Receptor phosphorylation is key for agonist-induced internalisation of M_1 muscarinic acetylcholine receptors

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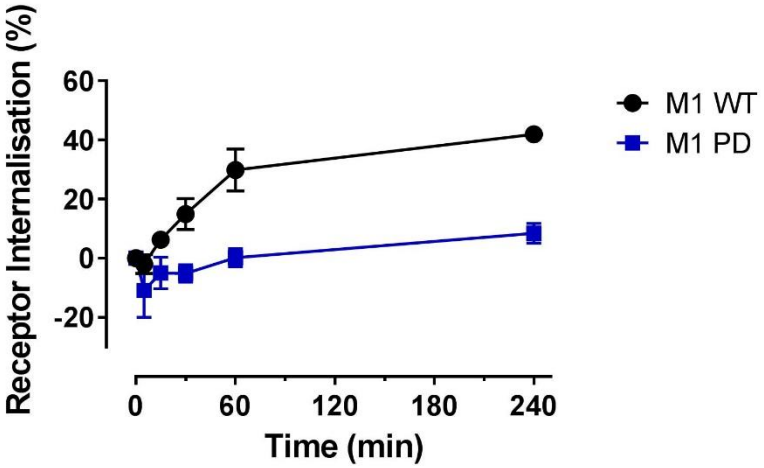
Introduction M_1 muscarinic acetylcholine receptor (M_1 mAChR) agonists and positive allosteric modulators improve cognition and enhance cholinergic signalling in Alzheimer's disease models[1-3]. However, these ligands can show a wide range of adverse effect liabilities associated with on-target overstimulation of the receptor[4]. Recently, our lab have demonstrated that transgenic mice expressing a phoshorylation-deficient mutant of the M_1 mAChR, are more sensitive to adverse responses, such as seizures, in response to administration of M_1-selective agonists. We hypothesise that M_1-agonists stimulating increased phosphorylation and internalisation may be less likely to induce receptor overstimulation and on-target adverse effects. In this study, we aim to understand the role of phosphorylation in agonist-induced internalisation specifically.

Method Cell monolayers of CHO cells expressing wild-type (WT) M_1 muscarinic acetylcholine receptors (mAChRs) or the phosphorylation-deficient mutant (PD) where all serine residues in the third intracellular loop and C-terminal tail are mutated to alanine, were stimulated for 0, 5, 15 and 30, 60 and 240 minutes with the muscarinic agonist carbachol (CCh; 100 µM). CCh-treated cells were transferred on ice, washed with ice-cold Krebs-Henseleit buffer and radiolabelled with saturating concentration of [^{3}H]-NMS (4 nM) overnight at 4°C to quantify cell surface expression. All data are expressed as means ± S.E.M. of three separate experiments (n=3) and were normalised as percentage of untreated cells for each assay.

Results In M_1 mAChR WT cells, four hours of CCh stimulation caused a reduction in cell surface receptors to to 58.4% ±2.2% compared to untreated cells, with most of the receptors (29.9% ±7.1%) internalising within the first hour (Fig. 1). In contrast, agonist treatment of cells expressing PD mutant of M_1 AChR caused no change in levels of surface receptors within the first hour of stimulation and 8.4% ±3.4% receptor internalisation after 4 hours , which may be associated with receptor desensitisation or constitutive internalisation rather than agonist-induced internalisation.



Conclusions Agonist-induced internalisation of M_1 mAChRs is mediated by receptor phosphorylation and it was prevented when receptor phosphorylation sites were removed. Receptor phosphorylation is crucial for agonist-induced internalisation and is likely contributing to an increased adverse effect liability of M_1-selective agonists in the M_1 PD mice.

Reference

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