Characterisation of agonists acting at the Muscarinic M_1 receptor

Efficacy and potency of agonists is sensitive to receptor expression/coupling so can vary in different systems. To aid agonist identification cell lines may be engineered to maximise agonist efficacy. However,this may causemaximum system effect (E_m) to be reached, so maximum efficacy (E_{max}) is compromised. Efficacy (tau) and affinity (K_A) can also be measured by analysing functional responses before and after a reduction of a proportion of receptors by use of an alkylating agent (such as phenoxybenzamine (PBZ)) (1). Here we show how alkylation can help define agonist parameters allowing identification of tool compounds to probe *in vivo*efficacy.

CHO cells overexpressing the human M_1 receptor (LeicesterUniversity, UK) were seeded at 15,000 cells/well in half-area 96-well plates in DMEM-alpha modification with 10 % FBS. 16 h later media was removed and 50 µL media supplemented with 1 % FBS ±1 µM PBZ (Sigma Aldrich UK) added. Cells were incubated (37 °C; 15 min) then washed with 50 µL of fresh 1 % serum mediaevery 10 min for 40 min. To measure G_q responses an IPone kit was used as per manufacturer's instructions (CisBio UK). Incubations were carried out for 30 min (37 °C). Data was fitted to an operational model of receptor depletion (Graph Pad Prism 5.04) (or operational model of partial agonism for AZD6088 using acetylcholine (ACh) as the full agonist) to define K_A and tau.Potency (pEC_{50}) was measured by fitting data to a concentration-response curve (variable slope).

AZD6088 appeared as a partial agonist in the absence of alkylation. Receptor alkylation caused a significant decrease in potency of ACh and GSK1034702 (Table 1). E_{max} of ACh was unaltered by alkylation whilst GSK1034702 became partial (reflected by tau). Data suggests AZD6088 potency is affinity-driven whilst GSK1034702 potency is efficacy-driven.

Table 1 –Potency (pEC_{50}), affinity (K_A) and efficacy (tau). Data is mean ± S.E.M from three independent experiments. pEC_{50} before and after alkylation was compared with an unpaired two-tailed t-test; *p<0.05 and ** p<0.01.

Compound	pEC50	pEC50 + 1 uM PBZ	Log K_A	Tau	Tau + 1 µM PBZ
ACh	7.41 ± 0.11	6.79 ± 0.12*	5.88 ± 0.42	69.67 ± 50.07	12.48 ± 7.06
GSK1034702	7.29 ± 0.06	6.33 ± 0.11**	6.11 ± 0.17	16.39 ± 3.89	1.39 ± 0.38
AZD6088	7.52 ± 0.15	N/A	7.28 ± 0.16	2.10 ± 0.34	N/A

M_1 agonists have beenreported to display profound differences in their efficacy in activatingdifferent signalling pathways *in vivo* (rat) (2) whilst decreases in M_1gene expression has been measuredinthe cortex ofhuman schizophrenicsubjects (3). Using the established approach of receptor alkylation we have identified tools that could be used to probe efficacy translation *in vivo*.Dataalsohighlights theimportance ofmeasuring efficacy– just by reducing receptor reserve GSK1034702 becomes a partial agonist in respect to ACh – an effect that could be misinterpreted as biased signalling.

1. Norel X et al.,(1996) *Br J Pharmacol* **119**: 149-57

2. Digby G et al., (2012) *J Neurosci* 32: **8**: 8532-8544

3. Seo MS et al., (2014) *Schizophr Res* **158** 247-254