"INFLUENCE OF THE ACCESSORY PROTEIN SET ON M3 MUSCARINIC RECEPTOR PHOSPHORYLATION AND G PROTEIN COUPLING"

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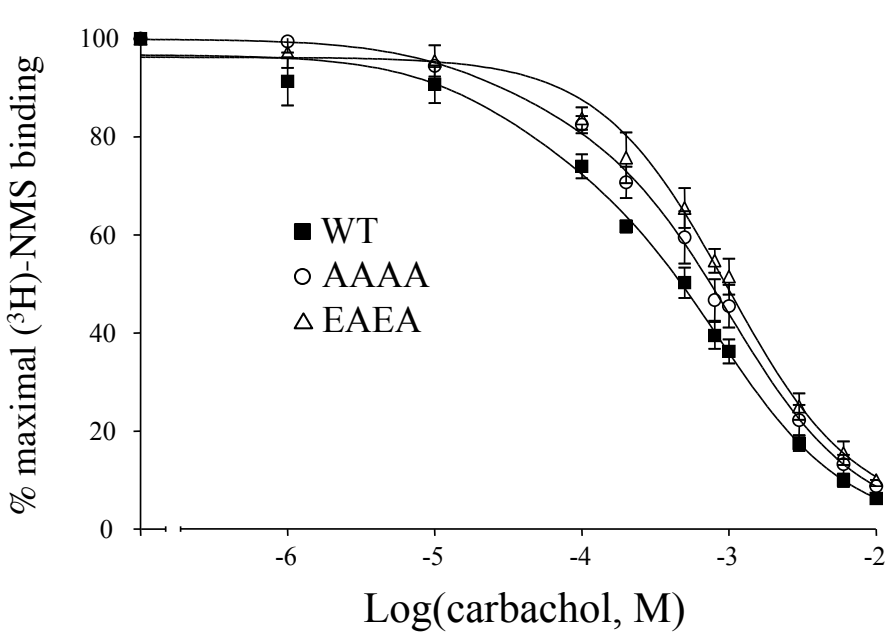
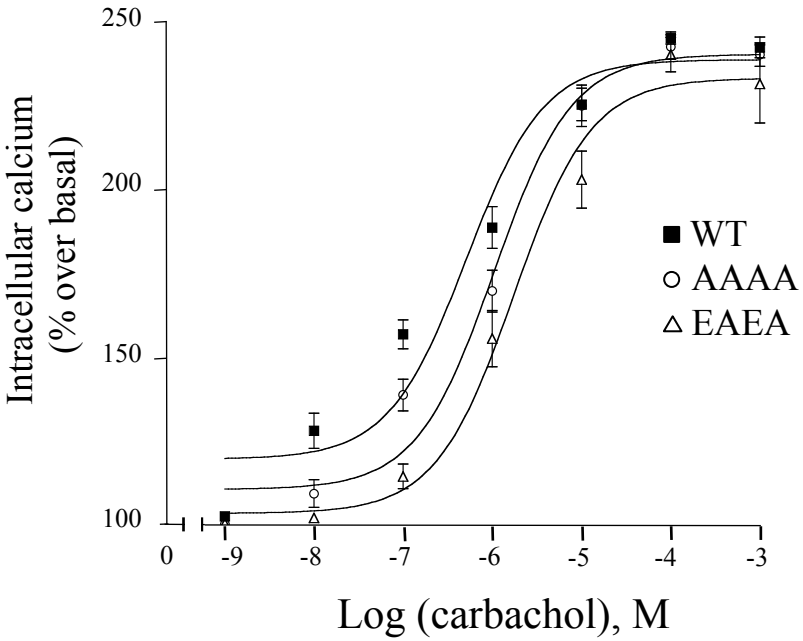
Supplemental Figure Legends

Figure 1 Influence of targeted M3-MR mutations on agonist binding and calcium mobilization. CHO cells were transfected with wild type M3-MR (WT) or M3-MR mutants containing ^{476}KRKR^{479} amino acid substitutions (AAAA or EAEA) and processed for radioligand binding assays (left panel) and measurements of intracellular calcium (right panel) as described in "Materials and Methods". Radioligand binding data are expressed as mean ± S.E. from four experiments with duplicate determinations. The agonist competition curves modeled to two sites in both wild type receptor and the receptor mutant containing ^{476}AAAA^{479} amino acid substitutions [wild type receptor - IC_{50H} = 35 uM LOG IC50 = 0.48 (24 ± 9.9 %), $IC_{50\,L}$ 910 uM LOG IC50 = 0.13; ^{476}AAAA^{479} M3-MR - IC_{50H} = 27 uM LOG IC50 = 0.72 (15 ± 8.1 %), $IC_{50\,L}$ 1000 uM LOG IC50 = 0.12]. Agonist competition curves for the receptor mutant containing ^{476}EAEA^{479} amino acid substitutions was best modeled to binding site of low affinity (900 uM LOG IC50 = 0.52). The calcium measurement data presented are representative of five independent experiments with triplicate determinations (EC_{50}: Wild type M3-MR = 0.3 ± 0.1 uM; ^{476}AAAA^{479} M3-MR = 0.9 ± 0.1 uM , $p<0.05$ compared to wild type; ^{476}EAEA^{479} = 2 ± 0.4uM, $p<0.01$ compared to WT).

Figure 2. Interaction of the M3-MR with endogenous SET in CHO-M3 transfectants in the presence and absence of agonist. CHOM3 cells were incubated with 100 uM carbachol or vehicle for 30 minutes, lysed in Nonidet P-40 buffer and the M3-MR was immunoprecipitated as described previously (Simon et al., 2006). The immunoprecipitates were separated on SDS-PAGE gel and immunostained with anti-M3-MR and anti-SET antibodies. The input lanes represent 1/100 of the lysate volume used for each immunoprecipitation (*IP*). The data are representative of two experiments.

SUPPLEMENTAL FIGURE 1





SUPPLEMENTAL FIGURE 2