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N-methyl-D-aspartate (NMDA)-evoked Ca^{2+} Mobilization via GTP-binding Proteins in Cultured Hippocampal Neurons

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Abstract Intracellular Ca^{2+} plays an important role as a second messenger in cellular functions in the brain. The increase in Ca^{2+} level can be accomplished mainly by two different mechanisms; influx from extracellular medium and release from intracellular stores (intracellular Ca^{2+} mobilization). Recently a metabotropic type of quisqualate receptor which is coupled to GTP-binding proteins (G proteins) and triggers intracellular Ca^{2+} mobilization has been identified. However, there have been no clear demonstrations showing an intracellular Ca^{2+} mobilization provoked by N-methyl-D-aspartate (NMDA). We have examined the possibility of intracellular Ca^{2+} mobilization induced by NMDA in fura-2-loaded hippocampal cultured neurons, and obtained the evidence that NMDA can trigger the release of Ca^{2+} from intracellular Ca^{2+} stores, by activating a glutamate receptor coupled to G proteins.

Key Words:N-methyl-D-aspartate, Islet-activating protein (pertussis toxin), Fura-2, Phospholipase C, Hippocampus

Introduction

Because glutamate is probably the most widely used excitatory neurotransmitter in the central nervous system, a detailed knowledge of glutamate receptor system is essential for our understanding of neuronal communication in the brain. L-Glutamate is assumed to interact with at least three classes of membrane receptor channels[1], each referred to its preferred pharmacological agonists; N-methyl-D-aspartate (NMDA), ∂-amino-3-hydroxy-5-methylisoxazole-propionic acids (AMPA), kainate. A metabotropic type of glutamate receptor, which is activated by quisqualate, also exists[2,3,4,5]. The NMDA receptor channel is permeable to both Ca^{2+} [6,7] and Na^{+} [8,9], gated by Mg^{2+} [10,11] and Zn^{2+} [12,13], and blocked competitively by ∂-amino-ω-phosphonocarboxylic acids[14] and non-competitively by phencyclidine[15] and MK-801[16]. Glycine, at submicromolar concentraion, exerts a positive modulatory effect on NMDA receptor channels[17], and NMDA receptors mediate the induction of long-term potentiation[18] in the hippocampus.

The glutamate-evoked formation of inositol phosphate in the brain appears to occur in response to activation of quisqualate and NMDA receptors[19], and a metabotropic quisqualate receptor has been extensively studied[2,3,20], although the exact effects of NMDA are controversial[2]. We will begin with a brief overview of a metabotropic quisqualate receptor, and the remainder of the view will discuss the intracellular Ca^{2+} mobilization (release of Ca^{2+} from intracellular calcium stores) evoked by NMDA.

Quisqualate-induced Ca^{2+} mobilization

It was demonstrated that two types of quisqualate-specific glutamate receptors exist on hippocampal neurons[4,21]. Activation of both types of receptors leads to characteristic changes in intracellular Ca^{2+} concentration. The first type of quisqualate receptor produces a Na^+-dependent depolarization resulting in the opening of voltage-sensitive Ca^{2+} channels. This receptor can also be activated by the quisqualate analogue AMPA[1]. The second type of response concerns the ability of quisqualate and glutamate to mobilize Ca^{2+} from intracellular stores. Various pieces of evidence make it highly likely that this type of quisqualate response is mediated by quisqualate-stimulated inositol 1, 4, 5-triphosphate (IP3) production and is coupled to G-proteins[3,4,5,21].

NMDA-induced Ca^{2+} mobilizatin

Quisqualate and NMDA receptors have been linked to phospholipase C activation in striatum[19] and in cultures of cerebellar granule cells[22], although in hippocampus exact effect of NMDA is controvertial[2]. The ability of NMDA receptor stimulation to activate phospholipase C is profoundly inhibited by physiological concentrations of extracellular Mg^{2+} [22]. The inhibition by Mg^{2+} is noncompetitive for the NMDA binding site and can be overcome by depolarization. In contrast, Mg^{2+} has little effect on the activation of phospholipase C through quisqualate receptors[22]. The effect of Mg^{2+} on the functional coupling of the NMDA receptor to phospholipase C is remarkably similar to its effect on the NMDA receptor associated channel, which is permeable to Ca^{2+} and Na^+ [23]. Recently, it was demonstrated that protein kinase C mediated a sustained increase of intracellular Ca^{2+} concentration by activation of NMDA receptors in hippocampal cultured neurons[24]. And we have demonstrated the first evidence of intracellular Ca^{2+} mobilization induced by NMDA in hippocampus[25]. In this report, we used a fluorescent Ca^{2+} indicator, fura-2[26,27] to investigate the changes of cytosolic Ca^{2+} concentration produced by NMDA in the presence or absence of extracellular Ca^{2+} ions in hippocampal cultured neurons, and examined the possibility of intracellular Ca^{2+} mobilization provoked by NMDA. In result, under Ca^{2+}-free conditions, NMDA (>100 μM) in the absence of glycine or NMDA ($>50\mu$M) in the presence of glycine produced intracellular Ca^{2+} mobilization, which was blocked by 2-amino-5-phosphnovaleric acid and reduced by islet-activating protein (pertussis toxin), and this NMDA-induced intracellular Ca^{2+} mobilization was potentiated by the entry of Ca^{2+} into the cells via NMDA receptor channels. These results indicate that there exists a metabotropic type of NMDA receptor, which is coupled to G proteins in hippocampus. In these responses, it is possible that NMDA may work through a new type of NMDA receptor. However, a more likely explanation may be that some, as yet, unidentified substance is secreted from nonneuronal cells as a result of NMDA stimulation and activates the neuronal second messenger system. Inhibition of long-term potentiation by islet-activating protein has been demonstrated[20,28]. The intracellular Ca^{2+} mobilization induced NMDA described in our report may contribute to the generation of long-term potentiation in hippocampus.

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