Korean J Physiol Pharmacol
Vol 7: 53−57, April, 2003

Mechanism of Glutamate-induced $[Ca^{2+}]_i$ Increase in Substantia Gelatinosa Neurons of Juvenile Rats

Sung Jun Jung, Jeong Sook Choi, Jiyeon Kwak[1], Jun Kim[1], Jong Whan Kim, and Sang Jeong Kim

Department of Physiology, College of Medicine, Kangwon National University, Chunchon; [1]Department of Physiology, Seoul National University College of Medicine, Seoul, Korea

The glutamate receptors (GluRs) are key receptors for modulatory synaptic events in the central nervous system. It has been reported that glutamate increases the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and induces cytotoxicity. In the present study, we investigated whether the glutamate-induced $[Ca^{2+}]_i$ increase was associated with the activation of ionotropic (iGluR) and metabotropic GluRs (mGluR) in substantia gelatinosa neurons, using spinal cord slice of juvenile rats (10∼21 day). $[Ca^{2+}]_i$ was measured using conventional imaging techniques, which was combined with whole-cell patch clamp recording by incorporating fura-2 in the patch pipette. At physiological concentration of extracellular Ca^{2+}, the inward current and $[Ca^{2+}]_i$ increase were induced by membrane depolarization and application of glutamate. Dose-response relationship with glutamate was observed in both Ca^{2+} signal and inward current. The glutamate-induced $[Ca^{2+}]_i$ increase at holding potential of −70 mV was blocked by CNQX, an AMPA receptor blocker, but not by AP-5, a NMDA receptor blocker. The glutamate-induced $[Ca^{2+}]_i$ increase in Ca^{2+} free condition was not affected by iGluR blockers. A selective mGluR (group I) agonist, RS-3,5-dihydroxyphenylglycine (DHPG), induced $[Ca^{2+}]_i$ increase at holding potential of −70 mV in SG neurons. These findings suggest that the glutamate-induced $[Ca^{2+}]_i$ increase is associated with AMPA-sensitive iGluR and group I mGluR in SG neurons of rats.

Key Words: Substantia gelatinosa neuron, Glutamate, Intracellular Ca^{2+} concentration, NMDA receptor, AMPA receptor, Metabotropic glutamate receptor

INTRODUCTION

Glutamate is well known as an excitatory neuro transmitter in the substantia gelatinosa (SG), which is a major site of termination of nociceptive primary afferents. Glutamate released from presynaptic nerve terminal evokes cellular response via binding to various glutamate receptors, such as ionotropic glutamate receptor (iGluR) and metabotropic glutamate receptor (mGluR). iGluR itself acts as an ion channel after glutamate receptor binding, while the activation of mGluR induces Ca^{2+} release from intracellular Ca^{2+} stores and modulates various voltage-dependent ion channel via the activation of intracellular secondary messenger.

Fast excitatory synaptic transmission in the SG is mediated mainly by release of glutamate onto postsynaptic AMPA and NMDA receptors (Yoshimura & Jessell, 1990). Ca^{2+} entry through NMDA receptors is known to activate Ca^{2+}-sensitive signaling cascades and potentiate synaptic transmission (McBain & Mayer, 1994). In addition, Ca^{2+} influx via NMDA receptor increases intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and consequently induces neurotoxicity (Hollmann & Heinemann, 1994; Jonas & Burnashev, 1995). Some AMPA and kainate receptors also have significant Ca^{2+} permeability (Hollmann & Heinemann, 1994; Jonas & Burnashev, 1995) and may activate pathways leading to synaptic strengthening (Gu et al, 1996; Jia et al, 1996; Mahanty & Sah, 1998). Less well known is a subpopulation of AMPA receptors that is Ca^{2+}-permeable, synaptically localized on dorsal horn neurons in culture (Gu et al, 1996), and expressed by dorsal horn neurons *in situ* (Nagy et al, 1994; Engelman et al, 1997, 1999).

mGluR in the SG is associated with phospholipase C (PLC), which hydrolyzes phosphate inositol diphosphate (PIP_2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). DAG activates protein kinase C (PKC), and resulting IP_3 releases Ca^{2+} from intracellular IP_3-sensitive Ca^{2+} store (Liberman & Mody, 1994; Nishiyama et al, 2000). Taken together, glutamate induces $[Ca^{2+}]_i$ increase, which can activate intracellular signaling cascades and evoke synaptic plasticity. However, it is not clearly elucidated which Ca^{2+} sources, such as NMDA receptor, Ca^{2+}-permeable AMPA receptor and mGluR, are associated with the glutamate-induced $[Ca^{2+}]_i$ increase in SG neurons.

Corresponding to: Sang Jeong Kim, Department of Physiology, College of Medicine, Kangwon National University, Hyoja 2 dong, Chunchon, Korea (Tel) +82-33-250-8820, (Fax) +82-33-242-7571, (E-mail) sangjkim@kangwon.ac.kr

ABBREVIATIONS: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, N-methyl-D-aspartate; DHPG, RS-3,5-dihydroxyphenylglycine; iGluR, ionotropic glutamate receptor; mGluR, metabotropic glutamate receptor; SG, substantia gelatinosa.

In the present study, we investigated the effects of glutamate on $[Ca^{2+}]_i$ of SG neuron and then characterized the Ca^{2+} sources in SG neurons which was associated with $[Ca^{2+}]_i$ increase. For this purpose, we recorded both $[Ca^{2+}]_i$ and ionic currents from SG neurons in the dorsal horn using conventional imaging techniques and whole cell patch clamp recordings in the spinal cord slice preparation of juvenile rats.

METHODS

Slice preparation

Sprague-Dawley juvenile (P8-P15) rats were anesthetized and decapitated, and the lumbosacral spinal cord was removed and placed in an ice-cold artificial cerebrospinal solution (ACSF; see below for composition of solutions) that had been bubbled with 95% O_2-5% CO_2. Laminectomies were performed, and the spinal cord was isolated and a segment from L4 to L6 was attached to agarose block (2.5% in ACSF). Transverse slices 300 μm thick were prepared with the use of a microslicer (Vibratome 3000, TPI). Slices were incubated for 1 hr at 32°C for recovery and then maintained at room temperature (20~24°C) in ACSF equilibrated with 95% O_2-5% CO_2.

Electrical recording

Patch clamp recordings in whole-cell configurations were employed to measure membrane potentials and ionic currents from neurons in SG. The neurons were visually identified using an fixed-stage microscope (Olympus, BX50 WI) with Nomarski optics and a 40× water-immersion objective. After identification of SG neuron, a patch pipette under positive pressure was placed on the surface of the neuron; release of pipette pressure and gentle suction allowed the giga-seal formation, and rupture of the membrane followed the additional suction, permitting whole-cell configuration. The recording electrodes were fabricated from Kimax-51 borosilicate capillary tubes (Kim ble, USA) by pulling on a microelectrode puller (Narishige, pp-83). Data were recorded and acquired using EPC-9 patch-clamp amplifier and Pulse 8.30 software (both from HEKA, Germany). Signals were filtered at 1 kHz and sampled at 3 kHz. Series resistance was typically less than 20 MΩ and was compensated by about 70%. Electrical recordings were done at room temperature. The liquid junction potential between bath and low chloride internal solution (12 mV) was corrected.

Measurement of intracellular Ca^{2+}

$[Ca^{2+}]_i$ was determined using microspectrofluorimetric techniques with Ca^{2+}-sensitive dye fura-2 (100 μM). In some study, membrane permeable fura-2/acetoxymethyl ester (fura-2AM, 10 μM) was used in external solution in order to load at cell. The fluorescence ratios were measured with alternative wavelength time scanning at room temperature (20~24°C) at excitation wavelength of 340 nm and 385 nm, and emission wavelength of 500 nm. We used monochromator (Optoscan, Cairn, UK) as light source for fluorescence. Camera and monochromator were controled by axon image workbench (Axon instruments, USA).

Solutions and materials

Slices were transferred to the recording chamber flooded with ACSF (in mM) 130 NaCl, 26.2 $NaHCO_3$, 1.25 NaH_2PO_4, 3 KCl, 1.5 $MgSO_4$, 2.5 $CaCl_2$, 10 glucose, 20 sucrose, pH 7.4, 320 mOsm. The patch pipette was filled with an internal solution containing (in mM) 126 K-gluconate, 10 NaCl, 1 $MgCl_2$, 11 EGTA, 10 HEPES, 2 NaATP, 0.1 MgGTP, pH adjusted to 7.3 with KOH. Solutions were applied with a multi-barrel delivery system in which five polyethylene tubes were packed into the end of a glass tube. The close apposition of the perfusion tip near the cells allowed fast exchange of bath within one second. Chemical reagents were purchased from Sigma, unless otherwise indicated.

Statistics

Values expressed in the text are mean±standard error of mean (s.e.m.) and the error bars in figures also indicate s.e.m.. The statistical significance of differences between the values was determined using the non-paired Students t-test and a P-value of <0.05 was considered to be statistically significant.

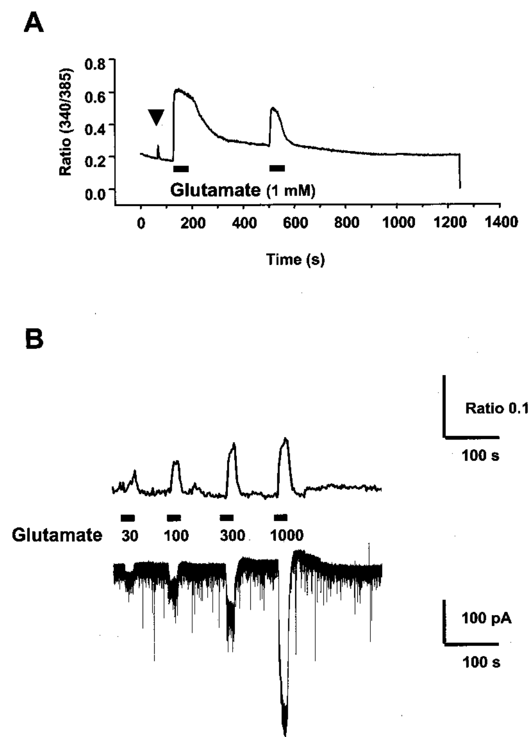

Fig. 1. (A) Membrane depolarization (▼, 0 mV, 1000 ms) of SGN induces intracellular Ca^{2+} increase, and extracellular application of glutamate (1 mM) induced repetitive increase of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). (B) Dose-response relationship of glutamate-induced $[Ca^{2+}]_i$ increase. Dose response relationship (30, 100, 300, 1000 μM) was simultaneously observed in Ca^{2+} signal and inward current (n=3). This increase in $[Ca^{2+}]_i$ was accompanied by whole cell inward current.

RESULTS

Glutamate-induced $[Ca^{2+}]_i$ increase

We measured $[Ca^{2+}]_i$ of SG neurons in whole-cell configuration using internal solution containing 100 μM Fura-2. At holding potential of −70 mV, application of glutamate (1 mM) induced an increase of $[Ca^{2+}]_i$, and the second application of glutamate also increased $[Ca^{2+}]_i$ in dose-dependent manner (Fig. 1). Simultaneously, glutamate-induced ionic current may be associated with the $[Ca^{2+}]_i$ increase. The amplitude of the glutamate-induced inward current also increased as the glutamate concentration increased. Although the $[Ca^{2+}]_i$ response was saturated at 300 μM, the glutamate-induced inward current was not saturated at glutamate 1 mM. The ED_{50} value of the $[Ca^{2+}]_i$ response and the response of the inward current were 99.8 μM and 412 μM, respectively.

Taken together, these results indicate that the activation of non-NMDA receptor or mGluR is associated with the glutamate-induced $[Ca^{2+}]_i$ increase, because NMDA receptor is not activated at −70 mV (Mayer et al, 1984).

Association with glutamate-induced $[Ca^{2+}]_i$ increase and glutamate receptors

The glutamate-induced $[Ca^{2+}]_i$ increase and inward current were reversibly inhibited by 6-cyano-7-nitroquinoxaline-2, 3-dione disodium salt (CNQX, 10 μM), an AMPA receptor antagonist (Fig. 2A). The glutamate-induced in ward current was 179 pA and reduced to 62 pA in the presence of CNQX, and the increment of the fluorescecence ratio reduced from 0.11 to 0.038. These results indicate that the activation of AMPA receptor is associated with the glutamate-induced $[Ca^{2+}]_i$ increase and inward current (n=4). However, the finding that all the glutamate-induced response was not blocked by CNQX suggests that there may exist another mechanism as well as the activation of AMPA receptor.

We tested the effect of another iGluR, NMDA receptor (n=3). At −70 mV of holding potential, the application of DL-2-amino-5-phosphonopentanoic acid (AP-5, 50 μM), an NMDA receptor antagonist, did not block the glutamate-induced $[Ca^{2+}]_i$ increase (Fig. 2B). Thus, we can exclude the association of NMDA receptor with the glutamate-induced $[Ca^{2+}]_i$ at resting membrane potential (−70 mV).

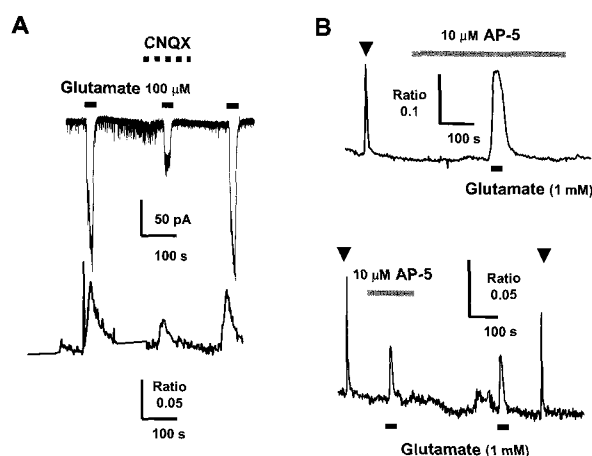

Fig. 2. Glutamate-induced Ca^{2+} transient is associated with AMPA, but not NMDA receptor. (A) CNQX (10 μM) blocked the glutamate-induced $[Ca^{2+}]_i$ increase. 100 μM glutamate increased Ca^{2+} signal and inward current. CNQX, a AMPA receptor blocker, reversibly decreased both Ca^{2+} signal and inward current to 52.5± 3.6% of control value (n=4). (B) At this holding potential of −70 mV, AP-5 (50 μM), a NMDA receptor blocker, did not affect the glutamate-induced $[Ca^{2+}]_i$ increase (n=3, 92.5±1.3%).

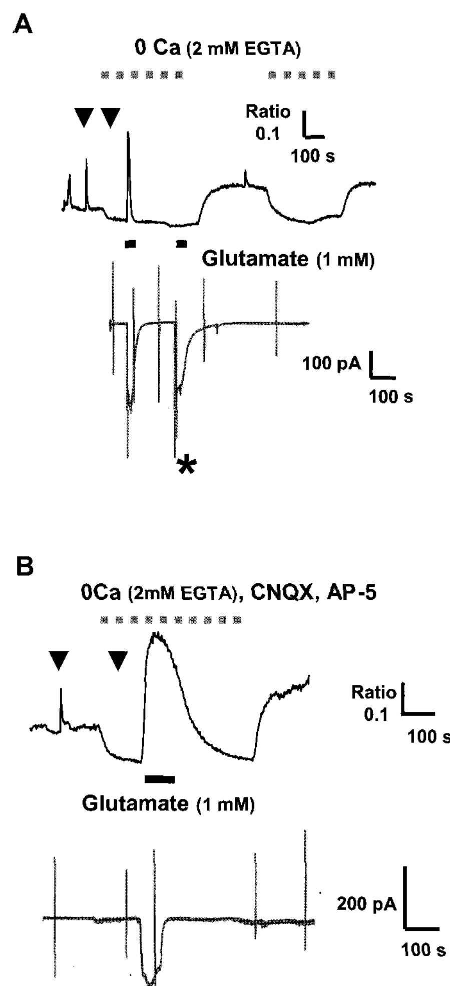

Fig. 3. The glutamate-induced $[Ca^{2+}]_i$ increase is associated with intracellular Ca^{2+} store. (A) The glutamate-induced $[Ca^{2+}]_i$ increase was observed in Ca^{2+}-free condition. One mM glutamate depleted Ca^{2+} response and the second application failed to induce the $[Ca^{2+}]_i$ increase, while the inward current was induced by the second application of glutamate (n=3). *indicates that the glutamate-induced $[Ca^{2+}]_i$ increase was not associated with the glutamate-induced inward current in Ca^{2+}-free condition. (B) Both the $[Ca^{2+}]_i$ increase and the glutamate-induced inward current in Ca^{2+}-free solution were not affected by iGluR blockers (10 μM CNQX and 50 μM AP-5, n=4).

Role of intracellular Ca^{2+} store in glutamate-induced $[Ca^{2+}]_i$ increase

We tested the glutamate-induced response in Ca^{2+} free condition to investigate the role of intracellular Ca^{2+} store in the glutamate-induced $[Ca^{2+}]_i$ increase. In the Ca^{2+}-free solution containing 2 mM EGTA, depolarization (▼, 0 mV) did not induce $[Ca^{2+}]_i$ increase, while glutamate-induced $[Ca^{2+}]_i$ increase was observed (Fig. 3A). Although the second application of glutamate did not increase $[Ca^{2+}]_i$, the amplitude of glutamate-induced inward current was similar to that of first response (n=3; Fig. 3A, *). These results suggest that the glutamate-induced $[Ca^{2+}]_i$ increase might not be associated with the glutamate-induced inward current in Ca^{2+}-free condition.

In order to exclude the involvement of iGluR (AMPA and NMDA receptor), we used the Ca^{2+}-free solution (2 mM EGTA) with CNQX (10 μM) and AP-5 (50 μM). As shown in Fig. 3B, the glutamate-induced responses were still observed (n=4). Thus, the glutamate-induced $[Ca^{2+}]_i$ increase may be related to Ca^{2+} release from intracellular Ca^{2+} store via activation of mGluR. Taken together, it suggests that the glutamate-induced $[Ca^{2+}]_i$ increase at -70 mV of holding potential is consisted of Ca^{2+} release from Ca^{2+} store as well as Ca^{2+} influx via Ca^{2+}-permeable AMPA receptor.

The effect of mGluR on glutamate-induced $[Ca^{2+}]_i$ increase

Next, we studied whether the activation of mGluR was associated with Ca^{2+} release from Ca^{2+} store in glutamate-induced $[Ca^{2+}]_i$ increase. Because Ca^{2+} release from Ca^{2+} store may result from the activation of mGluR, we tested effects of RS-3, 5-dihydroxyphenylglycine (DHPG, 10 μM, Sigma, USA), a group I mGluR agonis. Although the first application of DHPG induced $[Ca^{2+}]_i$ increase, the second application of DHPG induced no response (n=3, Fig. 4A). Since this experiment was conducted in whole-cell configuration, it was possible that cytosolic constituents could be diluted by internal solution and consequently second response could not be observed. Next, we used fura-2AM to maintain normal cellular function. In Fig. 4B, $[Ca^{2+}]_i$ increase after repetitive application of DHPG was observed in three cells (n=3). This implys that refill process of Ca^{2+} store may be washable in whole cell configuration. Thus, it could be concluded that the activation of group I mGluR was involved in the glutamate-induced $[Ca^{2+}]_i$ increase.

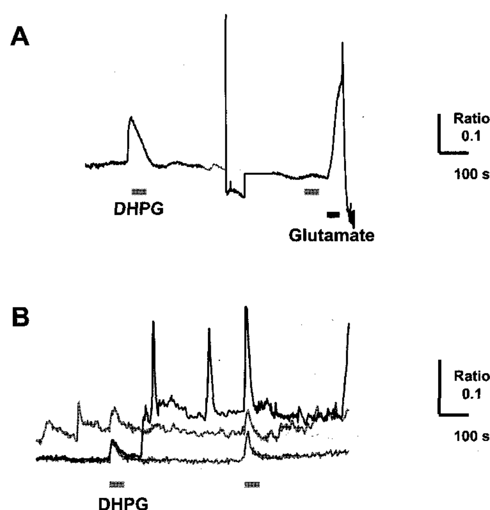

Fig. 4. The glutamate-induced $[Ca^{2+}]_i$ increase is associated with mGluR. (A) A selective mGluR agonist, DHPG, induced in a subgroup of SG neurons (n=3). This response was depleted by first trial of DHPG. (B) In fura-2AM loaded neurons, the DHPG-induced $[Ca^{2+}]_i$ increase was reproducible (n=3).

DISCUSSION

We have characterized $[Ca^{2+}]_i$ increases induced by glutamate in the SG neurons of juvenile rats. The $[Ca^{2+}]_i$ response to glutamate is a complex integration of Ca^{2+} influx through the membrane and Ca^{2+} mobilization from the intracellular Ca^{2+} store.

Glutamate-induced $[Ca^{2+}]$ increase by the Ca^{2+} influx through the membrane

When the membrane potential was clamped at around resting membrane potential, glutamate induced $[Ca^{2+}]_i$ increase in SG neurons of spinal dorsal horn. Although Ca^{2+} influx through NMDA receptor channel is well known to explain the glutamate-induced $[Ca^{2+}]_i$ increase (Mayer et al, 1984), Ca^{2+}-permeable AMPA receptor channel is the other explanation for the Ca^{2+} influx through the membrane (Hollmann et al, 1991; Hume et al, 1991; Burnashev et al, 1992; Geiger et al, 1995; Jia et al, 1996). NMDA receptor has voltage-dependency, and this channel is blocked by Mg^{2+} at resting membrane potential and depolarization removes the blockade of Mg^{2+} and enables the influx of Ca^{2+} through the membrane (Mayer et al, 1984). Therefore, Ca^{2+} influx in our voltage-clamp recording condition at around resting membrane potential can not be explained by the NMDA receptor channel. Ca^{2+} influx through the AMPA receptor which is permeable at resting potential is more preferable interpretation. The partial inhibition of the $[Ca^{2+}]_i$ increase by the AMPA receptor antagoinst supports the explanation that the influx of Ca^{2+} occurs through the AMPA receptor channel.

Ca^{2+} influx through the voltage-gated Ca^{2+} channel which is activated by local membrane potential depolarization by AMPA has been reported in cerebellar parallel fiber and Purkinje neuron synapse (Takechi et al, 1998), and the report was concerned with fast synaptic evoked synaptic current which induced local dendritic failure of voltage clamping. However, the chance of failure in voltage clamping in the present study was low, because of our relatively slow bath perfusion of glutamate. In addition, small membrane surface area of SG neurons, compared to large Purkinje neurons, also might have reduced the chance of clamping failure.

It has been reported that AMPA receptor without GluR2 subunit is permeable to Ca^{2+} in various central nervous system, including spinal cord dorsal horn (Hollmann et al, 1991; Hume et al, 1991; Burnashev et al, 1992; Geiger et al, 1995; Jia et al, 1996; Holly et al, 1999). The GluR2 (GluRB) subunit determines the Ca^{2+} permeability of these ligand-gated ion channels. GluR2 transcripts undergo RNA editing, producing a one amino acid change in the channel pore that decreases Ca^{2+} permeability (Burnashev et al,

1992). Recent studies suggest that one edited GluR2 subunit is sufficient to cause low receptor Ca^{2+} permeability (Washburn et al, 1997). AMPA receptors lacking GluR2 have Ca^{2+} permeability ratios up to P_{Ca}/P_{Na} = 3 (Hollmann et al, 1991; Hume et al, 1991; Burnashev et al, 1992; Geiger et al, 1995; Jia et al, 1996). In the present study, Ca^{2+} influx through the AMPA component suggests the presence of AMPA receptor channel which lacks of GluR2 subunit. Further molecular studies including single-cell RT-PCR may reveal the presence of GluR2 lacking AMPA receptor channel in SG neurons.

Ca^{2+} mobilization from intracellular Ca^{2+} store

As in many other central neurons, mGluR in addition to iGluR is present in SG neurons. Generally, mGluR is divided into three groups. The activation of group I receptor is linked to G-protein, and G-protein in turn activates PLC which produces IP_3 and DAG from PIP_2. IP_3 activates IP_3 receptor in the membrane of Ca^{2+} store and IP_3 activation can induce the mobilization of Ca^{2+} from intracellular Ca^{2+} stores (Finch & Augustine, 1998; Takechi et al, 1998). In our $[Ca^{2+}]_i$ measurement, significant amount of $[Ca^{2+}]_i$ increase was observed after the blocking of the Ca^{2+} influx through the AMPA receptor channel by the AMPA receptor antagonist. This Ca^{2+} signal was persistent in the absence of extracellular Ca^{2+}, implying that this Ca^{2+} was released from the store but not an influx through plasma membrane. In addition, mGluR group I agonist, DHPG, could induce Ca^{2+} increase, suggesting that the activation of mGluR by glutamate increased $[Ca^{2+}]_i$ by the Ca^{2+} mobilization from intracellular Ca^{2+} store.

The glutamate-induced inward current in Ca^{2+}-free condition was insensitive to AMPA channel antagonist. As mentioned before, NMDA can not be an interpretation at resting membrane potential. The possible interpretation of this current may be mGluR which activated inward current, as reported in other central neurons. In hippocapal pyramidal neuron and cerebellar Purkinje neurons, tetanic synaptic stimulation induces the mGluR mediated slow inward current (Tempia et al, 2001). Further studies on mGluR activated inward current will reveal the physiological significance of AMPA-insensitive inward current in SG neurons.

We observed the glutamate-induced changes in $[Ca^{2+}]_i$ from cell bodies of SG neurons in juvenile rats, and were able to clarify several aspects of the glutamate-induced Ca^{2+} signal. One route is Ca^{2+}-permeable AMPA channel and the other route is Ca^{2+} mobilization from intracellular Ca^{2+} store. This modulation of voltage-independent Ca^{2+} signal may have a role in synaptic plasticity in SG neuron synapse. The possibility and the significance of the Ca^{2+} signal to synaptic plasticity should be studied further in the dendritic tree of SG neurons, where the synaptic connection is present.

ACKNOWLEDGEMENTS

This work is supported by Korea Research Foundation Grant (KRF-2000-041-F00066).

REFERENCES

Burnashev N, Monyer H, Seeburg PH, Sakmann B. Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. *Neuron* 8: 189−198, 1992

Engelman HS, Albuquerque C, Lee CJ, Allen TB, MacDermott AB. Calcium permeable AMPA receptors expressed in laminae I and II of the postnatal rat spinal cord. *Soc Neurosci Abstr* 23: 1754, 1997

Engelman HS, Allen TB, MacDermott AB. The distribution of neurons expressing calcium-permeable AMPA receptors in the superficial laminae of the spinal cord dorsal horn. *J Neuroscience* 19(6): 2081−2089, 1999

Geiger JR, Melcher T, Koh DS, Sakmann B, Seeburg PH, Jonas P, Monyer H. Relative abundance of subunit mRNAs determines gating and Ca^{2+} permeability of AMPA receptors in principal neurons and interneurons in rat CNS. *Neuron* 15: 193−204, 1995

Gu JG, Albuquerque C, Lee CJ, MacDermott AB. Synaptic streng thening through activation of Ca^{2+}-permeable AMPA receptors. *Nature* 381: 793−796, 1996

Hollmann M, Hartley M, Heinemann S. Ca^{2+} permeability of KA-AMPA-gated glutamate receptor channels depends on subunit composition. *Science* 252: 851−853, 1991

Hollmann M, Heinemann S. Cloned glutamate receptors. *Annu Rev Neurosci* 17: 31−108, 1994

Hume RI, Dingledine R, Heinemann SF. Identification of a site in glutamate receptor subunits that controls calcium permeability. *Science* 253: 1028−10031, 1991

Finch EA, Augustine GJ. Local calcium signalling by inositol-1, 4,5-trisphosphate in Purkinje cell dendrites. *Nature* 396(6713): 753−756, 1998

Jia Z, Agopyan N, Miu P, Xiong Z, Henderson J, Gerlai R, Taverna FA, Velumian A, MacDonald J, Carlen P, Abramow-Newerly W, Roder J. Enhanced LTP in mice deficient in the AMPA receptor GluR2. *Neuron* 17: 945-956, 1996

Jonas P, Burnashev N. Molecular mechanisms controlling calcium entry through AMPA-type glutamate receptor channels. *Neuron* 15(5): 987−990, 1995

Liberman DN, Mody I. Regulation of NMDA channel function by endogenous Ca^{2+}-dependent phosphatase. *Nature* 369(6477): 235−239, 1994

Mahanty NK, Sah P. Calcium-permeable AMPA receptors mediate long-term potentiation in interneurons in the amygdala. *Nature* 394: 683-687, 1998

Mayer ML, Westbrook GL, Guthrie PB. Voltage-dependent block by Mg^{2+} of NMDA receptors in spinal cord neurons. *Nature* 309: 261−263, 1984

McBain CJ, Mayer ML. N-methyl-D-aspartic acid receptor struc ture and function. *Physiol Rev* 74: 723−760, 1994

Nagy I, Woolf CJ, Dray A, Urban L. Cobalt accumulation in neurons expressing ionotropic excitatory amino acid receptors in young rat spinal cord: morphology and distribution. *J Comp Neurol* 344: 321−335, 1994

Nishiyama M, Hong K, Mikoshiba K, Poo MM, Kato K. Calcium stores regulate the polarity and input specific of synaptic modification. *Nature* 408(6812): 584−588, 2000

Takechi H, Eilers J, Konnerth A. A new class of synaptic response involving calcium release in dendritic spines. *Nature* 396(6713): 757−760, 1998

Tempia F, Alojado ME, Strata P, Knopfel T. Characterization of the mGluR(1)-mediated electrical and calcium signaling in Purkinje cells of mouse cerebellar slices. *J Neurophysiol* 86(3): 1389−1397, 2001

Washburn MS, Numberger M, Zhang S, Dingledine R. Differential dependence on GluR2 expression of three characteristic features of AMPA receptors. *J Neurosci* 17: 9393−9406, 1997

Yoshimura M, Jessell T. Amino acid-mediated EPSPs at primary afferent synapses with substantia gelatinosa neurones in the rat spinal cord. *J Physiol (Lond)* 430: 315−335, 1990