Agonist-dependent Effects of a Constitutively Activating T372R Mutation on Dopamine D2L Receptor Signalling in Receptor Internalisation and cAMP Dependent Reporter Gene Assays

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T343R mutation of the dopamine D2 receptor (D2R) (short isoform) results in elevated constitutive activity (1). Here we investigate the equivalent T372R mutation, located in the third intracellular loop, in D2R (long isoform) (D2L) responses in cAMP-dependent reporter gene and receptor internalisation assays to compare different signalling endpoints and structural classes of agonist.

CHO cells stably expressed wild type (WT) or mutated (T372R) SNAP-tagged human D2L and CRE-secreted placental alkaline phosphatase (SPAP) reporter gene cDNAs. SPAP assays (2) were performed on serum starved cells, with 1h ligand pre-treatment prior to a 5h, 3µM forskolin stimulation. For receptor internalisation (3), cells on black-walled, clear bottomed, 96 well plates were labelled with 0.1µM SNAP-surface AF488 (30min), agonist treated for 1h at 37°C then fixed with paraformaldehyde. MDC IX Ultra plate-reader images were quantified by a granularity algorithm. Concentration response curves were fitted in GraphPad Prism v6 with 10µM quinpirole (QP) controls as the reference full agonist.

In WT cells, QP, bromocriptine (BC) and (+)-3-PPP were full agonists in the SPAP assay whereas dihydroergocristine (DHEC), (-)-3-PPP and aripiprazole (AP) were partial agonists (Table 1; order of potency: BC=DHEC>AP=QP>(-)-3-PPP=(+)-3-PPP). Only QP, (+)-3-PPP and BC (partial agonist) induced D2L internalisation. T372R mutation significantly increased the relative maximum response of AP, DHEC and (-)-3-PPP in the SPAP assay; furthermore AP, DHEC and (-)-3-PPP all now stimulated internalisation, while BC became a full agonist (Table 1). However T372R selectively *reduced* BC and DHEC potencies, by 28- and 5-fold respectively (SPAP), and by 35-fold (internalisation, BC).

Ligand	Receptor	SPAP		Internalisation	
		pEC_{50}	E_{max} (%10μM QP)	pEC_{50}	E_{max} (%10μM QP)
QP	**WT**	7.78 ± 0.25	100	6.51 ± 0.18	107.3 ± 5.0
	T372R	7.54 ± 0.41	100	6.81 ± 0.41	122.0 ± 6.6
AP	**WT**	8.02 ± 0.08	48.1 ± 0.5	NE	NE
	T372R	7.86 ± 0.28	98.9 ± 9.5*	7.22 ± 0.29	48.7 ± 9.4
BC	**WT**	9.33 ± 0.12	96.4 ± 3.6	8.65 ± 0.07	43.7 ± 9.5
	T372R	7.88 ± 0.28*	103.9 ± 11.4	7.11 ± 0.11*	119.4 ± 9.2*
DHEC	**WT**	8.97 ± 0.15	81.4 ± 3.7	NE	NE
	T372R	8.23 ± 0.04*	112.8 ± 7.1*	7.59 ± 0.09	77.5 ± 4.7
(-)-3-PPP	**WT**	6.63 ± 0.24	63.3 ± 10.7	NE	NE
	T372R	7.00 ± 0.09	109.4 ± 8.1*	5.95 ± 0.36	80.4 ± 9.9
(+)-3-PPP	**WT**	6.37 ± 0.29	100.3 ± 6.2	5.14 ± 0.12	82.9 ± 7.7
	T372R	6.76 ± 0.16	105.1 ± 10.0	6.27 ± 0.30*	122.5 ± 11.1*

Table 1: Summary of agonist responses in D2L WT and T372R expressing CHO cells. Data represent mean ± SEM of 4 experiments. NE: No effect; *P<0.05 cf WT (Student's unpaired t test).

The effects on D2L agonist pharmacology following T372R mutation are therefore generally consistent with increased constitutive activity in both assays. However, decreased potency of BC and DHEC in both assays suggests selectively impaired binding of these ergot derivatives to the T372R promoted receptor conformation. *Supported by a BBSRC CASE studentship and GSK.*

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