic determination (Marino, Rouse, Levey, Potter, & Conn, 1998; Rouse et al., 1999) or combined with patch–clamp techniques (Marino et al., 1998) has found the m1 receptor to be located postsynaptically on pyramidal cells and to be responsible for potentiating NMDA currents in the hippocampus, thereby increasing the excitability of pyramidal cells. Similarly, research has reported that M1 receptors modulate pyramidal cells directly through inhibiting M-currents (Hamilton et al., 1997), while others have reported the M1 plays no role in the M-current, but depolarizes pyramidals through both the I_h and the I_{cat} currents (Fisahn et al., 2002). These studies do not rule out the presynaptic localization of m1 mAChRs and their potential involvement in regulation of glutamatergic transmission.

One potential alternative route of action of muscarinic modulation may be through interneuron activity, as the activation of muscarinic receptors has been shown to depolarize some GABAergic interneurons in the hippocampus (McQuiston & Madison, 1999). This activity could result in ACh indirectly causing presynaptic inhibition by increasing GABA release and causing presynaptic inhibition via activation of $GABA_B$ receptors (Ault & Nadler, 1982; Dutar & Nicoll, 1988b; Poncer, McKinney, Gahwiler, & Thompson, 2000). ACh has been shown to cause indirect suppression of transmission at the mossy fiber synapses in CA3 through $GABA_B$ receptor activity (Vogt & Regehr, 2001).

To clarify the contribution of the m1 mAChR to suppression of glutamatergic suppression, we conducted a series of experiments utilizing mice with the m1 receptor genetically knocked out. While a full pharmacological profile of this phenotype would be a worthwhile project, our goal is to simply elucidate if the m1 mAChR has a substantive role in suppression of transmission in hippocampal circuits. The first experiment was designed to clarify the role of the m1 receptor and its potential contribution to presynaptic inhibition in stratum radiatum of CA1 compared to previous work from this laboratory using the ACh agonist muscarine. Further, the M2-selective agonist oxotremorine (Puolivali, Jakala, Koivisto, & Riekkinen, 1998; Ringdahl & Jenden, 1983a, 1983b) and purported M1-selective agonist MCN-A-343 (Caulfield & Birdsall, 1998; Davies, Scholes, Virdi, & Broadley, 2001; Wess, 2003) were also administered to allow comparisons and incorporation of behavioral effects in the neural models. However, the selectivity of

MCN-A-343 is highly debated, as reviewed by Caulfield and Birdsall (1998) and by Wess (2003) and the more recent Davies and associates research (Davies et al., 2001). Experiment two focused on use of antagonists to superficially test the contribution of the M2 receptor to ACh suppression by using the M2 antagonist gallamine as well as the potential indirect inhibition of transmission by ACh through $GABA_B$ receptor activity by utilizing a $GABA_B$ antagonist, SGS-742 (Green et al., 2000; Lacey & Curtis, 1994; Pozza, Manuel, Steinmann, Froestl, & Davies, 1999). Data included in this analysis have been previously presented in abstract form (Kremin, Gerber, Huang, Tonegawa, & Hasselmo, 2001; Kremin, Gerber, Huang, Tonegawa, & Hasselmo, 2002).

2. Materials and methods

All experiments utilized an m1 mAChR genetic knock out (KO) mouse (Gerber et al., 2001) with wild types (WT) used for control purposes. Animals varied in age from 10 to 19 weeks. Experiments were performed by two experimenters, with the experimenters blind as to KO or WT. Only after the data were collected were the subtypes identified and then the data were analyzed. Animals were deeply anesthetized with halothane, quickly decapitated, and the brains were removed in 4 °C ACSF (concentrations in millimolar: NaCl [124.0], KCl [2.5], $MgSO_4$ [1.3], dextrose [10.0], $NaHCO_3$ [26.0], KH_2PO_4 [1.2], and $CaCl_2$ [2.4]) oxygenated by bubbling 95% O_2/5% CO_2 through the solution. The brain was mounted on its dorsal surface in a manner which provided a 10–15° offset from horizontal to optimize preservation of the Schaffer collateral fibers and sliced in oxygenated 4 °C ACSF using a Campden vibroslicer. Slices were incremented in 400 μm steps, retaining the mid-septotemporal slices and hippocampal regions dissected from other tissue.

The slices of the hippocampus were stored in room temperature ACSF. After a minimum of 2 h, individual slices were transferred to the recording chamber (Fine Science Tools) and submerged in continuously flowing ACSF at 27–29 °C. Unipolar stimulating electrodes (WPI Inc.), placed in the stratum radiatum of CA1, were used to activate Schaffer collateral (SC) fibers to cause evoked potentials in stratum radiatum. Recording electrodes were pulled from 1 mm borosilicate capillary tubes (WPI Inc.) using a Sutter Instruments model P-87 pipette puller and filled with 2 M NaCl (3–6 MΩ resistance) and placed in CA1 stratum radiatum at a distance of 200–400 μm back toward CA3 from the recording site.

Paired-pulse stimulation was delivered with a 100 ms interstimulus interval, with pulse pairs applied every 10 s (Neuro Data Instruments PG4000 digital stimulator and SIU90 stimulus isolation unit). Data were acquired and recorded using a A-M Systems Model 1800 AC Amplifier, connected either to a custom AD board connected to a DOS computer running *nicepac* software written by Matt Wilson for initial dose–response data of Experiment 1 or to a Micro1401 ADC providing input to Spike2 software (Both Cambridge Electronic Designs) running under Windows 2000 for all other data.

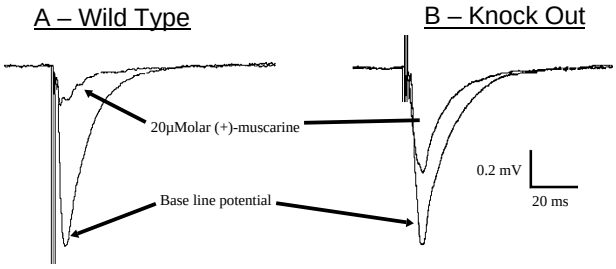
Once potentials were established, they were allowed to stabilize for 1 h before experimental runs. Procedures for each experiment vary after this point and are grouped into agonist studies (Experiment 1) and antagonist-challenge studies (Experiment 2). All perfusates were made using the ACSF to be utilized that day to insure uniformity, and all were oxygenated as above. All suppression measures are reported as mean percent suppression ± SEM. Data from slices failing to attain 80% of the baseline during wash were discarded. Rarely, individual potentials were contaminated with spontaneous EPSPs or population spikes and that EPSP was ignored and the next possible induced EPSP used. Observed power for all statistics was calculated using a value of .05 for α, and is reported to show the strength of the results with the small sample sizes.

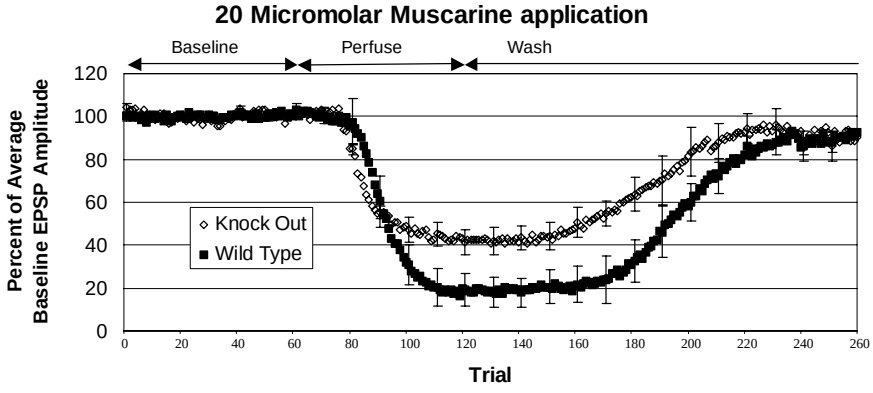
2.1. Experiment 1

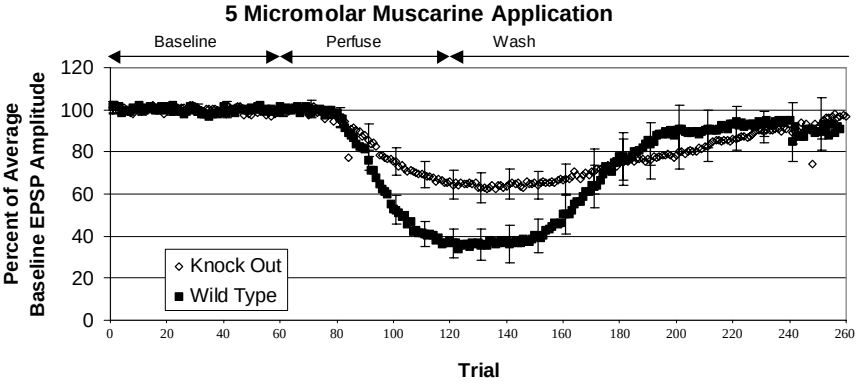
After the 1 h stabilization period, recording commenced with a 10 min baseline, followed by a 10 min perfusion of either 5, 10, 20, 50, and 100 μM (+)-muscarine Cl; 20 μM Oxotremorine sesquifumarate (an M2 preferential agonist); or 20 μM MCN-A-343 (an M1 preferential agonist) (all Sigma), followed by a 20 min washout period. Multiple trials were conducted on each slice, with order of application of the concentrations randomized, but no more than 1 trial of each concentration was conducted on a single slice. The average amplitude of 10 fEPSPs before perfusion was used to establish a baseline amplitude. The average of the first 10 trials after potentials plateaued under perfusion and wash were used to generate a percentage suppression value. Paired-pulse facilitation (PPF) values for each dose were calculated as a percentage of the baseline PPF to provide a within subjects control of variance. Percentage suppressions were analyzed using a MANOVA statistic (SPSS 11.0) for concentration and group, with planned comparisons of each dose and each group.

2.2. Experiment 2

These experiments used essentially the same techniques. Recording commenced with a 10 min baseline, followed by 15 min perfusion of 100 μM SGS-742 (gift of Saegis Pharmaceuticals), a selective $GABA_B$ antagonist, followed by 15 min perfusion of 100 μM SGS-742 with 20 μM (+)-muscarine Cl (Sigma and ICN), followed by 15 min of just 20 μM (+)-muscarine Cl, and then a 30 min wash to insure a return to within 85% of baseline. A 10 min baseline was then recorded, followed by perfusions of 10 min of 20 μM muscarine, followed by 15 min of 20 μM muscarine with 100 μM gallamine, a selective M2 antagonist, followed with a minimum 20 min washout. Analyses were conducted as above.







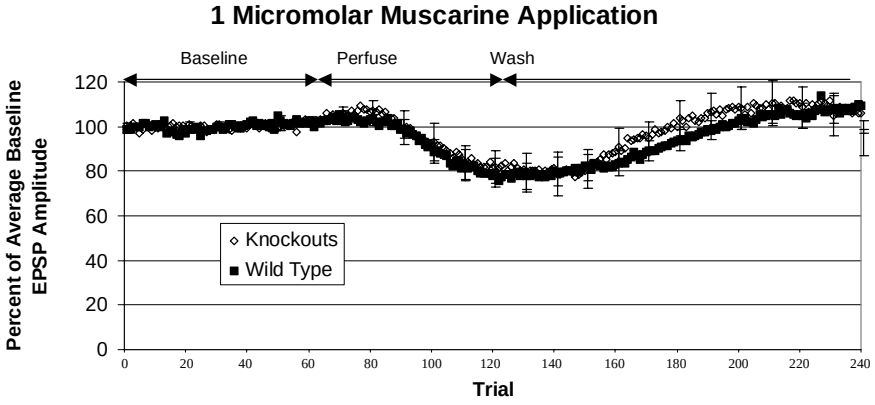


Fig. 1. Top: typical single EPSP traces from (A) WT and (B) KO with superimposed traces recorded before and during perfusion depict the suppression caused by 20 μM muscarine. Note that a much greater muscarinic suppression is obvious in the WT trace as compared to the KO traces (stimulus artifacts removed for clarity). Bottom: comparison of experimental runs of WT and KO mice at 20 (top), 5 (middle), and 1 (bottom) micromolar concentrations of (+)-muscarine chloride. Each trace shows averages across five runs from each group showing baseline, suppression and wash out periods. No differences are seen in the 1 μM runs, while the 5 and 20 μM concentrations show a clear decrease in suppression in the KO mice. The data also illustrate that muscarine alone can cause the enduring post-wash synaptic depression seen with non-specific cholinergic agonists. (±SE shown only every 10 trials for clarity.)

3. Results

3.1. Experiment 1

3.1.1. Effects of muscarine

As expected, there was a significant effect of muscarine concentration on synaptic transmission (4,63; $F = 17.875$, $p < .001$; power = 1.00) with increasing concentrations causing varied levels of suppression in CA1. Fig. 1 illustrates typical fEPSPs from WT (A) and KO (B) mice under baseline and 20 μM muscarine perfusion. Fig. 1 also illustrates the typical change in magnitude of synaptic potentials before, during, and after infusion of muscarine at 1, 5, and 20 μM concentrations in both WT and KO (averages of first five trials for each group and dose). Note also in Fig. 1 the long-term muscarinic depression seen even after prolonged washout after higher concentrations of muscarine perfusion. Fig. 2 illustrates the lesser suppression seen in KO as compared to WT during perfusion of 5, 20, 50, and 100 μM muscarine, as well as the lack of a difference at the 1 μM muscarine concentration.

Statistical analysis demonstrates a highly significant difference in muscarine-induced suppression between WT and KO groups (1,63; $F = 33.804$, $p < .001$; power = 1.000). This difference is clearly illustrated in Fig. 1 where the stronger suppression in the WT mice is clearly evident, as well as in Fig. 2, where muscarine infusion clearly causes a stronger suppression of glutamatergic transmission in the WT than in KO mice at all concentrations above 1 μM. The dose by group interaction was not significant (4,63; $F = 1.996$, $p = .106$; power = 0.567). These results suggest a role for the m1 receptor in inhibition of synaptic transmission.

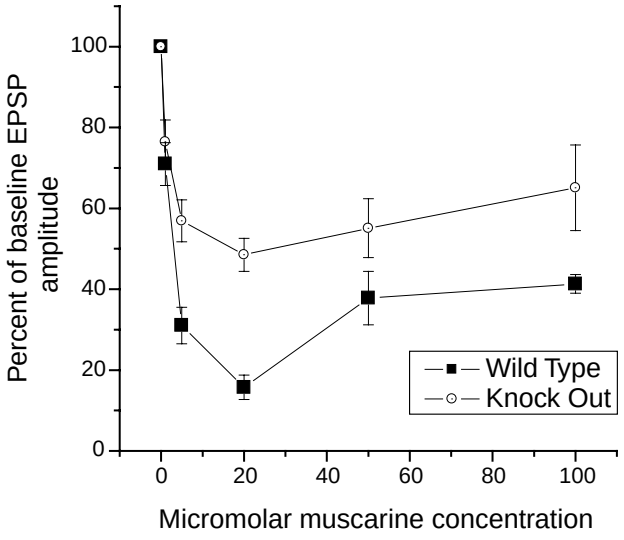


Fig. 2. Magnitude of presynaptic inhibition of EPSPs by muscarine at a range of concentrations expressed as a percentage of baseline EPSP amplitude. Error bars = *SE* while differences were not significant between WT and KO with 1, 50 or 100 μM muscarine, significantly less suppression was evident in the KO at 5 and 20 μM concentrations of muscarine.

Planned post hoc individual ANOVA tests showed significant differences between muscarinic suppression of transmission between WT and KO (Fig. 2) at 5 μM (WT = 68.97 ± 3.99, $n = 9$ and KO = 43.07 ± 5.20, $n = 8$, $F = 16.70$, $p = .001$; power = 0.968) and 20 μM (WT = 84.30 ± 4.08, $n = 8$ and KO = 51.48 ± 4.08, $n = 7$, $F = 43.20$, $p < .001$; power = 1.000) concentrations. The difference in suppression between WT and KO was not statistically significant at 1 μM (WT = 29.04 ± 5.30, $n = 10$ and KO = 22.52 ± 5.38, $n = 9$, $F = 0.532$; $p = .476$; power = 0.106), 50 μM (WT = 62.19 ± 6.59, $n = 5$ and KO = 44.90 ± 7.30, $n = 6$, respectively, $F = 2.97$, $p = .119$; power = 0.338), and 100 μM (WT = 58.74 ± 2.34, $n = 5$ and KO = 34.86 ± 10.55, $n = 6$, $F = 4.05$, $p = .075$; power = 0.436). The results for WT mice are similar to suppression values found in the rat hippocampus (Hasselmo & Schnell, 1994), and suggest a similar participation of presynaptic cholinergic modulation of transmission across these two species.

As can be seen in Fig. 2, we found that the 20 μM concentration deviates from a standard sigmoid dose–response curve. Prior research on cholinergic suppression has generally used log concentrations, and has not reported results using this concentration before. This result is similar to results we have found in stratum radiatum of CA3 in rat hippocampal slices in our lab for these same concentrations (Kremin and Hasselmo, unpublished data). Briefly, this alteration is suggestive of a dual function curve with an inhibition dose–response curve starting and predominating at lower concentrations before reaching a saturation point. A second excitatory dose–response curve appears at higher concentrations, partially countering the initial inhibitory function until an equilibrium of inhibition and excitation is attained at asymptotic levels of each. A reduced inhibition curve in the KO could also explain the slightly decreased inhibition in KO as compared to WT seen at the 100 μM concentration (Fig. 2), illustrating a shifting of one curve, as well as a lower asymptotic level of inhibition.

In the WT, the 20 μM concentration caused significantly greater inhibition than the 1 μM ($p < .001$), 5 μM ($p = .017$), 50 μM ($p = .004$), and 100 μM ($p = .001$) concentrations. The 1 μM concentration caused significantly less suppression than all other concentrations (all $p < .001$). The 5 μM concentration, however was not significantly different than the 50 μM ($p = .341$) or the 100 μM ($p = .154$). In the KO group, pair-wise comparisons failed to find the suppression from 20 μM significantly different from 5 μM ($p = .354$), 50 μM ($p = .499$) or 100 μM ($p = .094$) concentrations, with a significant differences found only between the 1 and 5 μM ($p = .026$), 20 μM ($p = .003$), and 50 μM ($p = .025$) concentrations (see Fig. 2).

3.1.2. Paired-pulse facilitation

Paired-pulse facilitation (PPF) did not vary significantly by group during baseline recordings (WT = 115.78 ± 2.39, $n = 37$ and KO = 122.72 ± 2.78, $n = 36$, $F = 3.59$, $p = .062$; power = 0.464). As can be seen in Fig. 3, however, during muscarinic perfusion, there was a significant effect of group

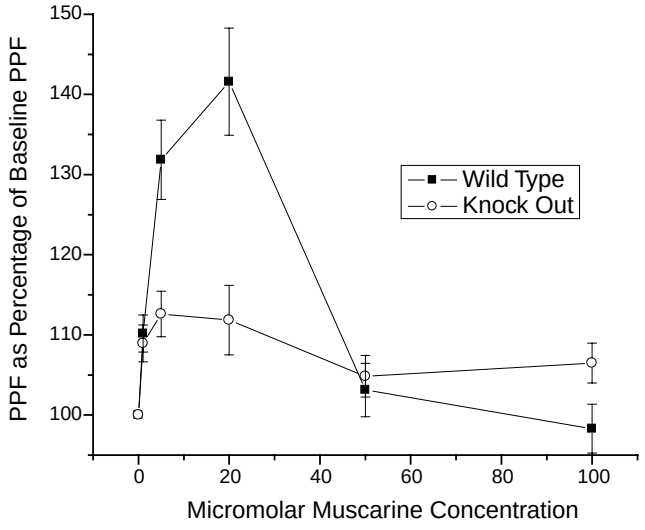


Fig. 3. Magnitude of paired-pulse facilitation (PPF) at a range of different concentrations of muscarine. WT mice showed significantly greater PPF than KO mice at the 5 and 20 μM concentrations, and highly dose-dependent differences between concentrations. KO mice still show a small but significant increase in PPF that is not dose dependent to a significant degree. As increased PPF has been suggested to be from presynaptic regulatory mechanisms, these results suggest a presynaptic location for the m1 receptor, and that the presynaptic inhibition has a limited effective dose range. Error bars = *SE*.

(WT vs. KO; 4,63, $F = 14.34$, $p < .001$), muscarinic concentration (1,63, $F = 9.90$, $p = .003$), and dose by group interaction (4,63, $F = 7.62$, $p < .001$). The differences in PPF between WT and KO at concentrations of 1 μM (WT = 110.17 ± 2.33, $n = 10$ and KO = 108.95 ± 2.29, $n = 9$, $F = 0.137$; $p = .716$; power = 0.064), 50 μM (WT = 103.13 ± 3.34, $n = 5$ and KO = 104.84 ± 2.61, $n = 6$, $F = .169$, $p = .691$; power = 0.066), and 100 μM (WT = 98.30 ± 2.24, $n = 5$ and KO = 106.49 ± 2.48, $n = 6$, $F = 4.44$, $p = .064$; power = 0.469) were not statistically significant. However, PPF during muscarinic perfusion was significantly greater in WT than KO at both 5 μM (WT = 131.85 ± 4.94, $n = 9$ and KO = 112.61 ± 2.83, $n = 8$, $F = 10.84$, $p = .005$; power = 0.868) and 20 μM (WT = 141.59 ± 6.70, $n = 8$ and KO = 111.84 ± 4.35, $n = 7$, $F = 12.99$, $p = .003$; power = 0.914) concentrations.

As depicted in Fig. 3, the WT displays a clear peak in the level of induced PPF at the 20 μM concentration. A significant effect of concentration was found in the WT group (4,32, $F = 15.08$, $p < .001$, power = 1.000). Post hoc Scheffe pair-wise comparisons showed the 20 μM induced PPF to be significantly greater than that of the 1, 50, and 100 μM concentrations, (all $p < .001$), but not significantly greater than 5 μM ($p = .650$). Likewise, 5 μM concentration caused significantly greater PPF than 1 μM ($p = .019$), 50 μM ($p = .008$), and 100 μM ($p = .002$). No significant difference in induced PPF was found between the 1 and the 50 μM ($p = .904$) or the 100 μM ($p = .580$) concentrations, and the difference in PPF between the 50 and 100 μM concentrations was also not significant ($p = .985$). As demonstrated in Fig. 3, an overall significant increase in PPF was found (1,31, $F = 40.05$, $p < .001$), while no significant differences between concentrations were found within the KO group (4,36, $F = 1.181$, $p = .339$; power = .325).

As drug induced increases in PPF are generally considered to be a presynaptic phenomenon, and as the KO do not show any changes in PPF across the concentrations while the WT show a very distinct and significant peak of cholinergically induced PPF, these results support the hypothesis that the m1 mAChR acts through a presynaptic locus and this dose-dependent, pre-synaptic inhibition is absent in the KO. The KO do show a small but significant increase in PPF during perfusion, but that does not vary by concentration, suggesting that the m2–m5 receptors play a role in the presynaptic effects as well.

3.1.3. Receptor subtype-specific agonists

As can be seen in Fig. 4, 20 μM MCN-A-343 exhibited a significant difference in suppression between WT (37.0 ± 8.7, $n = 3$) and KO (−3.7 ± 11.0, $n = 3$; $F = 25.131$, $p = .007$; power = .956), as would be expected from the absence of any M1 mAChRs in the KO mice, if MCN-A-343 is m1 selective. In contrast, oxotremorine sesquifumarate suppressed amplitudes to a similar degree in WT (51.8 ± 12.0, $n = 4$) and KO (39.17 ± 10.7, $n = 5$; 1,7, $F < 1$; power = .105; Fig. 4), and also failed to cause significant changes in PPF (WT, 115.1 ± 9.7; KO, 104.5 ± 1.2; 1,7, $F = 1.515$, $p = .258$) in both groups. Although, as stated earlier, the selectivity of subtype-specific agonists is debatable, the current data are presented for general comparison of these agonists' effects in a $m1^{-/-}$ phenotype to their actions as reported in non-genetically manipulated animals, and to allow generalizations from the pharmacological literature to the current study. Our data showing a significant lower magnitude of suppression and PPF in the KO mice compared to the WT mice clearly demonstrate a role of m1 receptors in presynaptic suppression of transmission.

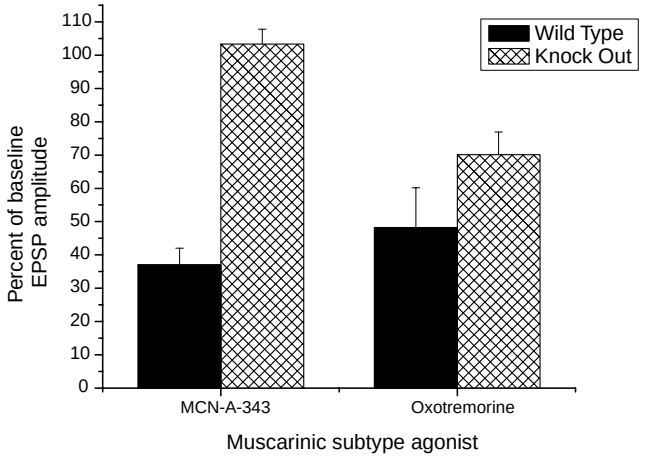


Fig. 4. Twenty micromolar MCN-A-343, a putative M1 selective agonist showed significant suppression in WT ($n = 3$) while failing to cause any suppression in KO ($n = 3$). Twenty micromolar Oxotremorine, a putative M2 agonist, did not show a significant difference in suppression between WT ($n = 4$) and KO ($n = 5$). Error bars = *SE*.

Our data on the sparing of some muscarinic suppression in the KO mice also, however, suggest involvement of other muscarinic subtypes. If oxotremorine primarily activates M2 receptors, then these findings suggest that M2 mAChRs mediate a similar and substantial component of the suppression of transmission in both WT and KO mice. As no significant effects of oxotremorine were found on PPF within either WT (1,6, $F=1.92$, $p=.22$) or KO (1,8, $F=1.90$, $p=.21$) mice, however, the data would suggest that this suppression is minimally through a presynaptic action, or that oxotremorine does not have a strong affinity for presynaptic m2 receptors.

3.2. Experiment 2

3.2.1. $GABA_B$ antagonist

As can be seen in Fig. 5, SGS-742 produced no significant changes in EPSP amplitude (1,12; $F=1.34$, $p=.27$) compared with baseline potentials, and no significant differences in potential amplitude between WT ($n=10$) and KO ($n=6$) groups (1,12; $F=0.90$) were found, suggesting that presynaptic $GABA_B$ receptors are not tonically active in these slice preparations. Further, no significant differences were found for the SGS-742 challenge of muscarinic inhibition of synaptic transmission (1,11; $F=0.03$) and pair-wise comparisons show no significant difference between PPF under muscarine or under SGS-742 challenge of muscarine ($p=.395$). This suggests that $GABA_B$ receptors are not involved in muscarinic suppression of the SC projection. That is, the muscarinic activation of interneurons is not contributing to suppression via activation of $GABA_B$ receptors. Although direct activation of $GABA_B$ receptors has been shown previously to suppress synaptic transmission at the Schaffer collaterals (Ault & Nadler, 1982; Colbert & Levy, 1992; Yanovsky, Sergeeva, Freund, & Haas, 1997), this does not appear to contribute to the suppression caused by muscarinic receptors. As expected, WT ($n=10$) and KO ($n=5$) showed a significant difference in presynaptic inhibition (1,11; $F=31.23$, $p<.001$) caused by muscarine, further supporting the findings of a significant difference found in Experiment 1.

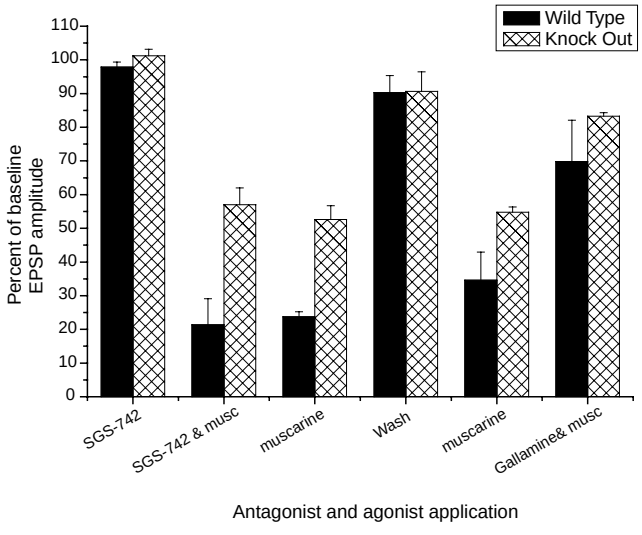


Fig. 5. Lack of effect of 100 μM SGS-742 (a $GABA_B$ antagonist) on the presynaptic inhibition caused by 20 μM muscarine in either WT ($n=10$) or KO ($n=6$) mice. SGS-742 alone also failed to alter synaptic transmission. Both groups exhibited differential suppression of muscarine as described in Experiment 1 and shown in Figs. 2 and 3. Gallamine produced a significant decrease in suppression in both WT and KO mice but the antagonism was not significantly different between WT and KO mice.

There may be important parallel effects of m1 mAChR and $GABA_B$ receptors. GABA blockade will sometimes result in spontaneous potentials, population spikes, and theta-like activity and this effect is blocked by the M1 antagonist pirenzipine, but not by an M2 antagonist (Konopacki, Golebiewski, Eckersdorf, Blaszczyk, & Grabowski, 1997). Intermittent population spikes, spontaneous potentials and a random short duration theta-like rhythmic activity were observed to some extent in all of the WT slices subjected to the combination of SGS-742 and muscarine, and were notably absent in the same slice before this perfusate was applied, and again were absent after this combination perfusate was changed. These effects however did not occur when the slices were perfused with SGS-742 alone, or in any other condition in any phase or part of these experiments. This effect was notably absent in all trials using KO mice. As this was an unexpected finding, sufficient experimental controls were not in place to accurately characterize or analyze these results, and they are therefore reported as an observation.

3.2.2. M2 mAChR antagonist

Gallamine challenge of muscarinic suppression, as shown in Fig. 5, did cause a significant decrease in muscarinic presynaptic inhibition of SC transmission (1,8; $F=8.19$, $p=.02$). However, PPF was not affected to a significant degree by gallamine challenge (1,8; $F=2.31$, $p=.17$), supporting our findings on oxotremorine and PPF. Also as depicted in Fig. 5, gallamine challenge in WT mice eliminated approximately 54% of the suppression seen from 20 μM muscarine, supporting a major and complimentary role in the suppression of transmission by the M2 subtype of receptor.

4. Discussion

4.1. Primary findings

Previous studies have suggested either m1 or m2 receptors are involved in presynaptic suppression of glutamatergic transmission in region CA1 of the hippocampus (Dutar & Nicoll, 1988a; Sheridan & Sutor, 1990). Here we show that both m1 and m2 mAChRs substantially contribute to the presynaptic inhibition of glutamatergic transmission from the Schaffer collaterals in CA1. Together, the oxotremorine data and the gallamine challenge clearly show a considerable role for the m2 receptor in the presynaptic inhibition of synaptic transmission. Even so, the highly reduced PPF seen in KO suggests that the m1 mAChR

contributes substantially to the presynaptic inhibition of glutamatergic transmission from the Schaffer collaterals in CA1. Our results provide evidence that the m1 mAChR is a primary contributor to this presynaptic inhibition, at least at the 5 and 20 μM concentrations. The lesser amount of suppression left may be attributed to combined effects of the m2, m3, m4, and m5 mAChR subtypes. Thus, the m1 receptor as well as other receptor subtypes have been shown to participate in the muscarinic presynaptic inhibition of synaptic transmission in stratum radiatum of CA1, which has been extensively described in pharmacological studies (Hasselmo & Schnell, 1994; Hounsgaard, 1978; Valentino & Dingledine, 1981).

The presynaptic inhibition caused by muscarinic receptors is accompanied by multiple post-synaptic effects of muscarinic receptors, including depolarization and reduction of spike frequency accommodation (Dutar & Nicoll, 1988a). These postsynaptic muscarinic effects are not incompatible with the presynaptic inhibition. Thus, m1 AChRs have been reported to be located postsynaptically and cause increased excitability in CA1 pyramidals (Rouse et al., 1999), but our results clearly demonstrate that their absence substantially decreases the presynaptic inhibition at the Schaffer collaterals caused by muscarine. Our results do not rule out the possibility that m1 receptors may have various effects at different loci, including postsynaptic effects on the dendrite or cell body, but our results clearly demonstrate that their absence decreases the presynaptic inhibition at the Schaffer collaterals caused by muscarine.

4.2. GABAergic involvement at $GABA_B$ receptors

The lack of effect of SGS-742 on the muscarinic suppression of transmission rules out the possibility that muscarinic suppression occurs through activation of $GABA_B$ receptors due to depolarization of GABAergic interneurons. It had previously been shown that disruption of both cholinergic and GABAergic input from the medial septum/diagonal band of Broca neurons to the hippocampus is required to severely impair hippocampal performance (Pang, Nocera, Secor, & Yoder, 2001). It has been shown that GABAergic interneurons are activated by muscarinic receptors (van der Zee, de Jong, Strosberg, & Luiten, 1991; van der Zee & Luiten, 1993), and the m1 receptor has been shown to modulate GABA release in the septal nuclei (Hasuo, Gallagher, & Shinnick-Gallagher, 1988), suggesting that muscarinic enhancement of GABA release could cause enhanced $GABA_B$ receptor mediated presynaptic inhibition of glutamatergic axons (Caillard, McLean, Ben-Ari, & Gaiarsa, 1998; Molyneaux & Hasselmo, 2002), or postsynaptic extra- and intra-synaptic inhibition by hyperpolarizing the pyramidal cells (Pham, Nurse, & Lacaille, 1998). Increased release of GABA and activation of $GABA_B$ receptors has been shown to mediate muscarinic suppression of the mossy fibers (Vogt & Regehr, 2001), but this does not appear to be the case for the muscarinic suppression in stratum radiatum of CA1.

4.3. GABAergic involvement at $GABA_A$ receptors

While the possibility of muscarinically induced GABA release acting through $GABA_B$ receptors to cause inhibition was specifically tested, potential $GABA_A$ receptor involvement is much more difficult to assess directly. Although we cannot directly refute an involvement of muscarinic-induced GABA release acting through $GABA_A$ receptors, we feel it is unlikely that this route contributes significantly to the specific suppression shown in this preparation. Previous work has also indicated that with low levels of stimulation, no phasic IPSPs are detectable in similar preparations (Sheridan & Sutor, 1990), and that high voltage stimulation is required to reliably trigger SR interneurons, and then only proximal (30–40 μm) to the stimulation site (Kozhemiakin, Draguhn, & Skrebitsky, 2004). As our stimulation levels are far below those reported values, and our recording site is an order of magnitude farther removed from the stimulation site than that required to reliably produce SR interneuron GABA release, we feel that such feed forward $GABA_A$ inhibition is very minimal and is therefore not a significant part of the suppression of synaptic potentials that we find. While there is no doubt that GABAergic inhibition plays crucial roles in regulating the dynamics of hippocampal activity, a wealth of past research has also shown that cholinergic suppression of SC transmission in CA1 SR such as that demonstrated here is not dependent on $GABA_A$ receptor activity (Dutar & Nicoll, 1988a; Hasselmo & Schnell, 1994; Hasselmo et al., 1995; Psarropoulou et al., 1998; Psarropoulou & Dallaire, 1998; Sheridan & Sutor, 1990). Even so, further studies beyond the scope of the current project will be required to fully and accurately define the involvement of the $GABA_A$ receptors' involvement.

4.4. Theoretical implications

Our results indicate that the m1 mAChR is directly, and substantially, involved in presynaptic inhibition of transmission at the Schaffer collaterals in CA1 of the hippocampus. Past research suggests that cholinergic modulation is vital to memory processes. It has been reported elsewhere that m1 KO mice have variable memory impairments in hippocampal dependent tasks (Anagnostaras et al., 2003; Miyakawa, Yamada, Duttaroy, & Wess, 2001). While the deficits may be attributable to hyperactivity (Gerber et al., 2001), lesions and manipulations of the hippocampus or fimbria fornix have also been reported to cause hyperactivity in rats (Whishaw & Jarrard, 1995). We suggest that the pattern of variable deficits across paradigms correlated with the varying task demands on memory support a role of the m1 receptor directly in the acquisition of the task, in addition to possible general effects of hyperactivity (Gerber et al., 2001). While the KO mice in other studies were unimpaired in a spatially guided water-maze task, they were impaired in a win-shift 8-arm radial arm maze task. This suggests that while additional suppression of ACh through

the m1 receptor is not vital for all hippocampal-dependent tasks, the higher level of suppression attained from functioning m1 modulation is important in a task that requires more complicated manipulations of information in memory.

Presynaptic inhibition of glutamatergic transmission can play a vital role in preventing interference from old memories during encoding of new information in models of hippocampal circuits (Hasselmo, 1999a, 1995; Hasselmo et al., 2002; Hasselmo & Schnell, 1994). The lessened suppression of transmission in m1 KO mice could result in interference from retrieval during encoding. This could cause a failure to adequately distinguish the individual arm visit episodes from each other not only on the particular trial, but also from previous trials on the same maze. This could be crucial in the case of the radial arm maze and interfere with the animal's ability to distinguish which arms have been visited. However, in the standard water-maze task this would not necessarily be problematic as each trial involves the same information (the same relation of the platform to extra maze cues).

The m1 KO mice have been shown to have intact LTP processes (Anagnostaras et al., 2003), and as they can learn the water-maze task, the absence of the M1 mAChR does not necessarily seem critical to learning or the formation of new memories at a fundamental level. As the task becomes more demanding, the KOs perform at a lower level. The theta rhythm has been shown to be dependent on m1 mAChRs (Golebiewski, Eckersdorf, & Konopacki, 1993). As m1 KO mice are impaired on the radial arm maze, it would seem that the m1 receptor may play a critical role in setting the dynamics of the hippocampus and that a substantial part of the m1 mAChR's role may be in the suppression of synaptic transmission as well as postsynaptic alteration in the pyramidal cells, and that this combination of actions could be involved in the acquisition and retrieval of complex tasks involving flexible representations of stimuli with a high potential for interference. In vivo EEG and place-cell recordings from behaving KO mice might also help elucidate this issue, but those experiments have not yet been performed.

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