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Effects of ondansetron on the activity of glutamate transporter type 3 and the modulation mechanism

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Ondansetron이 제 3형 글루탐산염 운반자의 활성도에 미치는 영향 및 조절 기전

지도 교수 도 상 환

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Effects of nicotine on the activity of glutamate transporter type 3 and the modulation mechanism

by

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A thesis submitted to the Department of Medicine in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Medical Science (Anesthesiology and Pain Medicine) at Seoul National University College of Medicine

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ABSTRACT

Background: The dysfunction of excitatory amino acid transporters (EAATs) is associated with neurologic disorders by increasing extracellular glutamate concentrations. Ondansetron, which is a serotonin antagonist used for anti-emesis, is known to provoke neurologic adverse events such as seizures. We investigated the effects of ondansetron on the EAAT type 3 (EAAT3, the major neuronal EAAT) and the role of protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3K).

Methods: EAAT3 was expressed in *Xenopus* oocytes following the injection of rat mRNA for EAAT3. Using the two-electrode voltage clamping method, the inward currents induced by L-glutamate were measured for 1 min. The trace of currents was integrated and described in microCoulombs (μC). Oocytes in study groups were exposed to ondansetron for 3 min. In addition, oocytes were exposed to PKC activator/inhibitors or PI3K inhibitors to elucidate the modulating mechanisms of ondansetron on EAAT3.

Results: Serial concentrations of ondansetron except 1 μM significantly decreased the activity of EAAT3 in a dose-dependent manner (IC_{50} = 8.57 μM, $p < 0.05$). In the kinetic study, 10 μM of ondansetron reduced V_{max} but not K_m compared to control group. Phorbol-12-myristate-13-acetate (PKC activator) abolished ondansetron-induced decrease in EAAT3 activity. Staurosporine and

chelerythrine, PKC inhibitors, significantly reduced the EAAT3 activity, but there were no interactions between ondansetron and PKC inhibitors. The two PI3K inhibitors (wortmannin and LY294002) decreased EAAT3 responses, although there were no differences among ondansetron, PI3K inhibitors, and ondansetron plus PI3K inhibitors groups.

Conclusions: Our results suggest that ondansetron decreases the activity of EAAT3 and this effect seems to be mediated by PKC and PI3K.

Keywords: Excitatory amino acid transporter type 3, glutamate, ondansetron, phasphatidylinositol 3-kinase, protein kinase C, *Xenopus* oocyte

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INTRODUCTION

Ondansetron is a 5-HT_3 receptor antagonist that is used for the prophylaxis and treatment of nausea and vomiting after surgery or chemotherapy. Ondansetron-induced seizures have been reported, but the related mechanism is not clear yet. The disinhibition of the central nervous system by blocking $GABA_A$ receptor was suggested as the related mechanism (1, 2). Because the 5-HT_3 receptor is a ligand gated ion channel, ondansetron may de- or hyperpolarize neuronal cells by changing ionic conductance or intracellular concentrations (3).

Glutamate is a major excitatory neurotransmitter in the central nervous system that is related to pain transduction and cognitive functions such as learning and memory (4). When the amount of extracellular glutamate increases, glutamate may cause neuronal damage (excitotoxicity). This phenomenon is related to neurologic diseases, including stroke, Alzheimer's disease, amyotrophic lateral sclerosis and epilepsy (5-8). Glutamate transporters (excitatory amino acid transporters, EAATs) keep the concentration of extracellular glutamate within physiological levels by transporting glutamate into neuronal or glial cells (5).

First cloned in 1992, five subtypes of EAATs have been identified. EAAT3 is the major neuronal glutamate transporter, and is abundant in the hippocampus, cerebral cortex, and basal ganglia (7). In addition to maintaining glutamate homeostasis, EAAT3 provides glutamate for the synthesis of GABA in neuronal cells. Dysfunction of EAAT3 has been linked

to seizures in a number of in vivo and in vitro studies (5, 9-11). However, no studies were conducted regarding the effects of ondansetron on EAAT3 activity.

The purpose of this study was to identify the effect of ondansetron on EAAT3 activity using the *Xenopus* oocyte expression system. Because serotonin is known to two important intracellular signaling molecules activate protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3K) signaling pathways (12, 13), we examined the involvement of PKC and PI3K as modulating mechanisms.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Seoul National University College of Medicine approved this study. Mature female *Xenopus laevis* frogs were purchased from Xenopus I (Dexter, MI, USA). Molecular biology and other chemical agents were purchased from Ambion (Austin, TX, USA) and Sigma (St. Louis, MO, USA). All the experiments were performed at the Biomedical Research Institute of Seoul National University Hospital.

1. Preparation of Xenopus oocytes

For the preparation of *Xenopus* oocytes, we followed the methods described by Do et al. (14). Operations were performed on ice after the anesthesia of frogs with 500 mL 0.2% 3-aminobenzoic acid ethyl ester in water to the level of no response to painful toe pinches. Ovarian lobules containing about 150–200 oocytes were removed from a 5 mm incision in the lower lateral abdomen and promptly placed in calcium-free OR-2 solution (containing in mM; NaCl 82.5, KCl 2, $MgCl_2$ 1, HEPES 5, and collagenase type Ia 0.1% [pH adjusted to 7.5]). The vitelline membrane of oocytes was removed, and shaking for about 2 h resulted in the defolliculation of oocytes. The oocytes were incubated in modified Barth's solution (containing in mM; NaCl 88, KCl 1, $NaHCO_2$ 2.4, $CaCl_2$ 0.41, $MgSO_4$ 0.82, $Ca(NO_3)_2$ 0.3, gentamicin 0.1, and HEPES 15 [pH 7.6]) at 18℃. We chose fully grown *Xenopus* oocytes in stage V or VI (15).

2. Expression of EAAT3 on Xenopus oocytes

Dr. M.A. Hediger (Brigham and Women's Hospital, Harvard Institute of Medicine, Boston, MA, USA) provided the rat EAAT3 complementary DNA (cDNA). The cDNA was subcloned using a commercial Bluescript-SKm vector. Plasmid DNA was linearized using the restriction enzyme Not I, and the messenger RNA (mRNA) was synthesized in vitro via a transcription kit. The resulting mRNA was quantified spectrophotometry, and was diluted in sterile water to the concentration of 30 ng/30 nL, and was injected into oocytes using an automated microinjector (Nanoject; Drummond Scientific Co., Broomall, PA, USA). The microinjected oocytes were incubated for 72 h at 18℃ prior to measuring currents.

3. Electrophysiological measurement

All experiments were performed at room temperature (21–23 ℃). Microelectrodes were prepared using a micropipette puller with 10 μl capillary glasses (Drummond Scientific Co., PA, USA). The tips of microelectrodes were modified to about 10 μm in diameter, which led to microelectrodes with a resistance of 1 to 5 MΩ when filled with 3 M KCl. To measure the current through the EAAT3 in the oocyte membrane, a defolliculated oocyte was placed in a concave recording chamber (a volume of 0.5 mL) and perfused with Tyrode's solution (containing in mM; NaCl 150,

KCl 5, $CaCl_2$ 2, $MgSO_4$ 1, dextrose 10 and HEPES 10 [pH 7.5]) at a rate of 3 mL/min before recording. The oocyte was voltage-clamped with two microelectrodes under a holding potential of -70 mV using a voltage clamp amplifier OC725-C (Warner Co., New Haven, CT, USA). Oocytes with a resting current greater than 0.6 μA were discarded. The inward currents induced by perfusion with L-glutamate were measured for 1 min at 125 Hz (first 5 s for baseline, 20 s for L-glutamate perfusion, and the remaining 35 s for washing-out with Tyrode's solution). The current traces were integrated and the resulting values were described as microcoulombs (μC), indicating the total amount of transported L-glutamate. Oocytes from at least three different frogs were used in each experiment.

4. Chemical treatment to Xenopus oocytes

To evaluate the dose-response effect of ondansetron on EAAT3 activity, ondansetron was diluted with Tyrode's solution to various final concentrations (1, 3, 10, 30, 100, 300, and 1000 μM). In the control group, oocytes were exposed to Tyrode's solution for 4 min. In the ondansetron groups, Tyrode's solution was perfused to oocytes for 1 min and then Tyrode's solution with ondansetron for the remaining 3 min before recording currents. Do et al. (14) showed that the median effective concentration of glutamate to trigger the EAAT3 response was 27.2 μM; therefore 30 μM L-glutamate was used to induce the current and sequential concentrations of L-glutamate (3, 10, 30, 100, and 300 μM) were perfused to oocytes in the control and ondansetron

groups to calculate V_{max} and K_m values of EAAT3 for L-glutamate.

To determine the involvement of PKC in EAAT3 activity, oocytes in both the control and ondansetron groups were exposed to 50 and 100 nM of phorbol-12-myristate-13-acetate (PMA, a PKC activator) at for 10 min before recording inward currents. In addition, oocytes were treated with staurosporine (2 μM for 1h) or chelerythrine (100 μM for 1h), two PKC inhibitors, to assess the effect of PKC inhibition on EAAT3. Wortmannin (1 μM for 1h) or LY294002 (50 μM for 1h), two PI3K inhibitors, were applied to the control and ondansetron-treated oocytes to determine whether the PI3K inhibitors affect EAAT3 activity.

5. Data processing and analysis

Because the expression of EAAT3 in oocyte membrane may be variable among batches, the measured data were sometimes to the control values from the same batch. For the statistical analysis, SPSS 18.0 (Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad, San Diego, CA, USA) were used. The results are shown as means ± S.E.M. and the one-way analysis of variance (ANOVA) with Student-Newman-Keuls correction or unpaired Student's *t*-test was utilized for multiple comparisons. The analyzed results were considered as statistically significant when the *p* values were less than 0.05.

RESULTS

When the oocytes were injected with EAAT3 mRNA, the application of L-glutamate caused inward currents, whereas no currents were recorded in oocytes without mRNA injection. In addition, when 100 μM of DL-threo-β-benzyloxyaspartate (a potent EAATs inhibitor) was applied with L-glutamate to the EAAT3 injected oocytes, inward currents were not measured nearly completely like bare oocytes without EAAT3 mRNA injection (data not shown). Through these experiments, we have confirmed the EAAT3 expression of oocytes. Exposure of oocytes injected with EAAT3 mRNA to 1–1000 μM ondansetron showed a dose-dependent decrease in EAAT3 responses to 30 μM L-glutamate, and 3 μM and higher concentrations of ondansetron showed significant differences from the control group (Fig. 1). The IC_{50} of ondansetron for reducing EAAT3 response was calculated to be 8.57 μM; therefore, 10 μM ondansetron was used in subsequent experiments.

Exposure of oocytes to 10 μM ondansetron for 3 min significantly reduced EAAT3 activity induced by 3, 10, 30, 100, and 300 μM L-glutamate (Fig. 2). Further analysis of the data showed that ondansetron decreased V_{max} with significance (1.4 ± 0.1 for the control vs. 0.9 ± 0.1 μC for ondansetron, $p < 0.05$). However, there was no difference in K_m values between the control and ondansetron groups (11.8 ± 2.8 for the control vs. 10.3 ± 3.7 μM, $p > 0.05$).

Treatment with PMA (100 nM for 10 min) increased EAAT3 activity with significance (1.00 ± 0.04 for the control vs. 1.22 ± 0.06 for PMA, $p < 0.05$),

which was in accordance with our previous reports (14, 16-18). When oocytes were exposed to PMA (100 nM) plus ondansetron, PMA recovered the attenuated EAAT3 activity by ondansetron (0.98 ± 0.05 for 100 nM PMA plus ondansetron, $p < 0.05$, Fig. 3). A lower concentration of PMA (50 nM) did not significantly increase EAAT3 activity compared with that in the control group, but it also restored the ondansetron-induced decrease in EAAT3 activity (1.00 ± 0.04 for the control, 1.11 ± 0.05 for 50 nM PMA, 0.82 ± 0.03 for ondansetron, and 0.94 ± 0.06 for 50 nM PMA plus ondansetron, Fig. 3).

Oocytes pre-treated with staurosporine or chelerythrine, two PKC inhibitors, showed significantly decreased EAAT3 responses (in staurosporine, 1.00 ± 0.06 for the control vs. 0.74 ± 0.04 for staurosporine, $p < 0.05$; in chelerythrine, 1.00 ± 0.04 for the control vs. 0.71 ± 0.04 for chelerythrine, $p < 0.05$, Fig. 4). However, no statistical differences in EAAT3 activity were detected among the PKC inhibitors, ondansetron, and a PKC inhibitor plus ondansetron groups (Fig. 4).

The pretreatment of oocytes with two PI3K inhibitors, wortmannin (1 μM for 1h) or LY294002 (50 μM for 1h), significantly reduced EAAT3 activity (in wortmannin, 1.00 ± 0.05 for the control vs. 0.80 ± 0.03 for wortmannin, $p < 0.05$; in LY294002, 1.00 ± 0.08 for the control vs. 0.78 ± 0.07 for LY294002, $p < 0.05$). However, there were no significant differences among oocytes exposed to PI3K inhibitors, ondansetron, and a PI3K inhibitor plus ondansetron (Fig. 5).

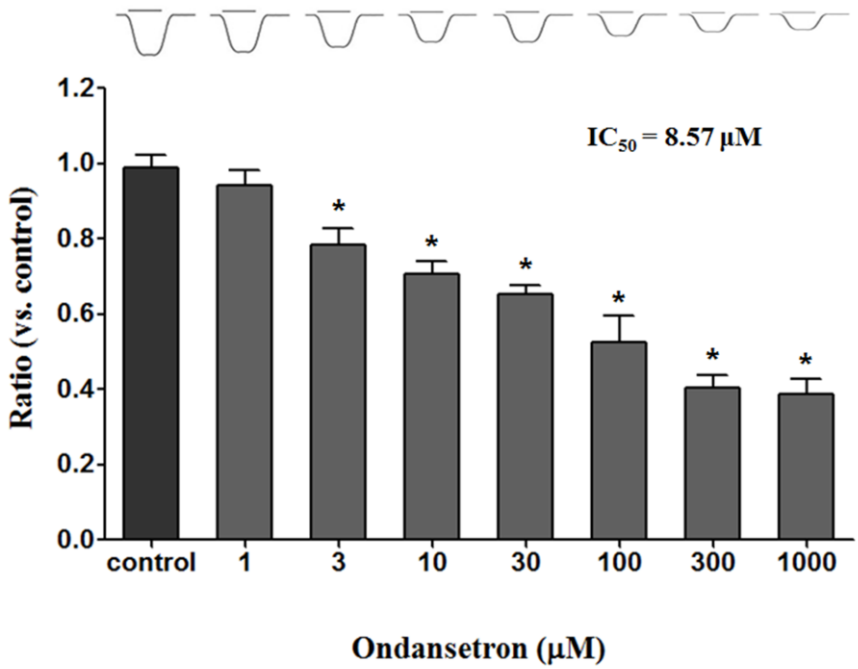

Fig. 1. The dose-responses of excitatory amino acid transporter type 3 (EAAT3) induced by 30 µM L-glutamate in the presence of various concentrations of ondansetron (1, 3, 10, 30, 100, 300, and 1000 µM). Oocytes were exposed to ondansetron for 3 min before response measurements were taken. Data are expressed as mean ± S.E.M. (n = 73 in the control group, n = 20–33 in ondansetron study groups). *p < 0.05 compared to the control group.

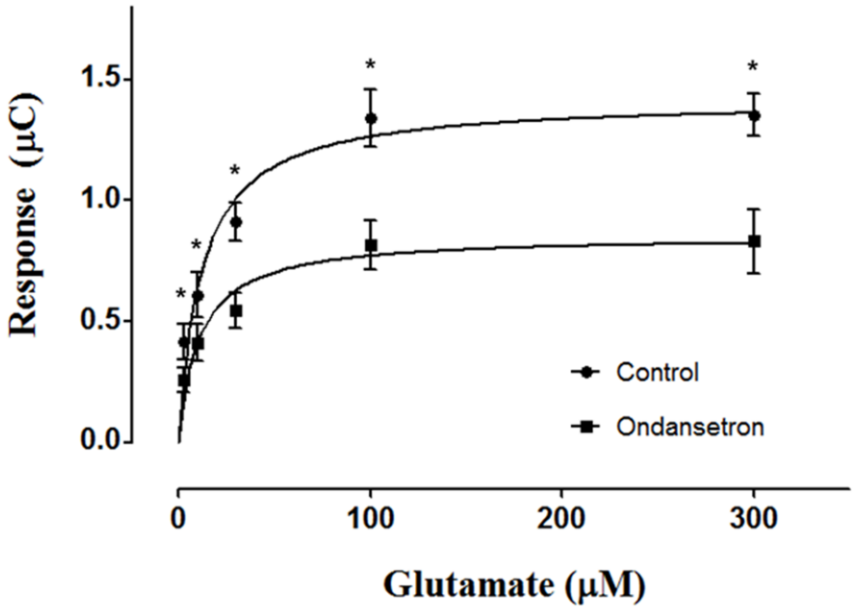

Fig. 2. Kinetic study of EAAT3 to L-glutamate in the presence or absence of 10 μM ondansetron for 3 minutes. In addition to the typical current responses induced by L-glutamate in the control group, oocytes treated with ondansetron showed decreased responses induced by 3, 10, 30, 100, and 300 μM L-glutamate with statistical significance. Data are expressed as mean ± S.E.M. (n = 15–27 in each group). *p < 0.05 compared to the corresponding control groups.

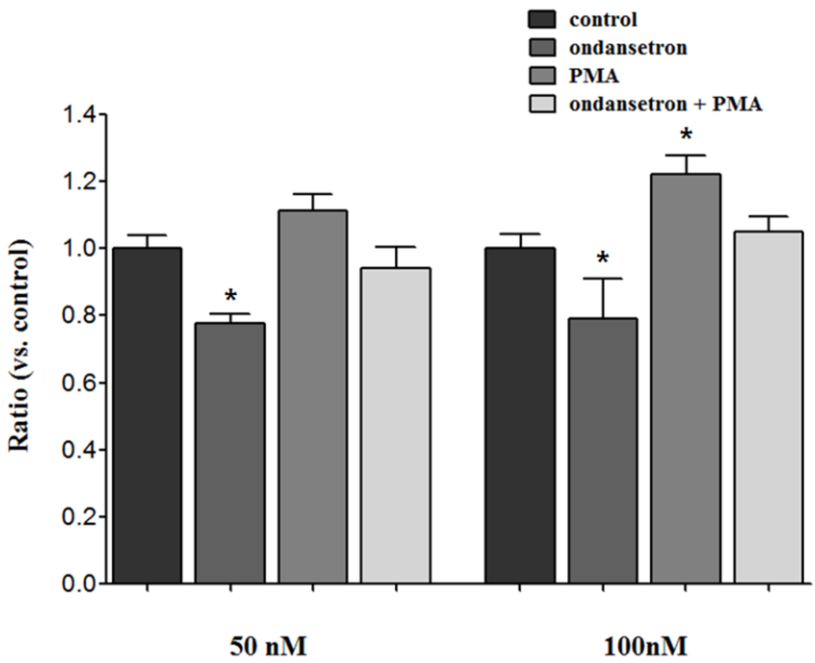

Fig. 3. Effects of protein kinase C (PKC) activation on EAAT3 activity in the presence or absence of 10 μM ondansetron for 3 min. The ondansetron-induced decrease in EAAT3 activity was attenuated when ondansetron-exposed oocytes were treated with 100 nM PMA for 10 min. A lower concentration of PMA (50nM), which showed no increase compared to the control group, also abolished the decreased EAAT3 activity by ondansetron. PMA, phorbol-12-myristate-13-acetate. Data are expressed as mean ± S.E.M. (n = 11–22 in each group). *$p < 0.05$ compared to the control group.

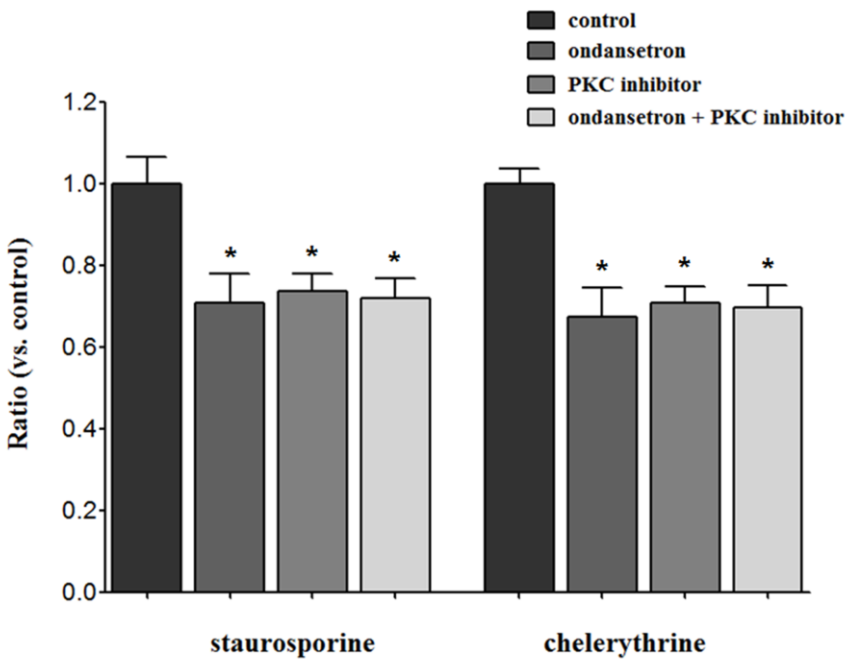

Fig. 4. Effects of PKC inhibition on EAAT3 activity in the presence or absence of 10 μM ondansetron for 3 min. Oocytes exposed to ondansetron, PKC inhibitor (2 μM staurosporine or 100 μM chelerythrine) or PKC inhibitor plus ondansetron showed a significant reduction in EAAT3 activity compared to the control group. However, no significant differences of EAAT3 activity were detected among oocytes treated with the PKC inhibitor, ondansetron, or both. Data are expressed as mean ± S.E.M. (n = 12–24 in each group). *$p < 0.05$ compared to the control group.

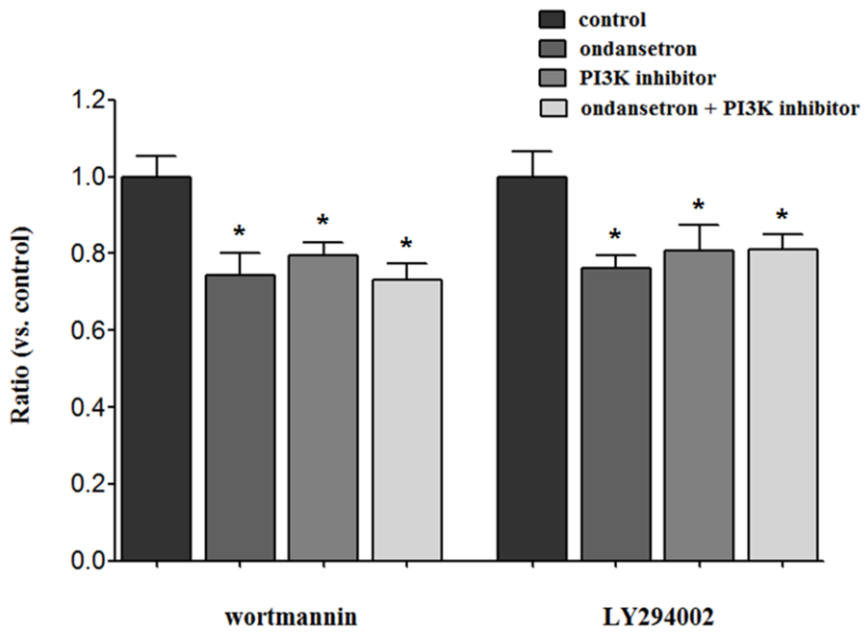

Fig. 5. Effects of phosphatidylinositol 3-kinase (PI3K) inhibition on EAAT3 activity in the presence or absence of 10 μM ondansetron for 3 min. Whereas preincubation of oocytes with PI3K inhibitors (1 μM wortmannin and 50 μM LY294002) for 1 h, reduced EAAT3 activity with statistical significance, the responses were not different among the ondansetron, PI3K inhibitors, or ondansetron plus PI3K inhibitors groups. Data are expressed as mean ± S.E.M. (n = 12–26 in each group). *$p < 0.05$ compared to the control group.

DISCUSSION

Our results show that ondansetron decreases EAAT3 activity in a dose-dependent manner and suggest that PKC and PI3K may work as modulating mechanisms. This is the first report about the effect of ondansetron on EAAT3 activity.

Ondansetron selectively inhibit 5-HT_3 receptors. These receptors are known to mediate nausea and vomiting in chemoreceptor trigger zone, in the vomiting center of reticular formation, and in the vagal afferents of enteric neurons (19, 20). Constipation, dizziness and headache are the most common adverse events. Recently, the U.S. Food and Drug Administration announced the risk of ondansetron causing QT prolongation and the single intravenous dose of ondansetron was limited up to 16 mg. This effect is mediated by blocking the human ether-a-go-go-related gene potassium channel in the myocardium, disturbing the rapid delayed rectifier potassium current (21).

Several studies proposed the relation of ondansetron to protein kinase pathways. Liu et al. showed the alleviating effect of ondansetron on hepatic injury through the p38 mitogen-activated protein kinase (MAPK) in a rat hemorrhagic shock model (22). In addition, they demonstrated that ondansetron suppresses platelet aggregation by decreasing agonist-induced IP3 production and MAPK phosphorylation, not involving 5-HT_3 pathway (23). Although it is not clear how ondansetron works on EAAT3 activity yet, our results imply that the attenuating effect of ondansetron on EAAT3 may be

mediated via PKC and PI3K.

Glutamate transport by EAATs is a process coupled with an exchange of sodium and potassium, and that is capable of making intracellular glutamate concentration 10,000-fold greater than in the extracellular space (24, 25). Glutamate transporters, especially EAAT3, contribute to the maintenance of GABA neurotransmission by supplying glutamate which is a precursor for the synthesis of GABA (26, 27). Kainic acid-induced status epilepticus has been shown to be related to the decreased expression of EAAT3 in a restricted area of the hippocampus in rats (11). Rothstein et al. (8) reported that the knockout of the EAAT3 gene with synthetic antisense oligonucleotide caused seizures in rats. Therefore, we hypothesized that ondansetron would have an effect on EAAT3 activity.

Ondansetron-related seizures have been reported in the literature, but the precise mechanism has not been elucidated. Sharma and Raina (28) presented a generalized tonic-clonic seizure after intravenous administration of 8 mg ondansetron in a patient on chemotherapy. In addition, one case reported a 4-year-old child who developed acute dystonia, hypoglycemia and seizure after the injection of ondansetron (29). Singh et al. (30) encountered seizures in three patients 12–22 min after intravenous injection of 4 mg ondansetron. As related mechanisms, it was suggested that ondansetron-induced suppression of GABA and the glycine response may explain the mechanism of ondansetron-related seizures in vivo (2, 31). 5-HT_3 receptor antagonists are known to inhibit the GABA actions by acting as inverse agonists at the benzodiazepine site of the $GABA_A$ receptor (1). Squires and Saederup (32) demonstrated that

ondansetron reverses the inhibitory effect of GABA in whole rat forebrain membranes.

On the other hand, there were two reports about the anti-convulsive effects of ondansetron in rats and mice. Balakrishnan *et al.* (33) reported that low-dose ondansetron reduced the incidence of maximal electric shock-induced seizures However, there was no protection in the high-dose ondansetron group, and the authors could not explain the protective mechanism of ondansetron. Recently, Jain *et al.* (34) showed the anti-convulsive effect of ondansetron in mice. In their study, ondansetron worked against electrically-induced seizures but it did not inhibit pentylenetetrazole-induced seizures, which are caused by the activation of N-methyl-D-aspartate receptors and the inhibition of GABA receptors (34). It was suggested that the neuro-protective action of ondansetron was due to alterations in the influx of cations (such as Na^+, K^+, and Ca^{2+}) via 5-HT_3 receptors, which prevent neuronal depolarization (34). However, this protective action against seizure might not have clinical significance, because 5-HT_3 receptors are distributed in low density in the cerebral cortex, hippocampus, and amygdala (35-37).

We performed a dose-response study with 1–1000 μM ondansetron, which was much higher than the clinical concentration of ondansetron in CSF. The concentrations of ondansetron in blood or cerebrospinal fluid (CSF) during seizures still remain unknown. However, clinical reports showed that common therapeutic doses of ondansetron can cause seizures (28-30). The therapeutic concentration of ondansetron in blood is 0.08–0.8 μM (38). In Colthup's study, the maximal concentration of ondansetron in blood after intravenous

injection of 8 mg ondansetron for 15 min in young and elderly groups were 0.23 μM and 0.31 μM, respectively (39). In addition, Simpson et al. (40) showed that ondansetron concentrations in CSF were less than 15% of that in blood. Therefore, very low concentrations of ondansetron may be related to seizures in vivo. In our dose-response study with the *Xenopus* oocyte expression system to evaluate EAAT3 activity in vitro, 1 μM ondansetron failed to reduce EAAT3 activity compared to the control group. We suppose that the discrepancy between concentrations of ondansetron in vivo and in vitro came from differences in the study models. In addition, high doses of ondansetron might be required to decrease EAAT3 activity because EAAT3 are mainly located on synaptic clefts.

Comparing with other types of glutamate transporters, EAAT3 is specific because only 20% of EAAT3 are present in the plasma membrane and the others present in the cytosol (41). In addition, the trafficking of glutamate transporter to the plasma membrane is modified rapidly that the estimated half-life of EAAT3 was around 5-7 min in C6 glioma and primary neuronal cultures (5, 42). Therefore, we think drug exposure for 3 min is enough to check the kinetic characteristic of EAAT3. EAAT3 activity is known to be controlled by two possible mechanisms; cell surface expression and/or the affinity of EAAT3 for glutamate (43, 44). Our kinetic study showed a significant decrease in V_{max} but not in K_m of EAAT3 for L-glutamate. This suggests that ondansetron suppresses the redistribution of EAAT3 from cytosol to the plasma membrane without changing the affinity of EAAT3 for L-glutamate.

PKC is one of the intracellular signaling enzymes and its activation is known to increase the trafficking of EAAT3 to the cell surface, which changes V_{max} of EAAT3 for L-glutamate (16, 45, 46). Of the isoforms of PKC, PKCα and PKCε are known to increase the activity of EAAT3 (43, 47, 48). PKCα facilitates the traffic rate in cell surface expression of EAAT3, whereas PKCε increases EAAT activity without those effects in C6 glioma cells (47, 48). In *Xenopus* oocytes, there are PKC isoforms of α, β1, β2, γ, δ and ζ, but not PKCε (49). Our study showed that the PKC activator abolished the action of ondansetron on EAAT3 and there was no additive or synergistic effect between ondansetron and the PKC inhibitor. Therefore, the effect of ondansetron on EAAT3 activity seems to be mediated by PKC.

PI3K is the key enzyme involved in the synthesis of 3-phosphoinositides, which have roles in the regulation of PKC activity as well as membrane trafficking (50). The addition of some phosphoinositides has been demonstrated to activate the ε, η, and ζ isoforms of PKC. PI3K activation facilitates the EAAT3 expression in the cell membrane (43, 51). Although PKC and PI3K are independent signaling molecules, the signaling pathways involving PKC and PI3K can interact with each other and PKC can work as a molecule downstream of PI3K (52). Ettinger et al. (53) reported that PI3K interacts with PKC isozymes. It is reported that wortmannin, a PI3K inhibitor, reduced EAAT3 expression level in biotinylation and immunoblotting experiments and induced intracellular clustering of EAAT3 in confocal microscopic analysis (15, 43). In this study, PI3K inhibitors decreased EAAT3 activity and the exposure of ondansetron pre-treated oocytes to PI3K

inhibitors did not show additive or synergistic suppression of EAAT3 activity. Therefore, PI3K may be involved as a modulator in ondansetron effect on EAAT3 activity.

The *Xenopus* oocyte expression system, introduced by Gurdon et al. (54), has been utilized as a unique method for the study of membrane proteins such as receptors and ion channels. Oocytes of *Xenopus* laevis frogs only have the lysophosphatidate receptor in the cell membrane but otherwise contain all the intracellular signaling apparatus of mammalian cells (55, 56). It is known that *Xenopus* oocytes can faithfully translate foreign DNA or mRNA into their corresponding proteins, and membrane proteins in *Xenopus* oocytes work similarly to those in human cells (24, 54, 57). Therefore, voltage clamping of *Xenopus* oocytes is more suitable for the study of a single subtype of EAATs than using synaptosome or nerve cells.

This study has some limitations. First, we did not identify direct expression level of EAAT3 at oocyte membranes using blotting analysis or immunostaining method. In addition, as a next step, an electrophysiological study using rat brain tissue slices could allow the recording of the native EAAT3 current to extrapolate our results to mammalian neuronal cells. Second, our study was focused on the effect of ondansetron on EAAT3 activity. Additional studies of EAAT1 and 2 to identify the selectivity of ondansetron are needed before specifying its effects on EAAT3.

In conclusion, ondansetron attenuates the activity of EAAT3. PKC and PI3K seem to modulate the effect of ondansetron on EAAT3 activity. This study may explain another mechanism for ondansetron-induced seizures.

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국 문 초 록

서론: Excitatory amino acid transporter (EAAT)는 외부의 글루탐산염 (glutamate)를 신경세포 안으로 이동시키는 과정을 통해 글루탐산염의 신경독성으로부터 신경세포를 보호한다. 항구토제로 사용되는 온단세트론 (ondansetron)은 발작과 같은 신경학적인 부작용을 초래하는 것으로 알려져 있다. 본 연구에서는 온단세트론이 신경세포에 주로 존재하는 EAAT3에 대한 영향과 그에 관련된 기전으로서 protein kinase C (PKC)와 phosphatidylinositol 3-kinase (PI3K)의 연관성을 밝히고자 한다.

방법: Rat EAAT3 mRNA를 *Xenopus* 난모세포에 미세주입 하여 EAAT3를 세포막에 발현시켰다. 2 electrode voltage clamp method를 사용하여 1분간 L-glutamate에 의해 발생하는 내향 전류의 변화를 측정하여 전하량을 구하였다. 난모세포에 정해진 농도의 온단세트론을 3분간 노출시킨 뒤 EAAT3 활성도 및 온단세트론에 의한 약동학적 변화 (*Km, Vmax*)를 알아보았다. 또한 PKC 활성제와 억제제, 그리고 PI3K 억제제로 oocyte를 전처지하여 PKC와 PI3K의 연관성을 확인하였다.

결과: 온단세트론은 3 μM 이상의 농도에서 농도의존적으로 EAAT3의 활성도를 감소시켰다 (IC_{50} = 8.57 μM, $p < 0.05$). 약동학적

연구에서 V_{max}는 온단세트론 처치군과 대조군 사이에 유의한 차이를 보였으나 K_m은 유의한 차이를 보이지 않았다. Phorbol-12-myristate-13-acetate (PKC 활성제)는 온단세트론에 의해 저하된 EAAT3의 활성도를 회복시켰다. PKC 억제제인 staurosporine과 chelerythrine은 EAAT3 활성도를 감소시켰으나, 온단세트론과 PKC 억제제와의 상호작용은 관찰되지 않았다. PI3K 억제제인 wortmannin and LY294002도 EAAT3의 활성도를 유의하게 감소시켰으나, 온단세트론과의 상호작용은 나타나지 않았다.

결론: 온단세트론은 EAAT3의 활성도를 감소시킨다. 이 과정에서 PKC와 PI3K가 관여하는 것으로 추정된다.

주요어: Excitatory amino acid transporter type 3, glutamate, ondansetron, phosphatidylinositol 3-kinase, protein kinase C, *Xenopus* oocyte

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