Investigating the functional interaction between the dopamine D_2 receptor (D_2R) and the dopamine transporter (DAT) using a novel plate-based assay

Disruption of synaptic dopamine (DA) levels is implicated in a number of neurological disorders such as Parkinson's disease and schizophrenia (1). DA release and reuptake are regulated by the short isoform of the D_2R (D_2S) and DAT co-expressed in pre-synaptic dopaminergic nerve terminals. Co-expression of D_2S with DAT has been reported to increase the uptake function of DAT (2). The aim of this study was to investigate whether co-expression of DAT and D_2R affected the function of either protein.

CHO K1 cells stably expressed DAT tagged with green fluorescent protein (GFP) alone, or with either SNAP-tagged D_2S (DAT-D_2S) or long isoform D_2R (D_2L) (DAT-D_2L). In assaying DAT function, cells on 96 well plates were incubated in buffer (HBSS, 50μM Brilliant Black BN) with ligand (20min, 37ºC), prior to addition of fluorescent DAT substrate 4-[4-(diethyl-amino)-styryl]-N-methylpyridinium iodide (ASP^+, 10 μM) (3). Fluorescent substrate uptake after 30min at 37ºC was quantified using a FlexStation3 (ex:485 nm, em:590nm). D_2R function was studied using CHO D_2S, D_2L, DAT-D_2S and DAT-D_2L membranes. Membranes were pre-incubated with ligand and 1μM GDP (1h, 21°C) in buffer (20mM HEPES, 10mM $MgCl_2$, 100mM NaCl, pH 7.4) before incubation with 200pM [^{35}S]GTPγS (20 min, 21ºC). Bound radioactivity was separated using GF/B filter plates, which were washed and dried before quantification of radioactivity (Packard TopCount). Concentration response curves were fitted in GraphPad Prism v6.

The rank order of potency of DAT inhibitors in the ASP^+ assay was consistent across DAT, DAT-D_2S and DAT-D_2L cell lines: IN>GBR>JHW>BP (**Table 1**). D_2R ligand effects were also consistent between cell lines: QP had no effect, AP and DA inhibited ASP^+ accumulation (**Table 1**), and BC increased accumulation at >10μM. In the [^{35}S]GTPγS assay, D_2R agonists showed no difference in pEC_{50} in D_2S, DAT-D_2S, D_2L and DAT-D_2L cell lines; QP: 6.82±0.23, 6.60± 0.07, 6.94±0.21, 6.54±0.10; BC: 8.76±0.18, 8.77±0.18, 8.84±0.19, 8.40±0.10; AP: no effect; and DA: 7.11±0.21, 6.78 ±0.08, 6.66±0.08, 6.53±0.11 respectively (n=5). The maximum responses to AP and DA (represented as % 10μM QP response) were similar in all cell lines, while BC Rmax decreased with DAT co-expression; D_2S 76±9, DAT-D_2S 51±3 and D_2L 70±11, DAT-D_2L 34±9% (*P*<0.05; unpaired t-test, Welch's correction). These data suggest that co-expression of either isoform of D_2R with DAT does not affect DAT inhibitor potency; however BC efficacy at theD_2S/L receptor is reduced with DAT co-expression, indicating an alteration in receptor function.

	ASP^+ Assay pIC_{50} Values		
Ligand	**DAT**	**DAT-D_2S**	**DAT-D_2L**
Quinpirole (QP)	NE	NE	NE
Bromocriptine (BC)	*	*	*
Aripiprazole (AP)	5.66 ± 0.16	5.54 ± 0.15	5.79 ± 0.16
Dopamine (DA)	5.00 ± 0.17	5.41 ± 0.12	5.14 ± 0.11
GBR-12909 (GBR)	7.57 ± 0.09	7.53 ± 0.11	7.55 ± 0.07
Indatraline (IN)	8.00 ± 0.19	8.06 ± 0.15	7.77 ± 0.15
Bupropion (BP)	6.32 ± 0.17	6.46 ± 0.07	6.39 ± 0.10
JHW007 (JHW)	7.59 ± 0.20	7.37 ± 0.06	7.39 ± 0.09

***Table 1:** ASP^+ assay data. Summary of D_2R agonist and DAT inhibitor responses in DAT, DAT-D_2S and DAT-D_2L expressing CHO cells. Data are shown as mean ± SEM; n=5-19. NE=no effect.* = Increase in ASP^+ accumulation at 10μM BC.*

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