078P POSSIBLE INVOLVEMENT OF PROTEIN KINASE C AND PHOSPHOLIPASE D IN μ-OPIOID RECEPTOR INTERNALIZATION IN HEK 293 CELLS

Elizabeth A. Johnson, Eamonn Kelly, Christopher P. Bailey & Graeme Henderson.
Department of Pharmacology, University of Bristol, Bristol BS8 1TD.

μ-Opioid receptors (MORs) exhibit rapid desensitization and internalization on exposure to some opioid agonists, but not to morphine (Bailey *et al.*, 2003a). We have recently shown that in mature rat locus coeruleus neurones, activation of protein kinase C (PKC) converts morphine into a MOR desensitizing agonist (Bailey *et al.*, 2003b). In this study we have investigated the role of protein kinase C (PKC) and phospholipase D2 (PLD2) in MOR internalization.

HEK 293 cells stably expressing T7-epitope tagged receptors of the MOR1 subtype (HEK 293MOR1) were used throughout. Internalization was measured by ELISA using a colourimetric alkaline phosphatase assay (Bailey *et al.,*2003a). Data are shown as means ± S.E.M of % internalization and were compared using unpaired Student's t-test.

A receptor saturating concentration of the opioid agonist morphine (30μM) produced only a low level of MOR1 internalization after 30 min (7 ± 1%, n=8). Phorbol 12-myristate 13-acetate (PMA, 1μM), an activator of PKC, itself caused MOR1 internalization (23 ± 2%, n=8) in the absence of any opioid agonist. This effect of PMA was antagonised by the PKC inhibitor GF 109203X but not chelerythrine (Johnson *et al*., 2003). PMA however, did not enhance morphine-induced MOR1 internalization (29 ± 2%, n=8) when these drugs were applied together.

Western blot analysis confirmed the presence in HEK 293MOR1 cells of all the conventional and novel isoforms of PKC except for PKCγ, the predominately neuronal isoform of PKC (Battaini., 2001). Transient expression of PKCγ in HEK 293MOR1 cells reduced PMA-induced MOR1 internalization (13 ± 3%, n=4, $p<0.05$) but not morphine-induced internalization (5 ± 2%, n=4, $p>0.05$). Also, PKCγ expression did not enhance MOR1 internalization in the presence of morphine and PMA (25 ± 1%, n=5, $p>0.05$). Transient expression of a dominant negative PKCγ mutant (K380R) had no effect on the internalization of MOR1 induced by morphine (6 ± 1%, n=4, $p>0.05$), PMA (26 ± 4%, n=4, $p>0.005$), or both drugs applied together (24 ± 1%, n=4, $p>0.05$).

Koch *et al,* (2003) suggested a requirement for PLD2 in morphine-induced MOR1 internalization. Expression of wild type PLD2 in HEK 293MOR1 cells had no effect on morphine-induced MOR1 internalization (8 ± 2%, n=4, $p>0.05$) and did not reveal any additional PKC-induced internalization when morphine and PMA were applied together (26 ± 3%, n=4, $p>0.05$).

These results suggest that in the absence of an opioid agonist MOR1 internalization can be enhanced by PKC activation. However, activation of PKC or PLD does not enhance morphine-induced MOR1 internalization in HEK 293MOR1 cells.

Bailey, C.P. *et al*., (2003a) *J. Neurosci.* 23, 10515-10520
Bailey, C.P. *et al*., (2003b) *PA_2 online,* Vol 1, Issue4, 180P
Koch, T. *et al.,* (2003) *J. Biol. Chem*. 278, 9979-85
Battaini, F., (2001) *Pharmacol. Res.* 44, 353-361
Johnson, E.A. (2003) *PA_2 online,* Vol 1, Issue4, 056P