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Agonist-dependent changes in the membrane organisation of the μ-opioid receptor in HEK293 cells

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The μ-opioid receptor (MOR) is a member of the opioid receptor family, known to be important in mediating the effects of analgesics such as morphine. Previous studies have shown that the mechanism of desensitisation and internalisation of the MOR by agonists such as morphine and DAMGO may be agonist-dependent (Johnson *et al.*, 2006). We have investigated this effect at the level of MOR membrane organisation using fluorescence correlation spectroscopy (FCS) to monitor changes in receptor diffusion and clustering following agonist stimulation.

Stable transfects of HEK293T cells expressing the human MOR tagged on its C-terminus with eGFP (HEK-MOR-GFP) were seeded onto 8-well chambered coverglasses in Dulbecco's Modified Eagle's Medium containing 10% foetal calf serum and 2 mM glutamine. Cells were washed twice in Hank's Buffered Saline Solution (HBSS), before exposure to receptor-saturating concentrations of morphine (30 μM) or DAMGO (10 μM) for 10 min at 37°C. FCS measurements were taken at 24°C on the upper cell membrane for 30s using a Zeiss Confocor 3 microscope, as previously described (Briddon *et al.*, 2004). Autocorrelation and photon counting histogram (PCH) analysis was performed using Zeiss AIM4.2 software, and data expressed as mean±s.e. mean of 'n' cells, from at least 3 experiments. Data were analysed by one-way ANOVA with post-hoc Dunn's test.

In HEK293T cells, in the absence of agonist, the MOR-GFP construct was expressed predominantly at the cell membrane. Exposure of HEK MOR-GFP cells to DAMGO (10 μM, 10min) but not morphine (30 μM, 10min) caused a significant re-distribution of the MOR from the membrane to punctuate cytoplasmic vesicles. FCS measurements indicated that stimulation by DAMGO caused a significant slowing of MOR diffusion (diffusion coefficient, D=0.29±0.04 and 0.10±0.02 $\mu m^2/s$, n=28 and 43, control and DAMGO, respectively; $P<0.01$). In contrast, morphine had no significant effect on receptor diffusion (D=0.25±0.03 $\mu m^2/s$, n=32). Exposure to either agonist did not significantly change the particle number (N=72±7, 74±7 and 66±6 μm^{-2}, control, morphine and DAMGO treated, respectively). Receptor clustering (as indicated by PCH analysis) was similar in control and morphine-exposed cells (molecular brightness, μ=16.3±0.9 and 16.2±1.7kHz counts per molecule s^{-1}, n=44 and 32, respectively). A component of similar brightness (μ=14.3±2.2kHz, n=32) was found in cells exposed to DAMGO. However, in 48% of these cells, a second substantially brighter component, consistent with clustering of receptors, was also found (μ=135±23kHz).

These data suggest that a short exposure to DAMGO causes significant changes in membrane organisation of the MOR, which may include clustering of the receptor prior to internalisation. Such changes are not seen following morphine exposure. This is consistent with previously reported differences in MOR desensitisation and internalisation seen with these two ligands.

Briddon, S.J. *et al.* (2004) Proc. Natl. Acad. Sci USA 101, 4673-4678.

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