Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol12Issue3abst245P.pdf

Characteristics of ERK1/2 activation by different μ-opioid receptor agonists

Elena Tsisanova, Eamonn Kelly, Graeme Henderson. [1]University of Bristol, Bristol, UK, [2]University of Bristol, Bristol, UK, [3]University of Bristol, Bristol, UK

In the current work the ability of μ-opioid receptor (MOPr) agonists with varying efficacy at MOPr (DAMGO, morphine, etorphine and oxycodone) to induce ERK1/2 activation was investigated. The effects of different kinase inhibitors on ERK1/2 signalling induced by these agonists was also studied.

For ERK activation HEK293-MOPr-expressing cells were pretreated for 5 minutes with agonist alone or with a combination of agonist and the CaMKII inhibitor KN93 (10 μM), the PI3 kinase inhibitor Wortmannin (0.1 μM), or the Src inhibitor PP1 (1 μM) in serum free media. Cell lysates were resolved by SDS-gel electrophoresis and Western blotting. The amount of phosphorylated ERK was monitored with a monoclonal antibody for phosphorylated ERK (P-p-44/42 MAPK anti-rabbit Ab) and was normalized to total ERK using an ERK antibody (p-44/42 MAPK anti-mouse Ab) (1). Bands on blots were analysed using ImageJ software. MOPr trafficking experiments were performed using primary monoclonal HA.11 mouse Ab clone 16B12 and anti-mouse AlexaFluor 488 secondary Ab. Phospho-ERK1/2 activation-immunofluorescence experiments were undertaken using an ERK antibody (p-44/42 MAPK anti-mouse Ab) and anti-rabbit AlexaFluor 555 secondary antibody. To visualize the nucleus, Hoechst 33258, pentahydrate (bis-Benzimide) was used.

Initial Western blotting experiments indicated that agonist-induced ERK activation peaked at 5-10 min. Following PTX treatment (100 ng/ml, overnight), ERK activation was decreased to the same extent (60-70% inhibition) for all agonists. Pretreatment with the PKC inhibitor Ro320432 (1 μM; 90 min) also inhibited agonist-induced ERK activation, but the extent of the inhibitory effect was agonist-dependent. Whereas DAMGO-induced ERK activation was inhibited by 24% ($p<0.05$), Endomorphin-2-induced activation was inhibited by 45% ($p<0.05$) and morphine-induced activation by 73% ($p<0.05$). Pretreatment with KN-93 inhibited ERK activation by all agonists to the same degree (~75 ± 1.1%, $p<0.05$). Pretreatment with wortmannin or PP1 also inhibited ERK activation by all agonists, but the extent of inhibition for each inhibitor was less (~70 ± 2.5% in case of wortmannin and ~30 ± 2.5%, $p<0.05$) than that seen with KN-93. These results suggest that, provided the kinase inhibitors are selective, a surprising number of kinases are involved in the MOPr-ERK signalling pathway in HEK293 cells. Internalization studies using immunofluorescence imaging revealed that high efficacy agonists DAMGO and etorphine induce more MOPr internalization than morphine and oxycodone which have lower efficacy. Cell imaging experiments of ERK1/2 activation revealed that following pretreatment with DAMGO or etorphine, phosphorylated ERK1/2 located more to the nucleus than in cytoplasm, whereas phosphorylated ERK1/2 following morphine and oxycodone treatment localized predominately to the cell cytoplasm. These differences may in part explain the different functional profiles of these drugs when administered *in vivo*.

1. Zheng, H et al (2007). Mol Pharm73(1), 178-190