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Kainate-Receptor Assay

Overview Sheet

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1. GLUTAMATE DEPENDENCE

Briefly, CHO cells were transfected with GluK2 or GluK2/5 and split for electrophysiological experiments after 24-48 h. During the electrophysiological experiment they were continuously perfused with buffer solution: 137 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Glucose, 10 mM HEPES. pH 7.4 (NaOH). Pipette was filled with a solution containing: 130 mM KCl, 1 mM $MgCl_2$, 5 mM EGTA, 10 mM HEPES, pH 7.2 (KOH). After the seal was obtained cells were clamped to -80 mV. GluK2 channels were activated with increasing concentrations of Glutamate 10 µM - 3000 µM (application interval: 30 s).
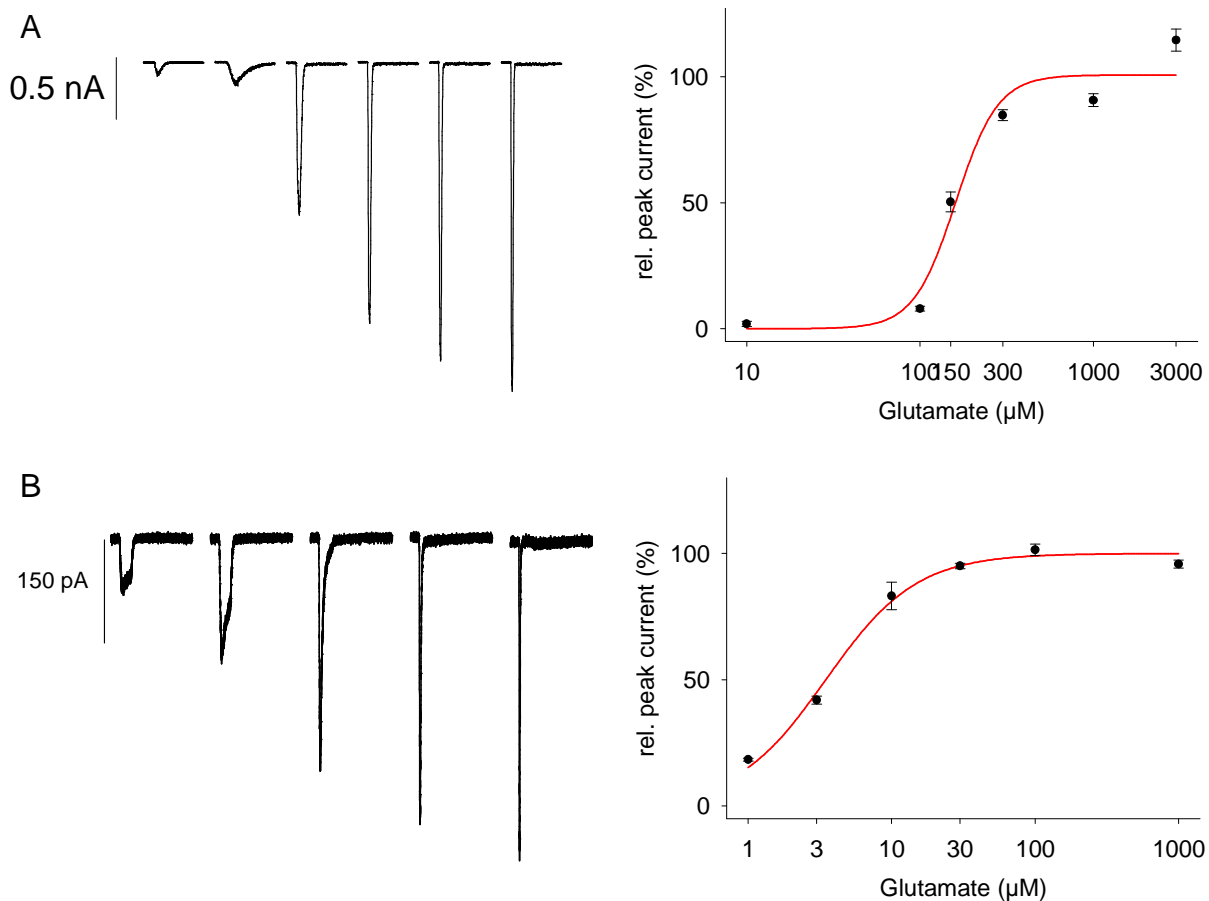

Figure 1: Effect of Glutamate on (A) GluK2 homomeric and (B) heteromeric GluK2/5 channels.

Table 1: Glutamate Dependence.

Receptor composition	EC_{50}	Hill coefficient
GluK2 homomer	159.32 ± 7.32 µM	3.58
GluK2 / GluK5 heteromer	3.47 ± 0.63 µM	1.37

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2.   ZK200775, A COMPTITIVE ANTAGONIST

ZK200775 is a competitive antagonist for that displays anxiolytic, anticonvulsant and muscle relaxant activity *in vivo*. Concentrations between 1.0 and 400 µM were tested in the presence of 80 µM Glutamate for GluK2 or 5.0 µM for GluK2/5.
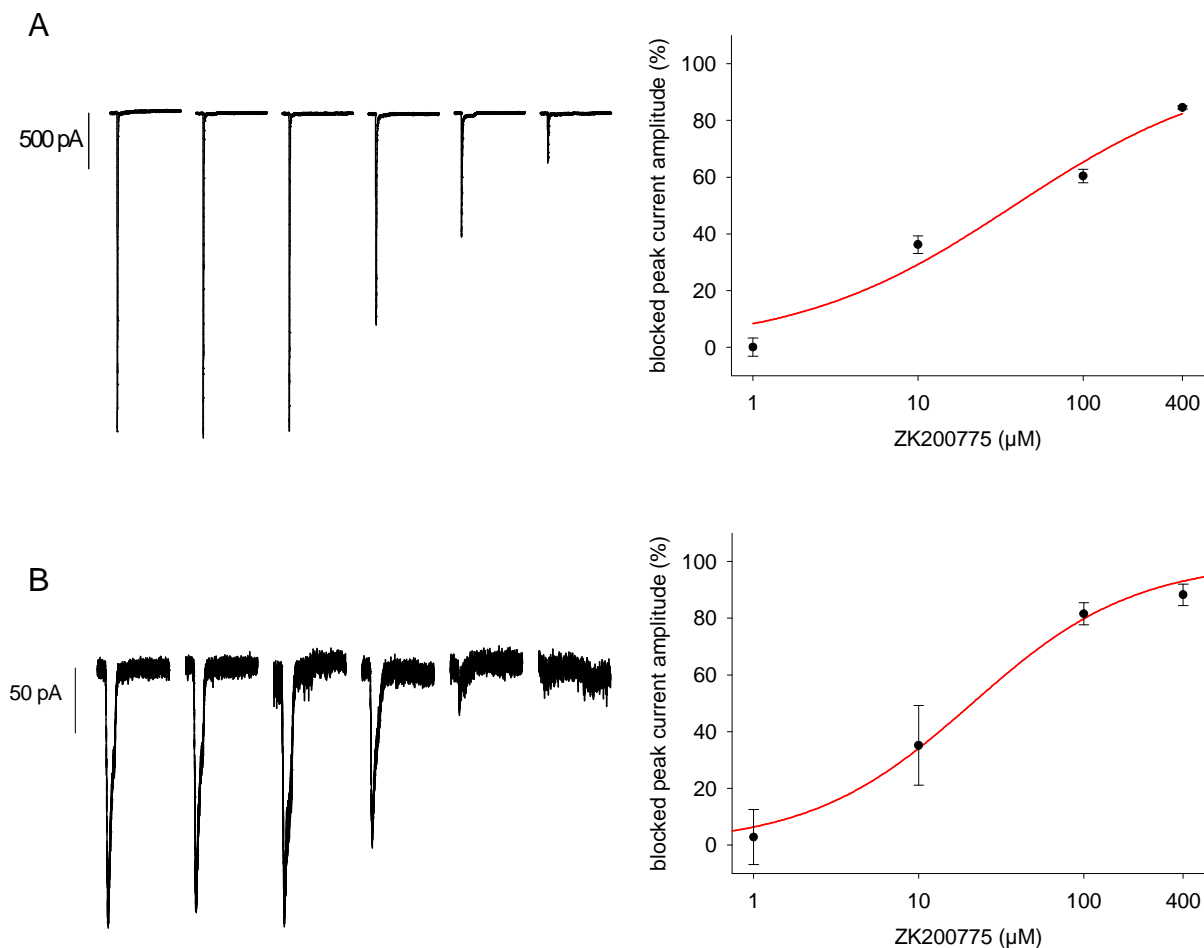

Figure 2: Effect of ZK200775 on (A) GluK2 homomeric and (B) heteromeric GluK2/5 channels. Application scheme: Glutamate, Glutamate, Glutamate + 1.0 µM ZK200775, Glutamate + 10 µM ZK200775, Glutamate + 100 µM ZK200775, Glutamate + 400 µM ZK200775

Table 2:

Receptor composition	IC$_{50}$	Hill coefficient
GluK2 homomer	38.24 µM	0.66
GluK2 / GluK5 heteromer	21.12 µM	0.88

3. CONTACT INFORMATION

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