**010P ASSEMBLY AND CELL SURFACE EXPRESSION OF KA-2 SUBUNIT-CONTAINING KAINATE RECEPTORS IN TRANSFECTED CELLS**

Gallyas, F. Jr. and Molnar, E. *(Introduced by Jane, D. E.)*

MRC Centre for Synaptic Plasticity, Department of Anatomy, School of Medical Sciences, University of Bristol, Bristol BS8 1TD

Kainate sensitive ionotropic glutamate receptors (KARs) are comprised of five distinct subunits (GluR5-7, KA-1/2). While some of these subunits can form functional homomeric receptors when expressed in cultured cells, most native receptor complexes found in brain are made up of two or more distinct subunits. Since the KA-2 KAR subunit is not functional when expressed alone but forms functional ion channels when expressed in combination with GluR5-7 subunits, it represents a useful marker for studying the assembly and surface targeting of heteromeric kainate receptors.

**AIM OF THE STUDY:** Identify key factors in the assembly process and cell surface targeting of KA-2 subunit containing heteromeric KARs.

HEK-293 cells were transiently transfected with various combinations of KAR (KA-2, GluR5-7) and other ionotropic glutamate receptor (GluR1, NR1) subunits using SuperFect (Promega) transfection system according to the manufacturer’s protocol. Two days after the transfection, the cell surface proteins were biotin labelled. Solubilised biotinylated (surface exposed) proteins were separated by streptavidin-agarose beads from intracellular protein components. The presence of glutamate receptor subunits in various fractions were assessed using immunoblotting with subunit specific antibodies to KA-2, GluR6/7, GluR1-4 and NR1. The β-tubulin content of each fraction was analysed to confirm the integrity of the cells during biotinylation.

Cells transfected with one glutamate receptor subunit alone or in combination with KA-2 produced essentially the same amounts of subunit proteins. None of the streptavidin-agarose isolated biotin-labelled samples contained β-tubulin, indicating that there was no contamination of the surface exposed protein samples with intracellular proteins. When expressed alone, KA-2 subunit was identified exclusively in intracellular membrane compartments, while this subunit was not detectable on the cell surface. The same pattern of distribution was observed when KA-2 was co-expressed with either NR1 or GluR1 subunits. In contrast, KA-2 was present both on the cell surface and in intracellular compartments when it was co-expressed with either GluR5, GluR6 or GluR7 KAR subunits. When expressed alone, GluR6 and GluR7 subunits showed positive immunoreaction in both surface expressed and intracellular protein fractions with the anti-GluR6/7 antibody. In contrast, the same subunits with an N-terminal myc-tag were only present in the intracellular protein fractions suggesting that the modification of their extracellular domain interfered with the normal assembly and/or cell surface targeting of these subunits.

The cell surface expression of the KA-2 subunit depends on the assembly with other KAR subunits. The modification of the N-terminal domain interfered with the cell surface expression and assembly of GluR6 and GluR7 containing KARs.