μ-OPIOID RECEPTOR DESENSITIZATION IN HEK 293 CELLS

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In HEK 293 cells, stably transfected with MOR1 receptor cDNA, a number of MOR agonists such as DAMGO, produce rapid MOR internalization, whereas morphine causes very little rapid MOR internalization (Bailey et al., 2003; Johnson et al., 2004). In the present study we have now investigated the desensitization of MORs in response to morphine and DAMGO in HEK 293 cells.

HEK 293 cells were stably transfected with T7-epitope tagged receptors of the MOR1 subtype (radioligand binding using [^{3}H]-diprenorphine showed the receptor density to be 174.8 ± 27.6 fmol mg^{-1}) and transiently transfected with both the Kir3.1 and Kir3.2A subunits of the G protein activated inwardly rectifying K^+ (GIRK) channel. MOR1 mediated GIRK channel activation was measured using the whole-cell patch clamp technique. Cells were kept in high (50mM) K^+ extracellular solution, and held at the K^+ equilibrium potential (-25mV). The holding potential was stepped to –60mV for 100ms every 5s and the current at the end of each step was recorded. In this way the inward K^+ current seen upon MOR activation could be measured, whilst K^+ accumulation inside the cell, which could result in run down of the current, was kept to a minimum. Agonists were applied for 10min, by which point the desensitization of the current response had reached a plateau. Desensitization was calculated by normalizing the current at the end of the 10min drug application to the peak current. Data are shown as mean ± S.E.M of % desensitization and were compared using unpaired Student's t-tests.

A receptor saturating concentration of morphine (30μM) elicited a peak current of 236 ± 80pA which rapidly desensitised by 80 ± 3% (n=4) over 10min. A receptor saturating concentration of DAMGO (10μM) elicited a peak current of 314 ± 95pA, which desensitized by 76 ± 3% (n=4) over 10 min. The desensitization in response to morphine and DAMGO was not significantly different ($p>0.05$). In addition, desensitization to submaximal agonist concentrations was studied. The responses to 1μM morphine or 1μM DAMGO desensitized by 59 ± 9% (n=4) and 50 ± 7% (n=4) respectively. As with experiments using receptor saturating agonist concentrations, the desensitization produced by 1μM morphine and DAMGO was not significantly different ($p>0.05$).

These results show that morphine and DAMGO cause equivalent levels of MOR desensitization in HEK 293 cells. This is in contrast to our previous work in rat locus coeruleus neurones where DAMGO, but not morphine, caused MOR desensitization (Bailey *et al*., 2003), and is also in contrast to the findings of Whistler *et al*, (1999), in which morphine was unable to induce desensitization in HEK 293 cells. However, these findings are consistent with a study in AtT20 cells where morphine- and DAMGO-mediated inhibition of Ca^+ channels desensitized to the same extent (Borgland *et al.*, 2003). In our previous studies on MOR internalization in HEK 293 cells, morphine caused significantly less MOR internalization than DAMGO (Bailey *et al*., 2003; Johnson *et al*., 2004). Our results show that the abilities of MOR agonists to cause desensitization and internalization do not necessarily correlate, and that the ability of morphine to induce MOR desensitization varies according to cell type.

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