Evaluation of the differing roles of transmembrane domain-5 serine residues in agonist activation at wild type and constitutively active dopamine D2 receptors

T372R mutation of the dopamine D2 receptor (long isoform, D2L) leads to constitutive activity. We previously identified that, while T372R mutation is accompanied by the expected increase in potency for many D2 agonists, the ergot derivative bromocriptine shows decreased potency but increased maximal response in functional assays (1). Here we explore whether changes in agonist interaction with the D2L binding site are responsible, focussing on the relative roles of transmembrane domain (TM) 5 serine residues implicated in binding catechol hydroxyl or ergot aromatic amine groups (2).

Stably transfected CHO K1 cells expressed a CRE-secreted placental alkaline phosphatase (SPAP) reporter gene, and the SNAP tagged D2L (WT) or D2L T372R receptors, additionally containing S193A, S194A or S197A mutations as indicated. SPAP assays were performed as described (3) to evaluate G protein dependent D2L responses, with 1h quinpirole (QP) or bromocriptine (BC) pre-treatment prior to a 5h forskolin (3μM) stimulation. In measurements of SNAP-D2L internalisation (4), cells were labelled with 0.1μM SNAP-surface AF488 (30min), agonist treated for 1h at 37°C then fixed. MDC IX Ultra plate-reader images were quantified by a granularity algorithm (4). Concentration response curves were fitted in GraphPad Prism v6 to determine pEC_{50} and R_{max} values (%max QP), as mean ± s.e.m (n = 3 – 4).

Table 1 shows that in the SPAP assay, S193A mutation had opposing actions on QP responses on the D2L WT and T372R background, increasing potency 16-fold in WT, but decreasing it 5 fold for T372R. S193A did not affect BC SPAP responses, but S197A reduced both QP and BC potency and the maximum response to BC relative to QP in D2L WT cells. However, while this inhibitory effect of S197A was maintained for QP when measuring D2L T372R SPAP responses, no serine mutation altered BC potency and maximum response mediated by the constitutively active receptor mutant. Equivalent effects were observed when monitoring SNAP-D2L or D2L T372R internalisation; for example, S193A mutation selectively decreased QP potency for T372R (pEC_{50} 5.8±0.2 [S193A, T372R] versus 6.8±0.4 [T372R]), but not for D2L WT responses (6.9±0.3 [S193A] versus 6.5±0.2 [WT]).

	WT				**T372R**			
	None	**S193A**	**S194A**	**S197A**	**None**	**S193A**	**S194A**	**S197A**
QP pEC_{50}	7.8±0.2	8.9±0.2*	7.9±0.1	6.7±0.1*	7.8±0.2	7.1±0.2*	8.1±0.1	7.1±0.1*
QP R_{max}	99±1	117±6	100±2	103±1	96±6	94±1	93±1	98±1
BC pEC_{50}	9.3±0.1	8.9±0.1	8.9±0.2	8.1±0.1*	8.3±0.2	8.3±0.3	8.6±0.2	8.0±0.1
BC R_{max}	100 ±4	124±7	105±5	64±5*	94±7	102±5	105±7	107±5

Table 1. The effects of serine mutation on QP and BC SPAP responses comparing the WT and T372R D2L receptor background. *P≤0.05 (One way ANOVA, and Dunnett's post test).

Thus, our results provide evidence that QP and BC interact differently with binding site serine residues in D2L WT and T372R receptors, and suggest that the constitutively active conformation promoted by T372R mutant is distinct from the agonist bound D2L WT. *Supported by a BBSRC / GSK CASE award.*

(1) Stott *et al.* (2014). pA2 online http://www.pa2online.org/abstracts/vol12issue3abst063p.pdf
(2) Krogsgaard-Larsen *et al*. (2014). *Neurochem Res*. **39**: 1997–2007.
(3) Baker JG (2010). *PLOS One* **5**: e15487.
(4) Kilpatrick LE et al. (2012). *Biochim. Biophys. Acta* **1823**: 1068.