Determination of ligand intrinsic efficacy at the dopamine D2S receptor using the Slack and Hall operational model

The Black and Leff operational model (OM) (1) is used to estimate functional ligand affinity (K_A) and efficacy (τ) but τ is a system dependent parameter, equal to the ratio of receptor concentration and convolved stimulus coupling efficiency. Moreover, this OM cannot account for a constitutively active receptor system. The Slack and Hall OM (2) incorporates constitutive activity and uses it to determine the system coupling efficiency (χ). Theoretically, this then allows separation of the ligand and system dependent components of τ, providing ligand intrinsic efficacy (ε) and χ determinations. Here we apply this approach to analyse dopamine D2 receptor (short isoform) (D2S) [^{35}S]-GTPγS binding assay data.

Membrane preparations were derived from a CHO K1 cell line expressing a SNAP-tagged human D2S receptor (B_{max}: 4.80±0.74pmol mg^{-1}). For [^{35}S]-GTPγS assays, membranes were incubated with agonist and one of four GDP concentrations (30nM–10μM; 1h, 21^{o}C) in buffer (20mM HEPES, 10mM $MgCl_2$, 100mM N-methyl-D-glucamine (NMDG), pH 7.4) before addition of 200pM [^{35}S]-GTPγS for 20min. Bound radioactivity was separated using GF/B filter plates, which were washed and dried before quantification of retained radioactivity (Packard TopCount). Data were baseline corrected using 1μM (+)butaclamol (inverse agonist) data to determine the level of D2S constitutive activity, and then globally analysed using the Slack and Hall OM (GraphPad Prism v6). Basal and E_{max} were globally shared, K_A and ε shared within agonist data sets and χ shared for each [GDP]. For [^{3}H]-spiperone binding, membranes were incubated (3h, 21^{o}C) with agonist and ~100pM [^{3}H]-spiperone in buffer (10mM HEPES, 1mM EDTA, 1mM EGTA, 6mM $MgCl_2$, 100mM NMDG, pH 7.4). Plates were filtered and counted as above. Data were analysed (GraphPad Prism) and fit to either one- or two-site binding models as determined by an F-test.

Fitting the Slack and Hall OM to [^{35}S]-GTPγS concentration response data derived ε and pK_A estimates (**Table 1**) with an order of affinity of aripiprazole = DHEC > bromocriptine > dopamine = quinpirole. Quinpirole, dopamine and bromocriptine were of equivalent intrinsic efficacy (in sodium-free buffer), as defined by logε values, while DHEC and aripiprazole had significantly lower logε compared to quinpirole ($P<0.05$; One-way ANOVA, Tukey's post test). [^{3}H]-spiperone binding assays in equivalent buffer indicated two-site competition by quinpirole and dopamine, and single site for the remaining agonists (**Table 1**). The same rank order of affinity was observed as for the functional pK_A values. Thus, the Slack and Hall OM can be used to analyse a constitutively active system to derive deconvolved ligand efficacy and affinity estimations, with functional pK_A values closely matching pK_is indicated by radioligand binding.

Ligand	logε	pK_A	pK_i low	pK_i high	%high
Quinpirole	1.36 ± 0.21	6.87 ± 0.14	5.70 ± 0.09	7.71 ± 0.17	30.6 ± 3.0
Dopamine	1.52 ± 0.16	7.05 ± 0.16	5.45 ± 0.06	7.88 ± 0.19	38.4 ± 3.1
Bromocriptine	1.28 ± 0.20	7.57 ± 0.12	7.90 ± 0.06	-	-
Dihydroergocristine	0.70 ± 0.04	8.09 ± 0.33	8.30 ± 0.03	-	-
Aripiprazole	0.74 ± 0.03	8.64 ± 0.52	8.68 ± 0.15	-	-

Table 1: *Summary of Slack and Hall OM fits and [^{3}H]-spiperone binding. Data are pooled (mean ± SEM; n=5). Where a single site binding model was used, data are expressed as pK_i low.*

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(1) Black JW and Leff P (1985) *Br J Pharmacol* **84**: 561–571
(2) Slack RJ and Hall DA (2012) *Br J Pharmacol* **166**: 1774–1792