Activation of Muscarinic M_4 Receptors Inhibits Dopamine D_1Receptor Signalling Pathways*In-Vivo*

The muscarinic M_4 receptor is an attractive therapeutic target for schizophrenia as revealed by Xanomeline, a mixed M_1/M_4 orthosteric agonist (1). Previous studies using transgenic mice have attributed Xanomeline's antipsychotic effects to M4 receptors located on D1-expressing GABAergic striatal neurones (2). Co-localisation of M_4 and D_1 receptors has been demonstrated on medium spiny neurons in striatal preparations through measuring the inhibitory effect of the $G_{i/o}$-coupled M_4 receptor on G_s-coupled D_1receptor mediated signalling cascade (3). This resulted in the overall modulation of dopaminergic neurotransmission. Although this interaction between D_1 and M_4 receptors has been characterised *ex-vivo*, demonstrating modulation of M_4 receptors *in-vivo* has proven challenging. The aim of this study was to confirm this D_1/M_4 converging signalling pathway in an*in-vivo* model.

The D_1 agonist, SKF82958 (s.c., dH_2O. Rat: 3 mg/kg, 10 mins. Mouse: 1 mg/kg, 15 mins), was used to activate the D_1-G_s coupled signalling cascade in maleadultSprague Dawley rats and in-house developed male transgenic mice expressing the human M_4 receptor.Following methods to preserve protein phosphorylation (4) increasesin cell signalling markers were detectable in the striatum, withrobust and reproducible cGMP and pCREB ELISA responses. Pre-treatment with Xanomeline (i.p., 0.5% methylcellulose)dose-dependently inhibited SKF82958 induced pCREB in both rat and mouse models (Fig 1A&B) and cGMP in mice (Fig 1C).

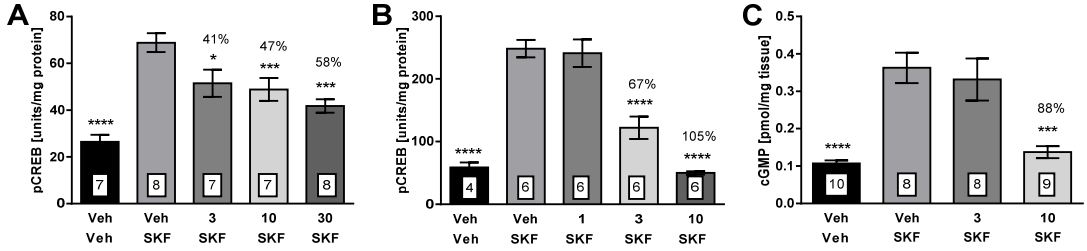

Figure 1. Effect of xanomeline on SKF82958 induced striatal pCREB in rat (A) and mouse (B), and cGMP in mouse (C). Data is expressed as pCREB units/mg protein or cGMP pmol/mg tissue relative to vehicle controls, analysed by one-way ANOVA with Dunnett's post-test compared to Veh-SKF control: *p<0.05, *** p<0.001 and **** p<0.0001. n number indicated in boxes.

This data, in combination with previous studies, suggests that *in-vivo* activation of the $G_{i/o}$-coupled M_4 signalling pathwaycan attenuateG_s-coupled D_1 induced signals. The present findings demonstrate signalling cross-talk between the M_4 and D_1 receptors *in-vivo* as verified by the measurement of pCREB and cGMP. The ability to stabilise phosphoproteins *in-vivo* has allowed the development of an assay that can reliably measure biologically relevant levels of phosphoprotein markers. This approach can confirm hypothesised signalling pathways and provide robust *in-vivo* assays for pharmacological screening.

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(3) Jeon J *et al*. (2010). *J Neurosci* **30**: 2396-2405

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