Molecular Pharmacology

Supplemental data

Evidence for non-canonical neurotransmitter activation: Norepinephrine as a dopamine D_2-like receptor agonist

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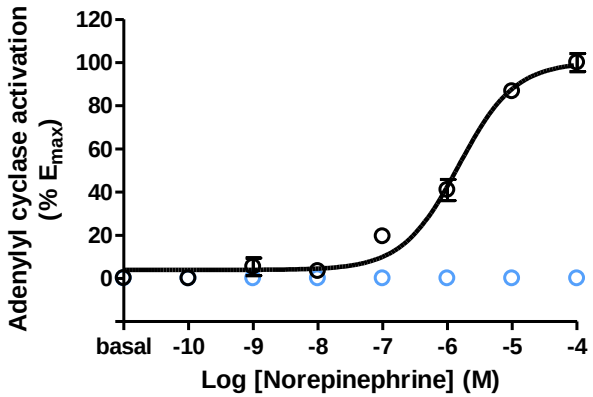
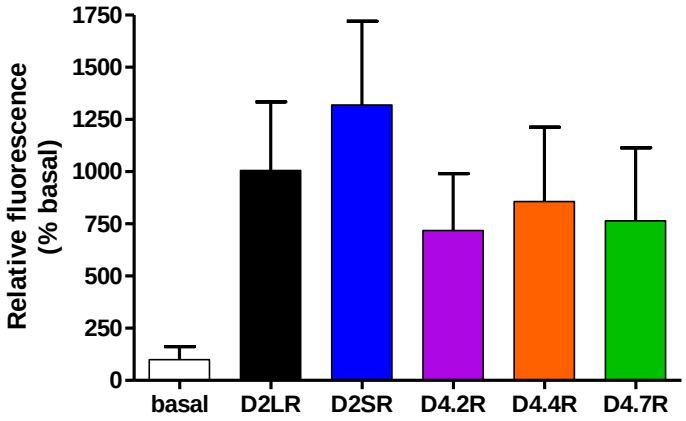
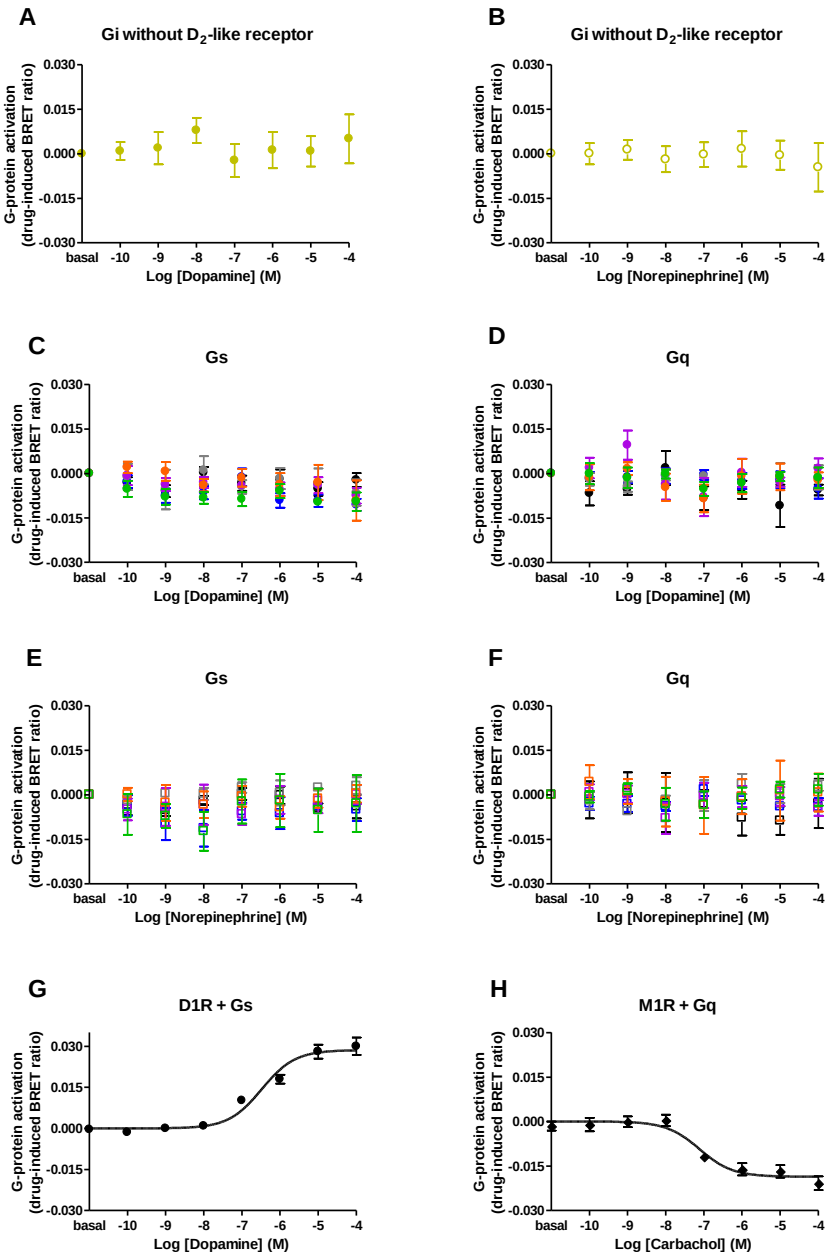


Supplementary Figure 1. Adenylyl cyclase activation induced by norepinephrine mediated by β adrenergic receptors in non-transfected HEK-293T cells. Dose dependent increase in cAMP without (black) and with the presence of specific β blocker propranolol (blue). Cells were pre-treated with propranolol (10 μM) 10 minutes prior to norepinephrine addition for the antagonist experiment (blue). Cells were treated with coelenterazine H followed by increasing concentrations of norepinephrine. After 10 minutes BRET was measured and basal BRET values were subtracted from BRET values for each norepinephrine concentration. Data represent means ± S.E.M. of 3 to 4 experiments performed in triplicate and are shown as a percentage of the maximal effect.

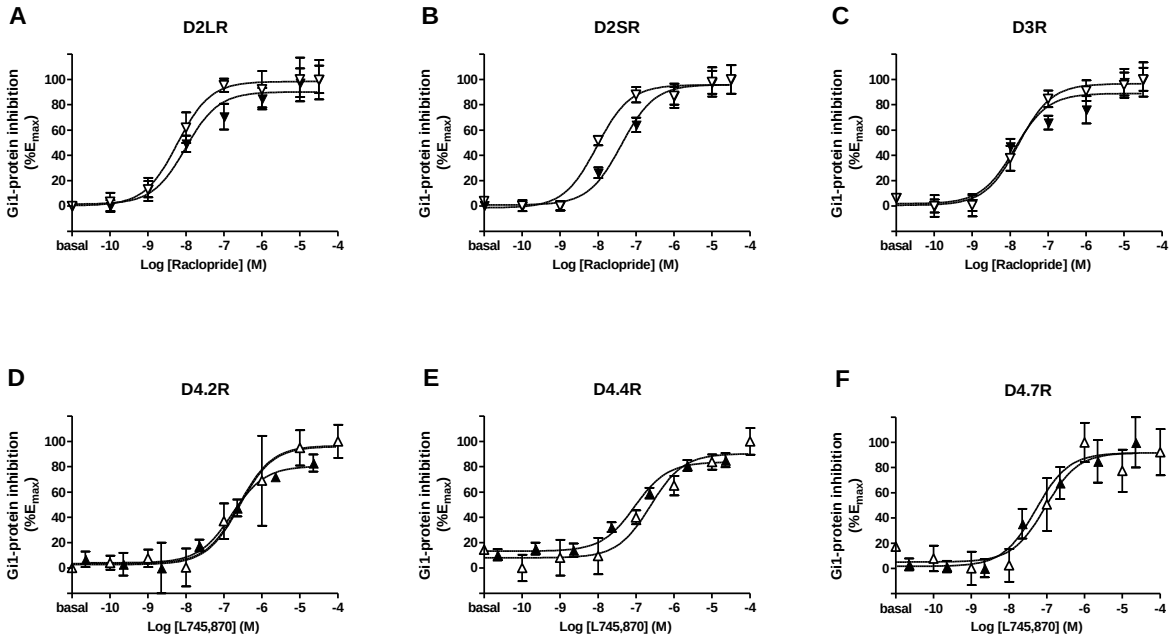


Supplementary Figure 2. Surface expression of the different D2R and D4R variants in cells used for Gi protein activation (transfected with Gαi1 subunit, unfused β_1 and γ_2-mVenus). As described in Materials and Methods, the surface levels of D2R and D4R variants were visualized by anti-flag immunostaining and detected by FACS. Data are normalized to non-specific staining from the non-transfected cells and shown in means ± S.E.M. of 3 to 4 experiments.



Supplementary Figure 3. G protein activation-BRET experiments showing the absence of G protein activation by dopamine (filled symbols) or norepinephrine (open symbols) in HEK-293T cells transfected with Gαi1-Rluc subunit without D_2-like receptors **(A-B)**, or transfected with Gαs-Rluc subunit **(C-D)** or with Gαq-Rluc subunit (**E-F)** with D2LR (black), D2SR (blue), D3R

(gray), D4.2R (purple), D4.4R (orange) or D4.7R (green) and with and the indicated D_2-like receptor. Cells were also transfected with β_1 and γ_2-mVenus. To show the validity of the Gs and Gq biosensors, cells were transfected with Gαs-Rluc subunit and dopamine D1 receptor (D1R) (**G**) or Gαq-Rluc subunit with muscarinic M1 receptor (**H**) together with unfused β_1 and γ_2-mVenus. Cells were treated with coelenterazine H followed by increasing concentrations of dopamine (**A**, **C**, **D**, **G**), norepinephrine (**B**, **E**, **F**) or carbachol (**H**). After 10 minutes BRET was measured as described in Materials and Methods. Basal BRET values in the absence of ligands were subtracted from the BRET values for each agonist concentration. Data were fit by non-linear regression to a sigmoidal dose-response relationship against the agonist concentration (**G**, **H**). Data are means ± S.E.M. of 3 to 4 experiments performed in triplicate



Supplementary Figure 4. Dose-dependent inhibition by selective D2R-D3R antagonist raclopride and D4R antagonist L745,870 of Gi protein activation in HEK-293T cells transfected with Gi protein (Gαi1 subunit, unfused β_1 and γ_2-mVenus) induced by dopamine (filled triangles) or norepinephrine (open triangles) mediated by **(A)** D2LR, **(B)** D2SR, **(C)** D3R, **(D)** D4.2R, **(E)** D4.4R or **(F)** D4.7R. Agonists (10 μM for D2LR, D2SR and D3R and 1 μM for D4.2R, D4.4R and D4.7R) were added 10 minutes after antagonist incubation. BRET was measured as described in Materials and Methods. BRET values for the agonist alone were subtracted from the BRET values for each antagonist concentration. Data were fit by non-linear regression to a sigmoidal dose-response relationship against the antagonist concentration and is shown as a percentage of the maximal inhibitory effect. Data are means ± S.E.M. of 3 to 9 experiments performed in triplicate.