ACTIVATION OF $G_{Q/11}$ AND $G_{I1\text{-}3}$ PROTEINS BY M_1 AND M_3 MUSCARINIC ACETYLCHOLINE (MACH) RECEPTORS: EVIDENCE FOR AGONIST-SPECIFIC RECEPTOR CONFORMATIONS

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The intrinsic activity of agonists to activate M_1 and M_3 mACh receptors stably expressed in CHO cells was assessed using two [^{35}S]-GTPγS binding methodologies. The total [^{35}S]-GTPγS binding assay measures the global activation of the cellular G protein population, however, through the use of pertussis toxin (PTx), to prevent receptor-mediated activation of G_i-like Gα subunits, inferences about the subclasses of G proteins activated can be made. Agonist-G protein activation profiles were further investigated using an antibody-capture technique (Akam *et al.*, 2001), which permits the potency or intrinsic activity of $G\alpha_{q/11}$ and $G\alpha_{i1\text{-}3}$ subunit activation in to be assessed.

Concentration-response curves constructed for total [^{35}S]-GTPγS binding stimulated by methacholine (MCh) in CHO-m1 membranes were biphasic comprising high- and low-affinity components (pEC_{50}s high, 6.23 ± 0.28; low, 4.33 ± 0.05). Pilocarpine failed to cause a significant increase in total [^{35}S]-GTPγS binding. Following pre-treatment of CHO cells with PTx (100 ng ml^{-1}, 24 h), monophasic increases in [^{35}S]-GTPγS binding stimulated by agonist were observed; with the EC_{50} correlating with the high affinity binding seen in control CHO-m1 membranes (pEC_{50}, 6.26 ± 0.06). Immunoprecipitation of $G\alpha_{q/11}$ following [^{35}S]-GTPγS binding yielded a similar pEC_{50} estimate (6.21 ± 0.13) for the MCh-stimulated response, supporting the hypothesis that the M_1 mACh receptor is efficiently couples to $G\alpha_{q/11}$. The improved sensitivity of the antibody-capture method revealed that pilocarpine robustly stimulated $G\alpha_{q/11}$-[^{35}S]-GTPγS binding (pEC_{50}, 6.00 ± 0.17). Assessment of $G\alpha_{i1\text{-}3}$-[^{35}S]-GTPγS binding revealed that only the most efficacious agonists could elicit responses above basal, with pilocarpine ineffective. Thus, agonist activation of the M_1 mACh receptor appears to follow a 'strength-of-signal' model, where agonists activate $G\alpha_{q/11}$ proteins most effectively, only high efficacy agonists active the relatively poorly-coupled $G\alpha_{i1\text{-}3}$ proteins.

Total [^{35}S]-GTPγS binding concentration-response curves constructed for agonist-mediated responses in CHO-m3 membranes were shallow, but could not reproducibly be better fitted to a two-site model. Following PTx treatment, maximal agonist-mediated total [^{35}S]-GTPγS binding responses were reduced indicating again that a heterogeneous population of G proteins is activated, but that the potency differences for PTx-sensitive and -insensitive Gα proteins is too small to allow their EC_{50}s to be determined. In agreement, the antibody capture method revealed that a subset of mACh receptor agonists could stimulate robust [^{35}S]-GTPγS binding to both $G\alpha_{q/11}$ and $G_{i1\text{-}3}$ proteins. Although pilocarpine did not stimulate a significant increase in [^{35}S]-GTPγS-$G\alpha_{q/11}$ binding it did increase [^{35}S]-GTPγS-$G\alpha_{i1\text{-}3}$ binding to approx. 50% of the response evoked by MCh. Therefore, with respect to the M_3 mACh receptor, our data provides some evidence for agonist-specific conformations with different Gα subunit activation profiles.

Akam, E.C. *et al*. (2001) *Br. J. Pharmacol.*, **132**, 950-958.