011P ACTIVATION OF EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK) AND C-JUN N-TERMINAL KINASE (JNK) BY GROUP I METABOTROPIC GLUTAMATE RECEPTORS

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Group I metabotropic glutamate receptors have been shown to activate extracellular signal-regulated kinase (ERK), a member of the mitogen-activated protein kinase (MAPK) family in native preparations (Peavy & Conn, 1999) and in model cell systems (Ferraguti et al., 1999; Thandi et al., 2002). The MAPK family also includes the stress-activated c-Jun N-terminal kinases (JNKs) and p38 kinases. The aim of this study was to investigate the activation of JNK by mGlu1a and mGlu5a receptors and compare this to the profile of ERK activation in the same cell background.

Chinese hamster ovary (CHO) cells expressing either human mGlu1a or mGlu5a receptors under the control of an inducible expression system were used as a model. Confluent cells were incubated in Krebs-Henseleit buffer (containing 5mM pyruvate and 3 units ml^{-1} of glutamic pyruvic transaminase), stimulated using quisqualate and then solubilized for use in *in-vitro* immunocomplex kinase assays to determine ERK and JNK activities (Wylie et al. 1999). Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple-range test at $p<0.05$ using Prism III software. Data are means ± s.e.m, n=3-5.

Both mGlu1a and mGlu5a elicited concentration-dependent, (pEC$_{50}$s, 5.78 ± 0.13 and 6.04 ± 0.15), and time-dependent increases in ERK activity. Maximal ERK activity occurred at 5 min and then declined towards basal at 30min.

The magnitude of the peak response was 4.9 ± 0.2 and 4.8 ± 0.4 fold-over-basal for mGlu1a- and mGlu5a receptor-expressing cells, respectively. The ERK response stimulated by mGlu1a was significantly attenuated by pertussis toxin (PTx; 100 ng ml^{-1}, 24 h) pre-treatment (by 80 ± 5%), whereas that seen in CHO-mGlu5a cells was unaffected by PTx. The mGlu1a, but not the mGlu5a receptor, also activated JNK activity in a time-dependent manner. Maximal JNK activation occurred at 30 min and declined towards basal at 2-3 h (peak response, 4.7 ± 0.13 fold-over-basal). JNK activation was unaffected by PTx pre-treatment. The JNK response was unaffected by the MEK1 inhibitor U0126 (1 μM), and both the ERK and JNK responses in both CHO-mGlu1a and -mGlu5a cells were unaffected by removal of intracellular Ca^{2+} by the addition of thapsigargin (2μM) and/or extracellular Ca^{2+} by placement in a calcium-free buffer with 100 μM EGTA.

The results suggest that these closely related mGlu receptors can couple to different classes of G protein to elicit ERK activation. Only the mGlu1a receptor significantly increases JNK activity in the CHO cell background, and in this case the response is PTx-insensitive suggesting diverging signalling pathways to ERK and JNK for the mGlu1a receptor.

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