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In vitro characterisation of a M1 muscarinic acetylcholine receptor (mAChR) ligand for positron emission tomography (PET) imaging.

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Introduction: The M1 mAChR is currently being pursued as a potential target for treatment of cognitive decline in neurodegenerative and neuropsychiatric disorders. Considering that PET imaging remains the gold-standard for studying drug-receptor in health and disease, use of PET- M1 mAChR radiotracer represents an important step in understanding M1 mAChR function to help develop drugs for disorders associated with M1. Thus, we have conducted an *in vitro* characterisation of LSN3172176, a recently reported M1 agonist (1) to explore its suitability as PET ligand.

Method: *In vitro* binding profile was assessed using [^{3}H]-NMS competition and [^{3}H]-LSN3172176 saturation binding. Functional *in vitro* profile of LSN3172176 at M1 mAChR was assessed using IP1 accumulation and ERK1/2 phosphorylation assays. Immunoprecipitation and immunoblotting with phospho-specific antibodies was used to assess the effect of LSN3172176 on M1 phosphorylation.

Results: There is equivalent affinity for M1 by the labelled ([^{3}H])-ligand (K_D=1.460 nM) and the unlabelled ligand (K_D=1.352 nM). LSN3172176 showed increased selectivity at the M1 mAChR (7-fold vrs M2, 300-fold vrs M3, 5-fold vrs M4, 6-fold vrs M5). Moreover, LSN3172176 had no effect on [^{3}H]-NMS displacement by atropine, suggesting LSN3172176 binds to an orthosteric pocket at the M1. LSN3172176 had 6000-fold and 4000-fold higher affinity at the M1 mAChR than ACh in human wild-type (WT) and DREADD respectively. At M1 WT, LSN3172176 was significantly more potent at inducing IP1 accumulation and ERK1/2 phosphorylation relative to ACh. In addition, LSN3172176 was about 10 times more potent at inducing phosphorylation of serine at position 228 (pS228) than ACh at M1 WT. At M1 DREADD, LSN3172176 induced pS228 but was about 100 times less potent relative to WT M1. Induction of pS228 at M1 mAChR is an indicator of receptor activation (2).

Conclusion: LSN3172176 retains its pharmacological properties after labelling, shows increased selectivity, affinity and potency at the M1 mAChR compared to ACh. Thus, LSN3172176 represents a promising ligand for PET imaging and *in vivo* characterisation is necessary to assess its suitability as M1-selective PET ligand.

References:

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