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Characterisation of an endogenous dopamine D1 receptor in U-2 OS cells

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***Introduction*:** U-2 OS cells are an osteocarcinoma derived cell line regularly used in biomedical research. U-2 OS cells are attractive because they are highly amenable to transfection/transduction and appear to lack many of the typically confounding functional responses of endogenous receptors found on other cell types (1). Here we report the characterisation of endogenous D1 receptors underpinning responses to dopamine in wild-type U-2 OS cells. Dopamine is a neurotransmitter which activates five different seven transmembrane G-protein coupled receptors. These receptors, namely D1, D2, D3, D4 and D5, are separated into two classes based on downstream second messenger signalling. The D1-like subfamily comprises D1 and D5 receptors coupled to $G_{\alpha s}$ which, when activated, increase intracellular cAMP via adenylate cyclase (2).

***Method*:** To pharmacologically profile dopaminergic responses in U-2 OS cells a subset of full agonists (dopamine, dihidrexidine hydrochloride, SKF81297 and SKF83822) and partial agonists (SKF77434 and SKF83959) were assayed as concentration-response curves using a homogenous time resolved fluorescence (HTRF) cAMP assay (Cisbio, UK). qPCR performed using DRD1 TaqMan probes (ThermoFisher Hs00265265_s1)

***Results*:** Agonists showed a rank order of potency and relative efficacies consistent with D1-like receptors. Table 1 shows pEC50 values derived from the cAMP response of wild-type U-2 OS cells treated with increasing concentrations of compound. % Intrinsic Activity was calculated relative to dopamine as 100% response. Similarly, Schild analysis using the highly selective D1/D5 receptor antagonist SCH23390 revealed a pA2 of 10.4. Importantly, the highly selective D1 positive allosteric modulator 'Compound A' (3) potentiated dopamine-mediated responses. In contrast, a range of D2-like receptor ligands (agonists and antagonists) failed to induce/attenuate responses in U-2 OS cells. To further confirm pharmacological responses were mediated by D1 receptors, quantitative PCR was carried out using D1 specific TaqMan probes.

***Conclusion*:** This investigation describes an endogenous $G_{\alpha s}$-coupled receptor expressed in U-2 OS cells and pharmacology consistent with that of D1 receptors. It is hoped that this will provide a useful tool when using U-2 OS as host cells for screening heterologous GPCRs that signal via cAMP. Moreover, this may facilitate the triage of compounds that act non-specifically on the cell background, are generic cAMP pathway modifiers or those which promiscuously activate other receptors.

***References*:** 1) Ames RS *et al.* (2011) *Recept. Channels* **10:** 117-124 2) Missale C *et al.* (1998) *Physiological Reviews* **78:** 189-225 3) Lewis MA *et al.* (2015) *J Pharmacol Exp Ther* **354:** 340-349

Table 1: pEC50 and % Intrinsic Activity from cAMP response of wild-type U-2 OS cells (n=3 +/- s.d.)

Compound	**pEC50**	**% Intrinsic Activity**
Dopamine	8.23 ± 0.2	102.25 ± 1.0
Dihidrexidine HCl	9.73 ± 0.2	99.59 ± 0.7

SKF81297	10.31 ± 0.4	96.56 ± 0.4
SKF83822	11.71 ± 0.4	97.07 ± 1.1
SKF77434	9.43 ± 0.3	25.40 ± 6.0
SKF83959	11.22 ± 0.3	30.01 ± 3.4