*Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol19Issue1abst039P.pdf*

**Characterization of the $M_4$ muscarinic acetylcholine receptor (mAChR) positive allosteric modulator, VU0467154, at chemo-genetically modified $M_4$ receptors**

P. Valuskova[1], S. J. Bradley[1], J. P. Conn[2], A. B. Tobin[1]. [1]Institute of Molecular, Cell and Systems Biology, University of Glasgow, Glasgow, United Kingdom, [2]Center for Neuroscience Drug Discovery, Vanderbilt University, Nashville.

**Introduction:** $M_4$ mAChRs play a crucial role in the control of basal locomotor activity (*1*), modulation of dopamine-dependent behaviours, neurotransmission and cognitive processes. Recently, the $M_4$ mAChR has been highlighted as a potential therapeutic target for neuropsychiatric disorders, such as schizophrenia, and pharmacological activation of $M_4$ mAChRs has been shown to alleviate some of the positive and cognitive symptoms associated with this disease (*2*).

**Method:** We have generated a transgenic mouse model whereby the wild-type $M_4$ receptor is replaced by a chemo-genetically modified $M_4$ mutant receptor ($M_4$ DREADD), where two mutations in the orthosteric binding pocket cause a loss of ACh activity but a gain in responsiveness to an otherwise inert ligand, clozapine-N-oxide (CNO). Here we conduct an *in vitro* pharmacological characterization of the $M_4$ positive allosteric modulator ($M_4$ PAM), VU0467154 (*3*), at $M_4$ DREADDs, to explore the utility of this ligand as a pharmacological tool in the $M_4$ DREADD mice.

**Results:** VU0467154 potentiated acetylcholine (ACh)-mediated displacement of [$^3$H]-NMS binding at the $M_4$ wild-type receptor but had no effect on affinity of ACh at the $M_4$ DREADD. To further examine the functional modulation mediated by VU0467154, we performed allosteric interaction studies between orthosteric ligands and VU0467154 at both $M_4$ wild-type and $M_4$ DREADDs. VU0467154 dose-dependently potentiated the ability of ACh and CNO to stimulate ERK1/2 phosphorylation at the $M_4$ wild-type receptor. At $M_4$ DREADD, VU0467154 enhanced the potency of ACh to stimulate ERK1/2 phosphorylation, however, had minimal effect on the action of CNO at the $M_4$ DREADD in the same pathway. Our initial behavioural experiments with $M_4$ DREADD mice revealed reduced cued and contextual fear conditioning responses that suggested the impairment of amygdala-based and hippocampal-based learning and memory, respectively. Our future experiments will be focused on restoration of the behavioural deficits associated with a lack of $M_4$ mAChRs after activation of $M_4$ DREADDs with CNO and/or $M_4$ PAM.

**Conclusions**: We have found that the $M_4$ PAM, VU0467154, can potentiate ACh-stimulation of ERK1/2 phosphorylation at the $M_4$ DREADD, highlighting the potential utility of this compound to restore ACh signaling in $M_4$ DREADD mice.

**References**:

1. J. Gomeza *et al.*(1999). *Proc. Natl. Acad. Sci. U.S.A.* **96**, 10483-10488.
2. M. Bubser *et al.*(2014). *ACS Chem Neurosci*. **5**, 920-942.
3. M. R. Wood *et al.(*2017). *Bioorg. Med. Chem. Lett.* **27**, 171-175 (2017).