Phosphorylation of the mu-opioid receptor by CaMKII and agonist-induced desensitization

Ying-Ju Chen, Eamonn Kelly, Graeme Henderson. University of Bristol, Physiology and Pharmacology, BS8 1TD, United Kingdom.

Several kinases have been proposed to be involved in agonist-induced desensitization of the mu-opioid receptor (MOPr)(ref). These include G-protein-coupled receptor kinase (GRK), protein kinase C (PKC) and calcium/calmodulin-dependent kinase II (CaMKII). In this study we have sought to identify the target amino acid residues for CaMKII on the intracellular loops (IL) and C terminal tail (CT) of MOPr and then to determine the role of this kinase in agonist-dependent MOPr desensitization.

An in vitro $^{32}$P-incorporation assay was used to identify potential CaMKII phosphorylation site(s) on GST fusion proteins of the intracellular regions of MOPr. To examine MOPr desensitization the human/rat MOPr was stably expressed in AtT20 cells. Whole cell patch clamp recordings of MOPr agonist-activated G-protein-activated inwardly rectifying potassium channel (GIRK) currents were made as previously described (Johnson et al., 2006). The decay of the agonist-activated current provides a real-time measure of receptor desensitization. To examine agonist-induced loss of cell surface MOPr in AtT20 cells an ELISA assay was used (Johnson et al., 2006).

GST fusion proteins of both the 3rd IL and CT of MOPr were phosphorylated in the presence of CaMKII. When Ser$^{261}$ or Ser$^{266}$ in the 3rd IL, or Thr$^{370}$ in the CT were mutated to Ala the degree of phosphorylation by CamKII was reduced by 51±6 %, 39±4 %, and 28±2 %, respectively. These data indicate that CaMKII can phosphorylate specific residues on GST fusion proteins containing the 3rd IL and CT of MOPr.

In AtT20 cells stably expressing wild type MOPr the CaMKII inhibitor KN93 (50 µM, applied to the cells via the pipette filling solution) reduced the desensitization induced by DAMGO (10 µM) from 65.6± 0.8% to 44.7± 1%, and by morphine (30 µM) from 63.2± 0.8% to 42.6 ± 1.1 % (n = 4 for each), respectively whereas the inactive analogue KN 92 (50 M) had no effect. In AtT-20 cells stably expressing a mutant MOPr in which the three putative CaMKII sites (Ser$^{261}$, Ser$^{266}$ and Thr$^{370}$) had been mutated to Ala only DAMGO-induced desensitization was reduced from 31.2± 0.9 % to 45% ± 0.9 % whereas morphine-induced desensitization was unaffected (n = 5 for both). Exposure to DAMGO (10 µM) resulted in a 52 ± 10 % (n = 6) loss of surface receptors, but this was reduced to 17 ± 16% loss (n = 6) when the cells were pre-treated with KN93 (3 µM, applied in the bathing solution 1 hour before DAMGO treatment).

These results demonstrate that CaMKII can phosphorylate the MOPr, and that procedures designed to prevent CaMKII-dependent phosphorylation reduce both MOPr desensitization and internalization.