New Characterization of Dihydroergotamine Receptor Pharmacology in the Context of Migraine: Utilization of a β-arrestin Recruitment Assay

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**In vitro screening for functional receptor activity of DHE and sumatriptan succinate methods**

Samples were added to cells and incubated for 90 or 180 minutes at 37 °C or room temperature, depending on the specific receptor, as noted, with specific incubation protocols outlined in **Supplemental Table 1**. For antagonist activity, preincubation temperatures were performed at the temperatures specified in **Supplemental Table 1**,per target. Incubation with 6× EC80 agonist was determined by the assay manufacturer and selected in order to provide a challenge that was low enough to be below the saturation point, so as to not bias the results, while still at a high enough challenge concentration to yield a robust signal-to-noise ratio. Further information is available from Eurofins Discovery Services upon request.

**Data analysis of functional receptor activity**

DHE and sumatriptan succinate activity were analyzed using CBIS data analysis suite (ChemInnovation, CA).

Agonist Mode

For agonist mode assays, percentage activity was calculated using the following formula:

% Activity = 100% × (mean RLU of DHE − mean RLU of vehicle control) / (mean MAX control ligand − mean RLU of vehicle control)

Activation of GPCRs by a compound acting as an agonist will result in an increase in β-arrestin recruitment to the target GPCR. To determine if DHE was potentially acting as an agonist to activate the receptor and induce β-arrestin recruitment the following factors were considered:

1. Is the DHE activity >30%?
   1. If so, is the DHE mean RLU > baseline RLU + 3 × baseline SD?
   2. Conversely, is the baseline RLU < DHE mean RLU – 3 × SD?
2. Is the DHE mean RLU / baseline RLU > 2?

If #2 is true and DHE activity is significantly different in cases 1a) and 1b), then the interaction is potentially significant.

Antagonist Mode

For antagonist mode assays, percentage inhibition was calculated using the following formula:

% Inhibition = 100% × (1 − (mean RLU of DHE − mean RLU of vehicle control) / (mean RLU of EC80 control − mean RLU of vehicle control))

Inhibition of GPCR activation by a compound acting as an antagonist of ligand binding will result in a decrease in the reference’s ability to induce β-arrestin recruitment to the target GPCR*.* To determine if DHE is potentially acting as an antagonist to inhibit receptor activation the following factors were considered:

1. Is the % inhibition > 50%?
   1. If so, is the compound mean RLU < EC80 RLU – 3 × EC80 SD?
   2. Conversely, is the EC80 RLU > compound mean RLU + 3 × compound SD?

If the inhibition is >50% and the compound activity is significantly different in cases a) and b), then the interaction is potentially significant.

**Radioligand competition binding assays**

Specific temperatures and incubation times for each target receptor assay outlined in the primary methods were pre-established by the manufacturer, based on assay optimization.

**Supplemental Table 1. Incubation Temperature and Times for Each Receptor Included in the Screening (1)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Family Name** | **Human Gene** | **Common Name** | **Assay Incubation Temperature** | **Assay Incubation Time** |
| 5-Hydroxytryptamine receptors | *HTR1A* | 5-HT1A receptor | 37 °C | 2 hours |
| *HTR1B* | 5-HT1B receptor | 37 °C | 2 hours |
| *HTR1F* | 5-HT1F receptor | 37 °C | 2 hours |
| *HTR2A* | 5-HT2A receptor | 37 °C | 2 hours |
| *HTR2C* | 5-HT2C receptor | 37 °C | 2 hours |
| *HTR5A* | 5-HT5A receptor | 37 °C | 2 hours |
| *HTR1E* | 5-HT1E receptor | 37 °C | 2 hours |
| Acetylcholine receptors | *CHRM1* | M1 receptor | 37 °C | 2 hours |
| *CHRM2* | M2 receptor | 37 °C | 2 hours |
| *CHRM3* | M3 receptor | RT | 2 hours |
| *CHRM4* | M4 receptor | 37 °C | 2 hours |
| *CHRM5* | M5 receptor | 37 °C | 2 hours |
| Adenosine receptors | *ADORA3* | A3 receptor | 37 °C | 2 hours |
| Adrenoceptors | *ADRA1B* | α-adrenergic1B (α1B-adrenoceptor) | 37 °C | 2 hours |
| *ADRA2A* | α-adrenergic2A (α2A-adrenoceptor) | RT | 2 hours |
| *ADRA2B* | α-adrenergic2B (α2B-adrenoceptor) | 37 °C | 2 hours |
| *ADRA2C* | α-adrenergic2C (α2C-adrenoceptor) | RT | 2 hours |
| *ADRB1* | β-adrenergic1 (β1-adrenoceptor) | 37 °C | 2 hours |
| *ADRB2* | β-adrenergic2 (β2-adrenoceptor) | 37 °C | 2 hours |
| Angiotensin receptor | *AGTR1* | AT1 receptor | RT | 2 hours |
| Apelin receptor | *AGTRL1(APLNR)* | APJ (Apelin receptor) | RT | 2 hours |
| Bombesin receptors | *BRS3* | BB3 receptor | 37 °C | 2 hours |
| *GRPR* | BB2 receptor | 37 °C | 2 hours |
| *NMBR* | BB1 receptor | RT | 2 hours |
| Bradykinin receptors | *BDKRB1* | B1 receptor | RT | 2 hours |
| *BDKRB2* | B2 receptor | 37 °C | 2 hours |
| Calcitonin receptors | *CALCR* | CT receptor | 37 °C | 2 hours |
| *CALCRL-RAMP1 (NA)* | CGRP receptor | RT | 2 hours |
| *CALCRL-RAMP2 (NA)* | AM1 receptor | 37 °C | 2 hours |
| *CALCRL-RAMP3 (NA)* | AM2 receptor | RT | 2 hours |
| *CALCR-RAMP2*  *(NA)* | AMY2 receptor | 37 °C | 2 hours |
| *CALCR-RAMP3*  *(NA)* | AMY3 receptor | RT | 2 hours |
| Cannabinoid receptors | *CNR1* | CB1 receptor | 37 °C | 2 hours |
| *CNR2* | CB2 receptor | RT | 2 hours |
| Chemerin receptor | *CMKLR1* | CMKLR1 (Chemerin receptor 1) | 37 °C | 2 hours |
| Chemokine receptors | *CCR1* | CCR1 | 37 °C | 2 hours |
| *CCR10* | CCR10 | 37 °C | 2 hours |
| *CCR2* | CCR2 | 37 °C | 2 hours |
| *CCR3* | CCR3 | 37 °C | 2 hours |
| *CCR4* | CCR4 | 37 °C | 2 hours |
| *CCR5* | CCR5 | 37 °C | 2 hours |
| *CCR6* | CCR6 | 37 °C | 2 hours |
| *CCR7* | CCR7 | 37 °C | 2 hours |
| *CCR8* | CCR8 | 37 °C | 2 hours |
| *CCR9* | CCR9 | 37 °C | 2 hours |
| *CX3CR1* | CX3CR1 | 37 °C | 2 hours |
| *CXCR1* | CXCR1 | 37 °C | 2 hours |
| *CXCR2* | CXCR2 | 37 °C | 2 hours |
| *CXCR3* | CXCR3 | 37 °C | 2 hours |
| *CXCR4* | CXCR4 | 37 °C | 2 hours |
| *CXCR5* | CXCR5 | 37 °C | 2 hours |
| *CXCR7* | CXCR7 | 37 °C | 2 hours |
| Cholecystokinin receptors | *CCKAR* | CCK1 receptor | RT | 2 hours |
| *CCKBR* | CCK2 receptor | RT | 2 hours |
| Class A orphans | *EBI2 (GPR183)* | GPR183 | RT | 2 hours |
| *GPR1 (CMKLR2)* | GPR1 (Chemerin receptor 2) | 37 °C | 2 hours |
| *GPR119* | GPR119 | 37 °C | 2 hours |
| *GPR35* | GPR35 | 37 °C | 2 hours |
| *MRGPRX1* | MRGPRX1 | 37 °C | 2 hours |
| *MRGPRX2* | MRGX2 | 37 °C | 2 hours |
| Complement peptide receptors | *C5AR1* | C5A receptor (C5a1 receptor) | RT | 2 hours |
| *C5L2 (C5AR2)* | C5L2 receptor (C5a2 receptor) | 37 °C | 2 hours |
| Corticotropin-releasing factor receptors | *CRHR1* | CRF1 receptor | 37 °C | 2 hours |
| *CRHR2* | CRF2 receptor | RT | 2 hours |
| Dopamine receptors | *DRD1* | D1 receptor | 37 °C | 2 hours |
| *DRD2L* | D2L receptor | 37 °C | 2 hours |
| *DRD2S* | D2S receptor | RT | 2 hours |
| *DRD3* | D3 receptor | 37 °C | 2 hours |
| *DRD4* | D4 receptor | 37 °C | 2 hours |
| *DRD5* | D5 receptor | 37 °C | 2 hours |
| Endothelin receptors | *EDNRA* | ETA receptor | RT | 2 hours |
| *EDNRB* | ETB receptor | 37 °C | 2 hours |
| Formylpeptide receptors | *FPR1* | FPR1 | 37 °C | 2 hours |
| *FPRL1 (FPR2)* | FPR2/ALX | 37 °C | 2 hours |
| Free fatty acid receptors | *FFAR1* | FFA1 receptor | 37 °C | 4 hours |
| *GPR120 (FFAR4)* | FFA4 receptor | 37 °C | 2 hours |
| Galanin receptors | *GALR1* | GALR1 receptor (GAL1 receptor) | 37 °C | 2 hours |
| *GALR2* | GALR2 receptor (GAL2 receptor) | 37 °C | 2 hours |
| Ghrelin receptor | *GHSR* | ghrelin receptor | 37 °C | 2 hours |
| Glucagon receptors | *GCGR* | glucagon receptor | RT | 2 hours |
| *GIPR* | GIP receptor | 37 °C | 2 hours |
| *GLP1R* | GLP-1 receptor | 37 °C | 2 hours |
| *GLP2R* | GLP-2 receptor | 37 °C | 2 hours |
| *SCTR* | secretin receptor | 37 °C | 2 hours |
| Glycoprotein hormone receptors | *FSHR* | FSHR receptor (FSH receptor) | 37 °C | 2 hours |
| *LHCGR* | LH receptor | 37 °C | 2 hours |
| *TSHR(L) (TSHR)* | TSH receptor | RT | 4 hours |
| Histamine receptors | *HRH1* | H1 receptor | RT | 2 hours |
| *HRH2* | H2 receptor | RT | 2 hours |
| *HRH3* | H3 receptor | 37 °C | 2 hours |
| *HRH4* | H4 receptor | 37 °C | 4 hours |
| Hydroxycarboxylic acid receptors | *GPR109A (HCAR2)* | HCA2 receptor | 37 °C | 2 hours |
| *GPR109B (HCAR3)* | HCA3 receptor | 37 °C | 2 hours |
| Kisspeptin receptor | *KISS1R* | kisspeptin receptor | 37 °C | 2 hours |
| Leukotriene receptors | *LTB4R* | BLT1 receptor | 37 °C | 2 hours |
| *OXER1* | OXE receptor | RT | 2 hours |
| Lysophospholipid (LPA) receptors | *EDG4 (LPAR2)* | LPA2 receptor | RT | 3 hours |
| *EDG7 (LPAR3)* | LPA3 receptor | RT | 3 hours |
| *GPR92 (LPAR5)* | GPR92 receptor (LPA5 receptor*)* | RT | 3 hours |
| Lysophospholipid (S1P) receptors | *EDG1 (S1PR1)* | S1P1 receptor | 37 °C | 2 hours |
| *EDG3 (S1PR3)* | S1P3 receptor | 37 °C | 2 hours |
| *EDG5 (S1PR2)* | S1P2 receptor | 37 °C | 2 hours |
| *EDG6 (S1PR4)* | S1P4 receptor | 37 °C | 4 hours |
| Melanin-concentrating hormone receptors | *MCHR1* | MCH1 receptor | 37 °C | 2 hours |
| *MCHR2* | MCH2 receptor | 37 °C | 2 hours |
| Melanocortin receptors | *MC1R* | MC1 receptor | 37 °C | 2 hours |
| *MC3R* | MC3 receptor | 37 °C | 2 hours |
| *MC4R* | MC4 receptor | 37 °C | 2 hours |
| *MC5R* | MC5 receptor | 37 °C | 2 hours |
| Melatonin receptor | *MTNR1A* | MT1 receptor | 37 °C | 2 hours |
| Motilin receptor | *MLNR* | motilin receptor | 37 °C | 2 hours |
| Neuromedin U receptor | *NMU1R* | NMU1 receptor | 37 °C | 2 hours |
| Neuropeptide B and W receptors | *NPBWR1* | NPBW1 receptor | RT | 2 hours |
| *NPBWR2* | NPBW2 receptor | 37 °C | 2 hours |
| Neuropeptide FF and AF receptor | *NPFFR1* | NPFF1 receptor | 37 °C | 2 hours |
| Neuropeptide S receptor | *NPSR1b (NPSR1)* | NPS receptor | 37 °C | 2 hours |
| Neuropeptide Y receptors | *NPY1R* | Y1 receptor | RT | 2 hours |
| *NPY2R* | Y2 receptor | 37 °C | 2 hours |
| *PPYR1 (NPY4R)* | Y4 receptor | 37 °C | 2 hours |
| Neurotensin receptors | *NTSR1* | NTS1 receptor | 37 °C | 2 hours |
| Opioid receptors | *OPRD1* | δ receptor | RT | 2 hours |
| *OPRK1* | κ receptor | 37 °C | 2 hours |
| *OPRL1* | NOP receptor | RT | 2 hours |
| *OPRM1* | μ receptor | RT | 2 hours |
| Orexin receptors | *HCRTR1* | OX1 receptor | 37 °C | 2 hours |
| *HCRTR2* | OX2 receptor | 37 °C | 2 hours |
| P2Y receptors | *P2RY1* | P2Y1 receptor | RT | 2 hours |
| *P2RY11* | P2Y11 receptor | 37 °C | 2 hours |
| *P2RY12* | P2Y12 receptor | RT | 2 hours |
| *P2RY2* | P2Y2 receptor | 37 °C | 2 hours |
| *P2RY4* | P2Y4 receptor | 37 °C | 2 hours |
| *P2RY6* | P2Y6 receptor | 37 °C | 2 hours |
| Parathyroid hormone receptors | *PTHR1 (PTH1R)* | PTH1 receptor | 37 °C | 2 hours |
| *PTHR2 (PTH2R)* | PTH2 receptor | 37 °C | 2 hours |
| Peptide P518 receptor | *GPR103 (QRFPR)* | QRFPR receptor | RT | 2 hours |
| Platelet-activating factor receptor | *PTAFR* | PAF receptor | 37 °C | 2 hours |
| Prokineticin receptors | *PROKR1* | PKR1 receptor | 37 °C | 2 hours |
| *PROKR2* | PKR2 receptor | 37 °C | 2 hours |
| Prolactin-releasing peptide receptor | *PRLHR* | PRRP receptor (PrRP receptor) | 37 °C | 2 hours |
| Prostanoid receptors | *CRTH2 (PTGDR2)* | PTGDR2 receptor (DP2 receptor) | RT | 2 hours |
| *PTGER2* | EP2 receptor | 37 °C | 2 hours |
| *PTGER3* | EP3 receptor | 37 °C | 2 hours |
| *PTGER4* | EP4 receptor | 37 °C | 2 hours |
| *PTGFR* | FP receptor | 37 °C | 2 hours |
| *PTGIR* | IP1 receptor (IP receptor) | 37 °C | 2 hours |
| *TBXA2R* | TP receptor | 37 °C | 2 hours |
| Protease activated receptors | *F2R* | PAR1 | RT | 2 hours |
| *F2RL1* | PAR2 | RT | 2 hours |
| *F2RL3* | PAR4 | 37 °C | 2 hours |
| Relaxin family peptide receptor | *RXFP3* | RXFP3 | 37 °C | 2 hours |
| Somatostatin receptors | *SSTR1* | SST1 receptor | 37 °C | 2 hours |
| *SSTR2* | SST2 receptor | 37 °C | 2 hours |
| *SSTR3* | SST3 receptor | 37 °C | 2 hours |
| *SSTR5* | SST5 receptor | 37 °C | 2 hours |
| Tachykinin receptors | *TACR1* | NK1 receptor | 37 °C | 2 hours |
| *TACR2* | NK2 receptor | 37 °C | 2 hours |
| *TACR3* | NK3 receptor | 37 °C | 2 hours |
| Thyrotropin-releasing hormone receptor | *TRHR* | TRH1 receptor | 37 °C | 2 hours |
| Urotensin receptor | *UTR2 (UTS2R)* | UT receptor | RT | 2 hours |
| Vasopressin and oxytocin receptors | *AVPR1A* | V1A receptor | RT | 2 hours |
| *AVPR1B* | V1B receptor | RT | 2 hours |
| *AVPR2* | V2 receptor | RT | 2 hours |
| *OXTR* | OT receptor | 37 °C | 2 hours |
| VIP and PACAP receptors | *ADCYAP1R1* | PAC1 receptor | 37 °C | 2 hours |
| *VIPR1* | VPAC1 receptor | RT | 2 hours |
| *VIPR2* | VPAC2 receptor | 37 °C | 2 hours |

Note: This table refers to receptor nomenclature at the time of assay performance. Information in parentheses refers to any updates in nomenclatures per IUPHAR guidelines (2).

DHE = dihydroergotamine mesylate; IUPHAR = International Union of Basic and Clinical Pharmacology; LPA = lysophosphatidic acid; NA = not applicable; S1P = sphingosine-1 phosphate; PACAP = pituitary adenylate cyclase-activating peptide; RT = room temperature; VIP = vasoactive intestinal peptide.

1. Eurofins. GPCR Assay Formats and Services Offered. <https://www.discoverx.com/services/drug-discovery-development-services/gpcr-screening-profiling-services/gpcrscan-gpcr-profiling/gpcrmax>. Accessed October 27, 2022.

2. IUPHAR GPCR nomenclature. <https://www.guidetopharmacology.org/GRAC/GPCRListForward?class=A>. Accessed October 18, 2023.