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IDENTIFICATION OF C-TERMINAL DOMAIN RESIDUES INVOLVED IN PROTEIN KINASE A-MEDIATED POTENTIATION OF KAINATE RECEPTOR SUBTYPE 6

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Abstract—Glutamate receptors are the major excitatory receptors in the vertebrate CNS and have been implicated in a number of physiological and pathological processes. Previous work has shown that glutamate receptor function may be modulated by protein kinase A (PKA)–mediated phosphorylation, although the molecular mechanism of this potentiation has remained unclear. We have investigated the phosphorylation of specific amino acid residues in the C-terminal cytoplasmic domain of the rat kainate receptor subtype 6 (GluR6) as a possible mechanism for regulation of receptor function. The C-terminal tail of rat GluR6 can be phosphorylated by PKA on serine residues as demonstrated using [γ-^{32}P]ATP kinase assays. Whole cell recordings of transiently transfected human embryonic kidney (HEK) 293 cells showed that phosphorylation by PKA potentiates whole cell currents in wildtype GluR6 and that removal of the cytoplasmic C-terminal domain abolishes this potentiation. This suggested that the C-terminal domain may contain residue(s) involved in the PKA-mediated potentiation. Single mutations of each serine residue in the C-terminal domain (S815A, S825A, S828A, and S837A) and a truncation after position 855, which removes all threonines (T856, T864, and T875) from the domain, do not abolish PKA potentiation. However, the S825A/S837A mutation, but no other double mutation, abolishes potentiation. These results demonstrate that phosphorylation of the C-terminal tail of GluR6 by PKA leads to potentiation of whole cell response, and the combination of S825 and S837 in the C-terminal domain is a vital component of the mechanism of GluR6 potentiation by PKA.

Key words: glutamate receptor, kainate receptor, phosphorylation, receptor regulation.

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Abbreviations: CaM kinase II, Ca^{2+}/calmodulin-dependent protein kinase II; GFP, green fluorescent protein; GluR, glutamate receptor; GluR6, kainate receptor subtype 6; GluR6ΔC-term, GluR6 truncated before S815; GluR6Δ855, GluR6 truncated before T856; GST, glutathione S-transferase; HEK, human embryonic kidney; iGluR, ionotropic glutamate receptor; LTD, long-term depression; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; PKA, protein kinase A; PKA_{cat}, protein kinase A catalytic subunit; PKC, protein kinase C; WT, wildtype.

Glutamate receptors (GluRs) are the major excitatory receptors in the vertebrate CNS and have been implicated in a number of normal CNS functions including synaptic plasticity, and pathological processes such as epilepsy, Parkinson's disease, schizophrenia, and ischemic cell death (Dingledine et al., 1999). GluRs are ligand-activated membrane receptors and consist of two large families: metabotropic (linked through G proteins to downstream second messengers) receptors termed mGluRs and ionotropic (current passing) receptors referred to as iGluRs (Dingledine et al., 1999). iGluRs have been subdivided based upon their agonist specificities and sequence homology into *N*-methyl-D-aspartate (NMDA), AMPA, and kainate receptors (Lerma, 2006). Each receptor is believed to be composed of a tetramer of subunits that oligomerize to form functional channels in the cell membrane (Dingledine et al., 1999). To date, five mammalian kainate receptor subunits (GluR5-7, KA1-2) have been identified based upon their high affinity for this compound (Bettler et al., 1990, 1992; Egebjerg et al., 1991; Werner et al., 1991; Herb et al., 1992).

Previous studies suggest that iGluR function may be modulated by protein phosphorylation (Raymond et al., 1993; Wang et al., 1993, 1994; Roche et al., 1996; Lee et al., 1998). Basal and induced phosphorylation of AMPA and kainate GluRs by protein kinase A (PKA), protein kinase C (PKC), and Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) has been demonstrated (McGlade-McCulloh et al., 1993; Moss et al., 1993; Raymond et al., 1993). Phosphorylation of NMDA receptors has similarly been reported (Chen and Huang, 1992; Tingley et al., 1993; Lieberman and Mody, 1994). Mutational studies, immunochemical assays, phosphoamino acid analysis and phosphopeptide mapping suggest that basal and kinase-dependent phosphorylation of GluRs occurs primarily on serine and threonine residues (Blackstone et al., 1994; Omkumar et al., 1996; Roche et al., 1996; Barria et al., 1997; Leonard and Hell, 1997; Mammen et al., 1997; Tingley et al., 1997; Lee et al., 1998). PKA-mediated potentiation of whole cell currents has been demonstrated in human embryonic kidney (HEK293) cells transiently transfected with rat GluR6 cDNA (Raymond et al., 1993; Wang et al., 1993). Raymond et al. (1993) demonstrated that intracellularly applied protein kinase A catalytic subunit (PKA_{cat}) increased the amplitude of glutamate-induced whole cell current. Although the specific site(s) of phosphorylation was (were) not identified, potentiation was abolished by a S684A point mutation and by inclusion of an inhibitor of PKA (PKI) in the pipette solution. Wang et al.

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(1993) observed potentiation of kainate-induced currents in GluR6-transfected HEK293 cells when PKA_{cat} was directly perfused into individual cells. A S684A point mutation decreased PKA_{cat}-induced current potentiation while a S666A mutation had no effect. The double mutation S684A/S666A abolished PKA_{cat}-induced potentiation. These experiments were reported at a time when the assumed transmembrane topology of the glutamate receptor placed the N- and C-termini and the S1 region in an extracellular location and S2 intracellularly (Hollmann et al., 1989; Keinänen et al., 1990). This topology assumed that the sites of interest in these studies (i.e. S684 and S666) were intracellular and were therefore exposed to PKA_{cat} and PKI. Subsequent immunocytochemical, phosphorylation, and *N*-glycosylation studies (Hollmann et al., 1994; Wo and Oswald, 1994, 1995a,b; Bennett and Dingledine, 1995) indicate an extracellular location for S2, thereby placing positions 684 and 666 in an extracellular location (Fig. 1). Reconciliation of the findings of Raymond et al. (1993) and Wang et al. (1993) with the corrected transmembrane topology (Hollmann et al., 1994; Wo and Oswald, 1994, 1995a) of the GluR6 subunit requires one or a combination of the following possibilities: (1) extracellular phosphorylation of sites 684 and 666 by kinases in the extracellular milieu (or by PKA released from lysed cells in the Raymond et al. (1993) paper), (2) intracellular phosphorylation of GluR6 en route from the endoplasmic reticulum (ER) to the cell membrane, or (3) the presence of one

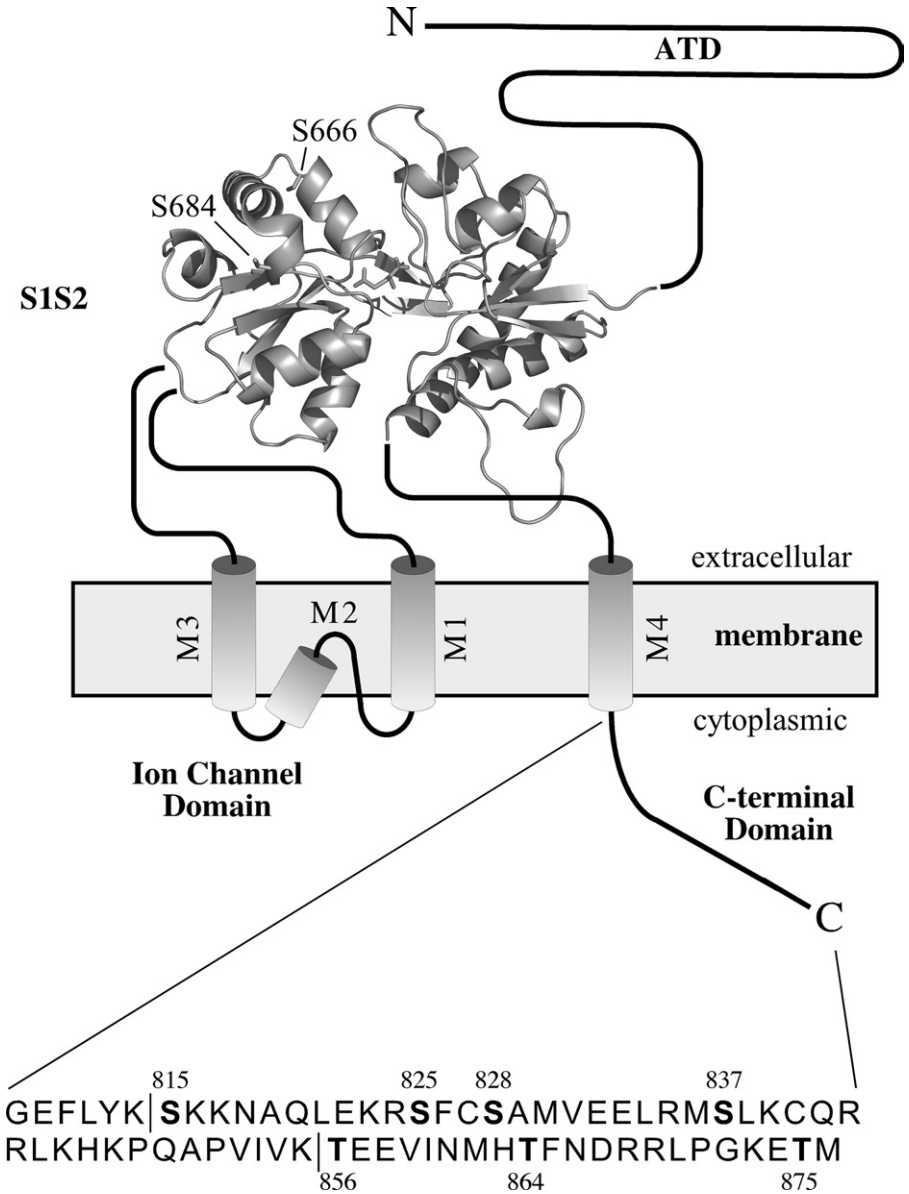

Fig. 1. Schematic of the domains of the GluR6 receptor. ATD refers to the amino terminal domain and M1 through M4 refer to membrane-associated regions of the protein. The structure shown is the S1S2 (extracellular ligand-binding domain of a GluR) of GluR6 (1S7Y; Mayer, 2005). The vertical lines in the sequence illustrate where the truncations were made for Fig. 3 (line before S815; GluR6ΔC-term) and Figs. 2, 4, and 5 (line before T856; GluR6Δ855).