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Excitotoxic injury profiles of low-affinity kainate receptor agonists in cortical neuronal cultures

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Abstract

Neurotoxic profiles of putative agonists for low-affinity kainate subtypes of L-glutamate receptors (GluR5-7) were determined in cultured cortical neurones. Rank order of neurotoxic potency (μM): (S)-5-iodowillardiine (9) ≈ (2*S*,4*R*,6*E*)-2-amino-4-carboxy-7-(2-naphthyl)hept-6-enoic acid (LY339434, 11) > (2*S*,4*R*)-4-methylglutamate (33) > kainate (100) > (*RS*)-2-amino-3-(hydroxy-5-*tert*-butylisoxazol-4-yl)propanoic acid (ATPA, 360). Using ionotropic glutamate receptor antagonists, neurotoxicity induced by kainate, ATPA and (*S*)-5-iodowillardiine appeared to involve a GluR5-7 component, unlike LY339434 and (2*S*,4*R*)-4-methylglutamate. These putative GluR5-7 agonists exhibited complex excitotoxic profiles highlighting the importance of studying native glutamate receptors.

Keywords: Kainate receptor, low-affinity; Cortical neurone, cultured; Excitotoxicity

L-Glutamate (Glu)-induced excitotoxicity is thought to be involved in the pathology of numerous neurological disorders and is mediated by various glutamate receptors, including kainate (KA) receptors of both high- (KA1-2) and low-affinity (GluR5-7) (review: Chittajallu et al., 1999). Based on evidence from investigations employing recombinant receptors, recently novel glutamate analogues (*RS*)-2-amino-3-(hydroxy-5-*tert*-butylisoxazol-4-yl)propanoic acid (ATPA), (*S*)-5-iodowillardiine, (2*S*,4*R*,6*E*)-2-amino-4-carboxy-7-(2-naphthyl)hept-6-enoic acid (LY339434) and (2*S*,4*R*)-4-methylglutamate, with selectivity for low-affinity kainate receptors, have been employed to examine the functional importance of GluR5-7 (Clarke et al., 1997; Jane et al., 1997; Small et al., 1998). GluR5-7 appear widely distributed through the primate neuroaxis (Carroll et al., 1998; Chittajallu et al., 1999) and are likely to play important roles in cognitive defects, epilepsy and neuropathologies (Bleakman and Lodge, 1998). As yet the activity of these novel ionotropic glutamate receptor agonists has not been fully evaluated at native glutamate receptors. This study describes the first evaluation of the neurotoxic profile of this group of putative GluR5-7 agonists relative to kainate at native receptors present in cultured cortical neurones by employing various ionotropic glutamate receptor antagonists.

Primary cortical cultures (> 95% neurones) were prepared from embryonic (days 14–16) C57Bl/6 mice and maintained as previously described (Cheung et al., 1998). Immunocytochemistry with an antibody recognizing GluR6/7 (Upstate Biotechnology, Lake Placid, U.S.A) demonstrated widespread neuronal localisation of low-affinity kainate receptors (data not shown). At day 6 in vitro cultures were exposed to drug treatments or vehicle for 24 h, after which cellular viability was determined by a 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay (Cheung et al., 1998). Raw MTT data were standardised relative to untreated control (100% cell viability) and 500 μM Glu (0% cell viability), and were expressed as mean ± S.E.M. Concentration–response curves were generated by nonlinear regression analysis (sigmoidal variable slope) using GraphPad PRISM™. One-way and two-way analysis of variance, followed by Student–Newman–Keuls test were used to determine significant ($P < 0.05$) differences among treatments.

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Each agonist caused a concentration-dependent decrease in cellular viability ($F(10,36) = 28.89$, $P < 0.0001$), which reached a maximum of approximately 25% of control at 1000 μM; except (2*S*,4*R*)-4-methylglutamate which caused nearly 100% cell death at 100 μM (Fig. 1A). There were significant differences among the agonist treatments ($F(4,36) = 5.96$, $P = 0.0021$) and calculated EC_{50} values revealed the following rank order of potency (μM): (*S*)-5-iodowillardiine (9) ≈ LY339434 (11) > (2*S*,4*R*)-4-methylglutamate (33) > kainate (100) > ATPA (360). Morphological observations employing phase-contrast microscopy confirmed the concentration-dependent neurotoxicity of all agonists (data not shown).

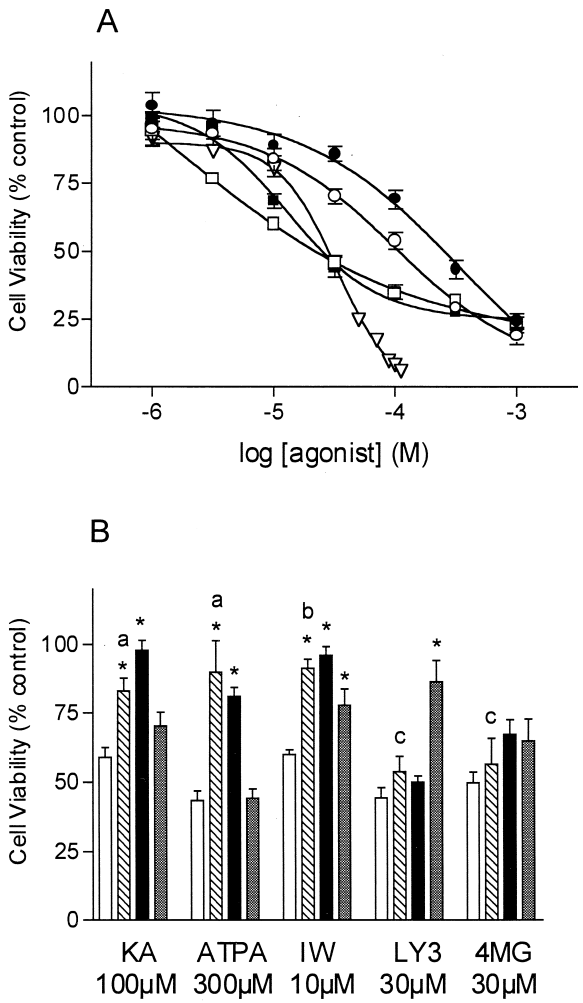

Fig. 1. (A) Concentration response curves for kainate (KA; open circle), ATPA (closed circle), (*S*)-5-iodowillardiine (IW; open square), LY339434 (LY3; closed square; 1–1000 μM) and (2*S*,4*R*)-4-methylglutamate (4MG; open triangle; 1–120 μM). (B) Bar graph showing cell viability at 24 h following treatments with agonists at approximate EC_{50} concentrations (calculated from above) in the absence and presence of ionotropic glutamate receptor antagonists: agonist alone (open bar), agonist + CNQX (lined bar), agonist + 20 μM GYKI 52466 (dark shaded bar), agonist + 10 μM MK-801 (light shaded bar). Treatments where 10 μM, 20 μM and 50 μM CNQX have been used in the presence of an agonist are indicated by a, b, and c respectively. $^{*}P < 0.05$ when compared to agonist alone. Cell viability at 24 h, standardised against no treatment (100% control) and 500 μM glutamate (0% control). Values are mean ± S.E.M., $n = 6–12$ across 2–3 independent cultures.

In further experiments, ionotropic glutamate receptor antagonists were utilised to analyse the pharmacological profile of the putative GluR5-7 agonists. The non-*N*-methyl-D-aspartate (NMDA) receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and the α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor antagonist 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI 52466; Bleakman and Lodge, 1998) attenuated the decrease in cell viability caused by kainate (100 μM), ATPA (300 μM) and (*S*)-5-iodowillardiine (10 μM; Fig. 1B) suggesting that their toxicities are mediated via AMPA and kainate receptors, including a component likely to be via low-affinity kainate receptors. A higher concentration of CNQX (20 μM) was required to elicit significant neuroprotection against (*S*)-5-iodowillardiine-induced toxicity when compared to kainate or ATPA (10 μM), suggestive of a lower selectivity of (*S*)-5-iodowillardiine for kainate receptors.

LY339434 is a ligand reported to have affinity for recombinant GluR5, with some AMPA and NMDA receptor activity (Small et al., 1998). GYKI 52466 (20 μM), and CNQX (50 μM) failed to attenuate LY339434-induced (30 μM) neurotoxicity (Fig. 1B). In contrast, the NMDA receptor antagonist (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine (MK-801, 10 μM) provided substantial neuroprotection (Fig. 1B), while producing minor neuroprotection for (*S*)-5-iodowillardiine-induced toxicity. Relative to the data obtained with ATPA and kainate in the presence of MK-801, these findings indicate that NMDA receptors are involved in the toxicity induced by LY339434 and (*S*)-5-iodowillardiine.

Evidence of extensive neuronal death resulting from (2*S*,4*R*)-4-methylglutamate at a concentration nearly 10 times less than that of other agonists and the failure of the individual antagonists under these experimental conditions to attenuate the toxicity supports a hypothesis that (2*S*,4*R*)-4-methylglutamate may cause excitotoxicity by an alternative mechanism. This mechanism might result from the ability of (2*S*,4*R*)-4-methylglutamate to compete as a substrate for glutamate transporters (Vandenberg et al., 1997), thereby increasing extracellular glutamate and causing extensive excitotoxicity indirectly.

Of the agonists tested, ATPA appeared more selective for low-affinity kainate receptors, but was the least potent. Kainate and (*S*)-5-iodowillardiine, in addition to ATPA, seemed likely to possess activity at low-affinity kainate receptors (Clarke et al., 1997), and hence the novel GluR5/AMPA receptor antagonist (3*S*,4a*R*,6*R*,8a*R*)-6-[2-(1(2)*H*-tetrazol-5-yl)ethyl]decahydroisoquinoline-3-carboxylic acid (LY293558; Bleakman and Lodge, 1998; Chittajallu et al., 1999) was employed to further characterise these putative agonists. LY293558 (10 μM) attenuated neurotoxicity caused by the approximate EC_{50} con-

centrations of kainate (150 μM), ATPA (250 μM) and (*S*)-5-iodowillardiine (30 μM) by 55%, 40% and 25%, respectively ($F(3,15) = 5.98$, $P = 0.0098$; data not shown). LY293558 antagonised only half of the (*S*)-5-iodowillardiine-induced toxicity, unlike the almost complete blockade seen for kainate and ATPA, further supporting the involvement of an NMDA receptor-mediated component of (*S*)-5-iodowillardiine-induced neurotoxicity.

These data reveal for the first time that this novel group of putative GluR5-7 agonists possess quite different excitotoxic patterns in cultured cortical neurones consistent with diverse pharmacological selectivities, although specific interactions with low-affinity kainate receptors were difficult to determine. Overall our findings demonstrate the importance of investigations at native GluR5-7 in conjunction with analyses at recombinant receptors, and highlight the need for the development of drugs with improved selectivity to further understand the involvement of low-affinity kainate receptors in neurological disorders.

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