|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **pEC50** | **Emax** | ***n*** |
| **Arrestin recruitment (HEK)** | M1-WT | 5.7 ± 0.1 | 104 ± 1 | *5* |
| M1-PD | 5.4 ± 0.1 | 40.7 ± 2.4\*\*\* | *5* |
| **Internalisation (HEK)** | M1-WT | 6.4 ± 0.1 | 92.5 ± 2.1\*\*\* | *5* |
| M1-PD | 5.8 ± 1.9 | 22.5 ± 5.4 | *5* |
| **Gq activation (HEK)** | M1-WT | 6.4 ± 0.1 | 100.0 ± 0.0 | *5* |
| M1-PD | 7.6 ± 0.1\*\*\* | 108.0 ± 7.5 | *5* |
| **IP1 (HEK)** | M1-WT | 6.4 ± 0.1 | 107.5 ± 7.3 | *7* |
| M1-PD | 6.7 ± 0.2 | 98.6 ± 7.3 | *5* |
| **IP1 (neurons)** | M1-WT | 4.8 ± 0.2 | 96.3 ± 9.5 | *3* |
| M1-PD | 4.9 ± 0.1 | 89.6 ± 4.3 | *3* |

**Tables**

**Table 1 M1-PD shows equivalent G protein-mediated responses compared to M1-WT, but impaired arrestin recruitment and receptor internalisation.** Potency and maximum effect of agonist stimulated β-arrestin-2 recruitment, receptor translocation to early endosomes, Gq recruitment and IP1 accumulation at M1-WT or M1-PD receptors. Agonists used for HEK cells and neurons were ACh or CCh, respectively. Data are expressed as the means ± S.E.M. of 3-7 independent experiments. \*\*\* Indicates p<0.001 compared to WT.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Gq activation** | | **Arrestin recruitment** | | **Log Bias Factor**  **Gq – Arrestin** |
|  | **Log10(τ/KA)** | **ΔLog10(τ/KA)** | **Log10(τ/KA)** | **ΔLog10(τ/KA)** | **ΔΔLog10(τ/KA)** |
| **M1-WT** | 6.4 ± 0.08 | 0.00 ± 0.11 | 6.12 ± 0.29 | 0.00 ± 0.40 | 0.00 ± 0.42 |
| **M1-PD** | 7.36 ± 0.10 | 0.95 ± 0.12 | 5.76 ± 0.13 | -0.36 ± 0.32 | 1.32 ± 0.34 |

**Table 2 Bias calculations for Gq activation or arrestin recruitment at the M1-PD receptor.** M1-PD receptors expressed in HEK cells show preferential signalling bias (ΔΔlog10(τ/KA)) towards Gq activation versus arrestin recruitment, using BRET biosensors. Data are presented as means ± S.E.M., with M1-WT receptors used as the reference. Statisical analysis carried out by t-test comparing Log10(τ/KΑ) values between M1-WT and M1-PD, indicates a statistical difference in Gq activationp<0.001.