Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol12Issue1abst028P.pdf

Agonist Regulation of Muscarinic M_2/M_3 Receptor Heteromer and M_2 Homomer Stability, but not of the Corresponding M_3 Homomer

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Muscarinic receptors (M_1-M_5) belong to class A of the G protein coupled receptor (GPCR) family. There is growing evidence that many GPCRs exist as dimers or higher-order oligomers (**1**) and muscarinic receptors are no exception (**2**). Herein, as for the co-existence of homomers and heteromers of the dopamine D_2 and D_3 receptors (**3**) we demonstrate such combinations of co-expressed human M_2 (hM_2WT) and a RASSL (Receptor Activated Solely by Synthetic Ligand) form of the human M_3 receptor (hM_3RASSL) using N-terminal SNAP and CLIP tags in combination with homogeneous time resolved FRET (HTRF) (**3**). Stable Flp-In™ T-REx™ 293 cell lines able to inducibly express each of these receptor forms upon addition of doxycycline, and a cell line able to express both hM_3RASSL constitutively and hM_2WT in a doxycycline inducible manner were generated.

In these cells both hM_3RASSL and hM_2WT were detected after treatment with different concentrations of doxycycline via Western Blots using tag-specific antibodies. Radioligand binding using [^{3}H]-QNB indicated that similar amounts of hM_2WT and hM_3RASSL were expressed following induction with 5 ng.ml^{-1} doxycycline; B_{max} (no dox) = 2603 ± 200 fmol.mg protein^{-1}; B_{max} (+ dox) = 5465 ± 244 fmol.mg protein^{-1}). Following induction with doxycycline each of hM_2WT and hM_3RASSL homo-oligomers and hM_2WT-hM_3RASSL heteromers were identified. Unlike the corresponding homo-oligomers in cells expressing either receptor alone, occupancy of hM_2WT-hM_3RASSL heteromers with the hM_2WT agonist carbachol resulted in a marked, time and concentration-dependent (pIC_{50}= 5.2 ± 0.25) decrease in detected heteromers and a concomitant, concentration-dependent (pEC_{50} = 5.5 ± 0.2) increase in hM_2WT homomers. The formation of hM_2WT-hM_3RASSL heteromers was significantly decreased (P=0.007) by 1.2 fold in the presence of 1 mM carbachol, and by 1.3 fold when 1 mM carbachol was added in the presence of 100 µM CNO (P=0.037). There was a 2.3 fold increase detected in the hM_2WT homomers, in the presence of 1mM carbachol (P=0.0002) or 1 mM carbachol and 100 µM CNO together (P=0.001).

Despite the presence of hM_2WT-hM_3RASSL heteromers the functional pharmacology of hM_2WT and hM_3RASSL receptor specific agonists (carbachol and clozapine N-oxide respectively) were largely unaltered.

1. Milligan G, Mol Pharmacol 84:158, 2013
2. Alvarez-Curto E et al, J Biol Chem 285:23318, 2010
3. Pou C et al, JBC 287:8864, 2012