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Full Length Research Paper

N-acetylaspartylglutamate (NAAG) exhibits anti-inflammatory effects on carrageenan-induced paw edema model of inflammation in rats

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The present study was designed to evaluate anti-inflammatory effects of *N*-acetylaspartylglutamate (NAAG), as a presynaptic mGlu3 receptor agonist on carrageenan-induced paw edema in rats. NAAG was administered intraperitoneally (10 mg/kg) 20 min before the intraplantar injection of the carrageenan. Neutrophil infiltration (MPO activity), lipid peroxidation (MDA assay), free radical scavenging activity (SOD and GPx assay), IL-1β, TNF-α, and PGE_2 levels were assessed in the paw tissue of the NAAG treated rats when compared with the control rats. Results showed that intraperitoneal administration of the NAAG significantly reduced maximum paw volume by 52.5% and total inflammatory response by 56.9%, 4h after the induction of inflammation ($p < 0.001$). Injection of the NAAG resulted in a marked reduction of MPO activity in the inflamed paw by 80.2% in comparison with the control group ($p < 0.01$). Moreover, NAAG not only reduced the MDA levels to 54.3%, but also enhanced the SOD activity in the NAAG treated group up to 25% when compared with the control group. Levels of IL-1β, TNF-α, and PGE_2 in the NAAG treated group were respectively reduced by 72.4%, 23%, and 13%. Generally, the NAAG activates mGlu3 receptors on sensory neurons, resulting in reduction of cAMP levels and inhibition of glutamate release. Overall, our results suggest that NAAG activation of mGlu3 receptors on the sensory neurons negates the effects of PGE_2 and reduces the sensory neuron communication of inflammation.

Key words: *N*-acetylaspartylglutamate, glutamate, inflammation, carrageenan, cytokines.

INTRODUCTION

N-acetylaspartylglutamate (NAAG) serves as a cotransmitter with several small amine transmitters, including glutamate and is widely distributed in the central and peripheral nervous systems of mammals. NAAG acts as an agonist at metabotropic type II glutamate receptors, particularly mGluR3, and this would provide reduction of glutamate release; because activation of mGluR3 appears to reduce the synaptic release of glutamate (Neale et al., 2000; Yamamoto et al., 2004). Inflammation causes elevated glutamate release from primary afferents leading to increased activation of excitatory amino acid receptors in peripheral tissue (Yang and Gereau 2003; Liu et al., 2009; Miller et al., 2011). The peripheral roles of metabotropic glutamate receptors (mGluRs) are supported by morphological studies, demonstrating that all the three groups of mGluRs are expressed in the peripheral afferents (Zhou et al., 2001; Chen et al., 2009).

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Physical trauma or anoxia causes excessive glutamate release from neurons (Neale et al., 2005) and subsequently the neurotransmitter interaction with G-protein-coupled metabotropic glutamate receptors results in a cascade of inflammatory events including: activation of phospholipase C or adenylyl cyclase, production of arachidonic acid, activation of cyclo-oxygenase 2 (COX-2), and generation of prostanoids, such as prostaglandin E_2 (PGE_2) (Bazan, 2001; Yang and Gereau, 2002; Bauer et al., 2008; Zibell et al., 2009).

Since glutamate contributes to the inflammatory processes and NAAG reduces the level of glutamate in the peripheral and central nervous system by acting at mGluR3, we aimed at evaluating the protective effects of NAAG in a rat model of acute local inflammation (carrageenan-induced paw edema). We measured the levels of the inflammatory and biochemical factors including myeloperoxidase (MPO), malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), cytokine levels (IL-1β and TNF-α), and PGE_2 in paw tissue of the rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (180 to 200 g) were used in this study. The animals were given food and water *ad libitum*. They were housed in the Animal House of the Tabriz University of Medical Sciences at a controlled ambient temperature of 25 ± 2°C and a 12 h light/12 h dark cycle. The present study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Tabriz University of Medical Sciences, Tabriz-Iran.

Carrageenan-induced paw edema

Rats were randomized into 2 groups of control and treatment consisting of 6 rats each. The animals received an intraperitoneal injection of either 500 µl of normal saline or 500 µl of NAAG solution in saline (10 mg/kg) 20 min before intraplantar injection of 100 µl of carrageenan 1% (w/v) in the right hind paw (Bilici et al., 2002).

The volume of the paw was measured by plethys-mometer immediately prior to the carrageenan injection and then at hourly intervals from 1 to 4 h afterward. Data were expressed as a percentage of increase in the paw volume and were compared with those of pre-injection values. After measurement of the paw edema, the rats were sacrificed by an overdose of pentobarbital and then the inflamed hind paws were excised with a guillotine.

MPO activity

Carrageenan edema was induced as described earlier. The rats were sacrificed 4 h later by an overdose of pentobarbital and the inflamed hind paws were excised with a guillotine. MPO activity was assayed according to the method of Bradley et al. (1982). The inflamed paw tissues were finely chopped in 1 ml of 50 mM potassium phosphate buffer (pH = 6), containing 0.5% hexa-decyl-trimethyl-ammonium-bromide (HTAB). The chopped tissues were homogenized (50 mg/ml) in phosphate buffer (50 mM) (pH = 6) containing 0.5% HTAB for 5 × 45 s at 1 min intervals at 8500 rpm. The homogenates were sonicated for 10 s, frozen and thawed 3 times, then sonicated for further 10 s and centrifuged at 3000 rpm, in 5°C for 30 min. The supernatant (100 µl) was added to 2.9 ml of phosphate buffer (50 mM; pH = 6) containing 0.167 mg/ml of O-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. Five minutes later the reaction was stopped by adding 0.1 ml of 1.2 M hydrochloric acid. The absorbance was measured spectrophotometrically at 400 nm. The concentrations were calculated by using calibration curve and were expressed as miliunits of MPO in 100 mg weight of wet tissue (mU/100 mg).

MDA assay

Carrageenan edema was induced as described earlier. The rats were sacrificed 4 h later by an overdose of pentobarbital and the inflamed hind paws were excised with a guillotine. Lipid peroxidation in the rat paw tissues was quantified by determination of the MDA levels according to the method of Olgen and Coban, (2003). The tissues were homogenized in 1.15% KCl to achieve a 10% (w/v) homogenate. The homogenates were centri-fuged and 1 ml of each supernatant was added to a mixture containing 3 ml of O-phosphorous acid (1%) and 1 ml of thiobarbituric acid (TBA; 0.67%) in an aqueous solution. The reaction mixture was heated for 60 min up to 95°C, and then was cooled in a room temperature. Then, 3 ml of n-butanol was added to each test tube, the tubes were shaken vigorously and then centrifuged. The absorbance of n-butanol phase was measured spectrophotometrically at 532 nm and the amount of thiobarbituric acid reactant substances (TBARS) was calculated from a calibration curve and reported as nmol MDA/100 mg tissue.

SOD and GPx assays

Carrageenan edema was induced as described earlier. The rats were sacrificed 4 h later by an overdose of pentobarbital and the inflamed hind paws were excised with a guillotine. The rat paw tissues were homogenized in 1.15% KCl solution, 10% (w/v). The homogenates were centrifuged and the supernatants were used for SOD and GPx assays. SOD activity was measured according to the method of Paoletti et al. (1986). SOD accelerates the dismutation of the toxic superoxide radical produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase to generate the superoxide radicals reacting with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The SOD activity was then measured by the degree of inhibition of the formazin production. The absorbance was measured at 505 nm; one unit of SOD was defined as the amount of the enzyme that caused a 50% inhibition of the INT reduction.

GPx catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), the oxidized glutathione is imme-diately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm was measured. GPx activity was determined according to the method of Paglia and Valentine (1967). Tissue GPx and SOD were measured by Randox kit (UK) and were reported as Unit/mg protein.

Determination of IL-1β and TNF-α and PGE_2 levels in rat paw tissue

The rat paw tissues were collected 4 h after inducing the inflammation by carrageenan and were homogenized (1 g/4 ml) in extraction buffer containing 1 mM of phenylmethylsulfonyl fluoride, 1 µg/ml o f aprotinin and 0.05% Tween-20 in phosphate-buffered

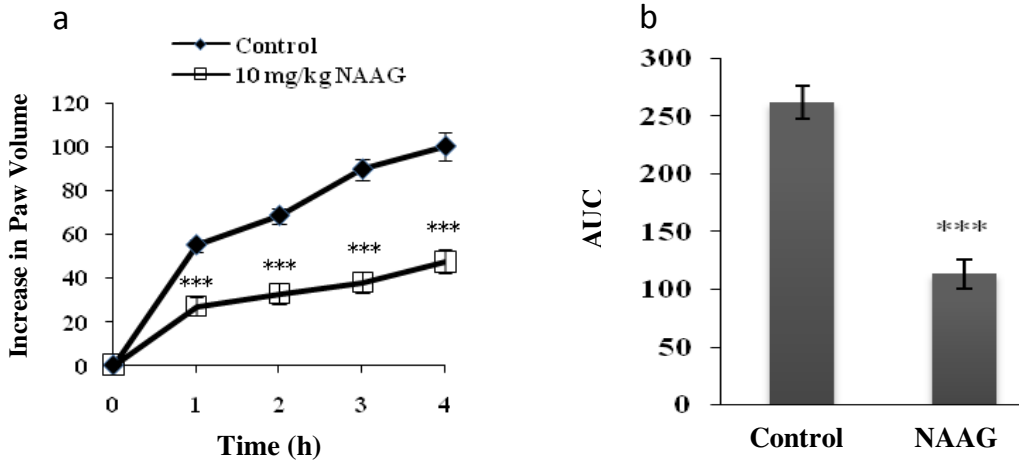

Figure 1. The effect of intraperitoneal injection of NAAG (10 mg/kg) on carrageenan-induced paw edema in rats. NAAG was injected 20 min before induction of inflammation by carrageenan; (a) Results are expressed as percentage of increase in paw volume from control (pre-drug) values; (b) Total edema responses measured as area under the curve (AUC) of the treated rats compared to control. Each point represents mean ± SEM of six rats per group. Asterisks indicate significant changes from the control value (***$p < 0.001$).

saline (PBS). For measurement of PGE_2, the paw tissues were added to a lysis solution (1 g/4 ml) containing 80% methanol, 20% saline and 1 mM indomethacin. Tissues were homogenized on ice with a polytron and centrifuged at 5000 ×g for 15 min. The supernatants were stored at -80°C until analysis. IL-1β and TNF-α level in the super-natants were determined (Magari et al., 2003) using ELISA kits specific for rat IL-1β and TNF-α (eBioscience, USA). PGE_2 level was determined using a PGE_2 EIA kit (Cayman Chemical Company, USA). The sensitivities of the assays for IL-1β, TNF-α, and PGE_2 were 31, 39.1, and 7.8 pg/ml, respectively.

Statistical evaluation

Data were presented as mean ± standard error of the mean (SEM). We assessed carrageenan-induced inflam-mation data by one-way analysis of variance (ANOVA) and the significant differences were examined by the Tukey post-hoc test. The mean values were compared using the two-tailed t-test as appropriate. P-values < 0.05 were considered statistically significant.

RESULTS

Effects of NAAG on carrageenan-induced paw edema

The protocol involved intraperitoneal injection of 10 mg/kg of NAAG 20 min before induction of the inflammation by intraplantar injection of 100 µl carra-geenan 1%. Induction of acute inflammation in control rats resulted in a significant increase in the paw volume, 1 h after the intraplantar injection of the carrageenan reaching a peak of inflammation after 4 h. In the control group, at the 4th hour, the paw volume increased by 99.99 ± 6.62% in comparison with pre-carrageenan control value. The inhibition of edema formation by NAAG with a dose of 10 mg/kg was prominent at all hours ($p < 0.001$) after the carrageenan injection (Figure 1a). Intraperitoneal injection of rats with 10 mg/kg NAAG 20 min before induction of the inflammation resulted in a significant reduction in the paw volume by 52.5% after 4 h ($p < 0.001$) (Figure 1a). Total inflammatory response measured as area under the curve (AUC) was significantly reduced in the NAAG (10 mg/kg) treated rats by 56.9% ($p < 0.001$) compared to the control group (Figure 1b).

Effect of NAAG on MPO activity

The intraplantar injection of the carrageenan into the rat hind paw induced an inflammation (swelling and erythema) that was maximal by the 4th hour following the carrageenan administration and produced a time-dependent polymorphonuclear leukocytes (PMN) accumulation into the paw tissue. Intraperitoneal injection of a single dose of NAAG (10 mg/kg) led to a marked reduction of MPO activity, indicative of reduced neutrophil accumulation in the inflamed paw (from 146.46 ± 16.37 mU/100 mg tissue to 29 ± 6 mU/100 mg tissue, n = 6) that means more than 80% reduction in PMN influx ($p < 0.01$) (Figure 2).

Effect of NAAG on MDA level

To determine the lipid peroxidation, MDA levels were measured in hind paw tissue homogenates. The intra-plantar injection of the carrageenan into the rat hind paw

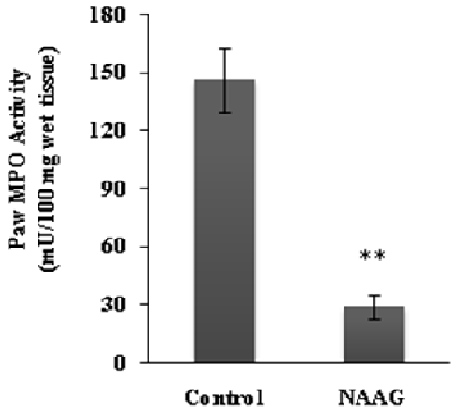

Figure 2. The effect of intraperitoneal injection of NAAG (10 mg/kg) on MPO activity in the paw of rats. Inflammation was induced in the paw by intraplantar injection of carrageenan 20 min after intraperitoneal injection of NAAG. Data represented as mean ± SEM of six rats per group. Asterisks indicate significant changes from the control value (**p < 0.01).

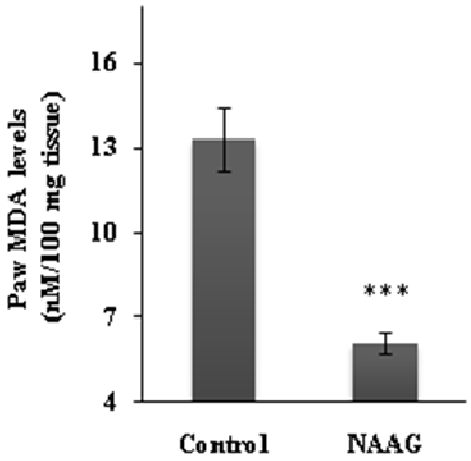

Figure 3. The effect of intraperitoneal injection of NAAG (10 mg/kg) on MDA level in the paw of rats. Inflammation was induced in the paw by intraplantar injection of the carrageenan 20 min after intraperitoneal injection of NAAG. Data represented as mean ± SEM of six rats per group. Asterisks indicate significant changes from the control value (***p < 0.001).

induced the inflammatory response that was maximal by the 4th hour following carrageenan administration. At this time point, the level of MDA in the control group (carrageenan) was 13.32 ± 1.15 nmol/100 mg tissue. Intraperitoneal administration of NAAG (10 mg/kg) diminished MDA level from 13.32 ± 1.15 nmol/100 mg tissue to 6.08 ± 0.35 nmol/100 mg tissue in NAAG treated group. MDA level in the NAAG treated group was reduced by 54.36% in comparison with the control group (p < 0.001) (Figure 3).

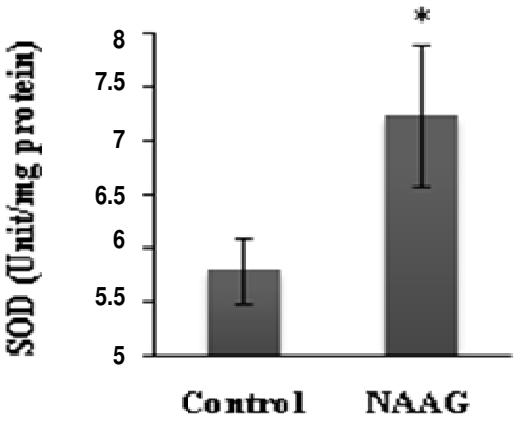

Figure 4. The effect of intraperitoneal injection of NAAG (10 mg/kg) on SOD activity in inflamed paw of rats. Inflammation was induced in the paw by intraplantar injection of the carrageenan 20 min after intraperitoneal injection of NAAG. Data represented as mean ± SEM of six rats per group. Asterisks indicate significant changes from the control value (*p < 0.05).

Effect of NAAG on SOD activity

SOD activity was measured in the hind paw tissue homogenates. The intraplantar injection of the carrageenan into the rat hind paw induced the inflammatory response that was maximal by the 4th hour following carrageenan administration. At this time point, SOD activity in the control group (carrageenan) was 5.79 ± 0.31 unit/mg protein which was increased to 7.24 ± 0.65 unit/mg protein in the NAAG (10 mg/kg) treated group. This revealed that treatment by NAAG restored free radical-scavenging activity of inflamed rat paw tissue. SOD activity in NAAG treated group was increased up to 25% in comparison with that in the control group (p < 0.05*)* (Figure 4).

Effect of NAAG on GPx activity

GPx is a free radical scavenging enzyme cleaving hydrogen peroxide and lipid peroxide. GPx activity was measured in the hind paw tissue homogenates. The intraplantar injection of the carrageenan into the rat hind paw induced the inflammatory response that was maximal at the 4th hour following carrageenan administration. At this time point, GPx activity in the control (carrageenan) and the NAAG groups was 4.44 ± 0.17 and 4.64 ± 0.13 unit/mg protein, respectively. This

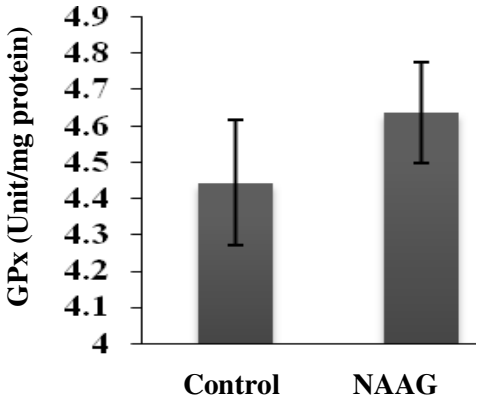

Figure 5. The effect of intraperitoneal injection of NAAG (10 mg/kg) on GPx activity in inflamed paw of rats. Inflammation was induced in the paw by intraplantar injection of the carrageenan 20 min after intraperitoneal injection of NAAG. Data represented as mean ± SEM. There was no significant change in the GPx activity compared with the control group.

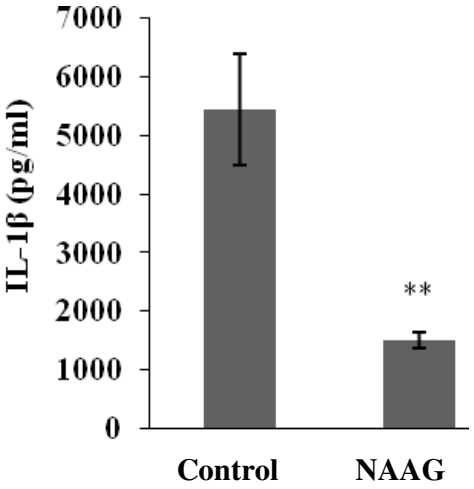

Figure 6. The effect of intraperitoneal injection of NAAG (10 mg/kg) on IL-1β level in inflamed paw of rats. Inflammation was induced in the paw by intraplantar injection of the carrageenan 20 min after intraperitoneal injection of the NAAG. Data represented as mean ± SEM of six rats per group. Asterisks indicate significant changes from the control value (**p < 0.01).

result showed that administration of the NAAG did not modify the GPx activity (Figure 5).

Effect of NAAG on IL-1β level in the inflamed paw

The effect of NAAG (10 mg/kg) on the levels of IL-1β in the inflamed paws was examined 4 h after the induction of inflammation. As shown in Figure 6, treatment of rats with the NAAG (10 mg/kg) resulted in a pronounced reduction of IL-1β level in the inflamed paw tissue (from 5446.4 ± 957.6 to 1506.2 ± 137.5 pg/ml) and this means more than 70% reduction of IL-1β level in the NAAG treated group in comparison with the control group ($p < 0.01$) (Figure 6).

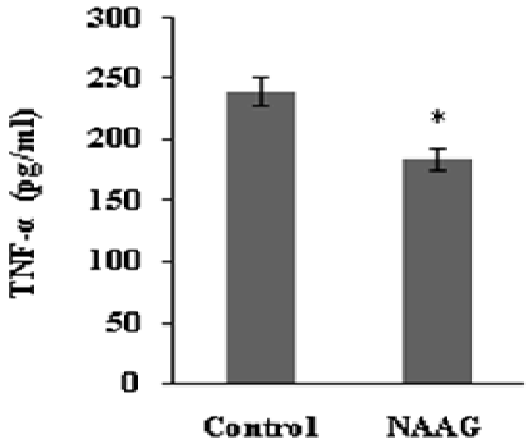

Figure 7. The effect of intraperitoneal injection of NAAG (10 mg/kg) on TNF-α level in inflamed paw of rats. Inflammation was induced in the paw by intraplantar injection of the carrageenan 20 min after intraperitoneal injection of the NAAG. Data represented as mean ± SEM of six rats per group. Asterisks indicate significant changes from the control value (*p < 0.05).

Effect of NAAG on TNF-α level in the inflamed paw

The effect of NAAG (10 mg/kg) on the levels of TNF-α in the inflamed paw was examined 4 h after the induction of inflammation. As shown in Figure 7, treatment of rats with the NAAG (10 mg/kg) resulted in a significant reduction of TNF-α level in the inflamed paw tissue (from 240 ± 11.1 to 184.8 ± 8.8 pg/ml). TNF-α level in the treated group was reduced by 23% in comparison with that in the control group ($p < 0.05$) (Figure 7).

Effect of NAAG on PGE_2 level in inflamed paw

The effect of NAAG (10 mg/kg) on the levels of PGE_2 in the inflamed paws was examined 4 h after the induction of inflammation. As shown in Figure 8, treatment of rats with NAAG (10 mg/kg) resulted in a significant reduction of PGE_2 level in the inflamed paw tissue (from 5.9 ± 0.08 ng/100 ml to 5.11 ± 0.15 ng/100 ml). PGE_2 level in the NAAG treated group was reduced by 13% in comparison with that in the control group ($p < 0.05$) (Figure 8).

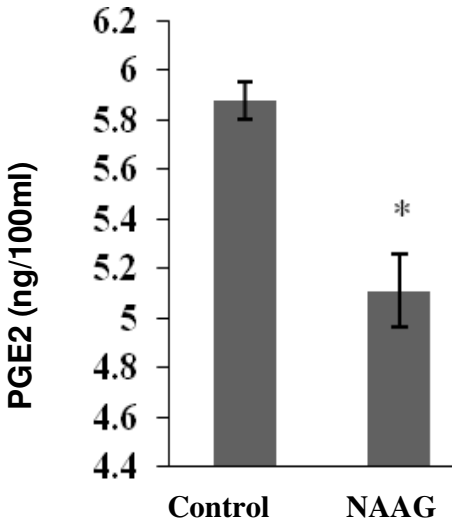

Figure 8. The effect of intraperitoneal injection of NAAG (10 mg/kg) on PGE_2 level in inflamed paw of rats. Inflammation was induced in the paw by intraplantar injection of the carrageenan 20 min after intraperitoneal injection of the NAAG. Data represented as mean ± SEM of six rats per group. Asterisks indicate significant changes from the control value (*$p < 0.05$).

DISCUSSION

NAAG is present throughout the mammalian central and peripheral nervous systems. Compared to the numerous amounts of data explaining the role of NAAG in the central nervous system (CNS) and nociceptive responses, the biological role of the NAAG in the peripheral tissues and inflammatory responses is still unknown. NAAG is located in the peripheral nerve and N-acetylated α-linked acidic dipeptidase (NAALADase); glutamate carboxypeptidase II (GCPII), a peptidase that cleaves NAAG to glutamate and N-acetylaspartate, is found primarily in the peripheral nerve (Miller et al., 2011). NAAG selectively activates group II mGluRs with a preference for mGluR3 and results in inhibition of the glutamate release (Neale et al., 2005). Our results suggest that the intraperitoneal injection of the NAAG (10 mg/kg) attenuates the peripheral inflammatory response induced by the carrageenan injection. NAAG was injected 20 min prior to the induction of inflammation by carrageenan and its significant anti-inflammatory effect was detectable at both phases of the carrageenan model. The first phase (1 h) involves the release of histamine and serotonin and the second phase (over 1 h) involves the release of prostaglandin-like substances, infiltration of neutrophils, production of neutrophil-derived free radicals, such as hydrogen peroxide, superoxide and $OH^{\bullet}$ radicals and the release of other neutrophil-derived mediators (Bilici et al., 2002; Capuozzo et al., 2011). We attribute the anti-inflammatory effect of NAAG to the action of this neuropeptide on the presynaptic mGlu3 receptors. Activation of NAAG on the presynaptic mGlu3 receptors reduces the levels of cAMP and Ca^{2+} influx into the nerve endings and reduces the activity of postsynaptic potentials resulting in lower amounts of glutamate release per action potential (Neale et al., 2005). It has been known that the elevated glutamate release from the peripheral afferent nerve fibers caused by inflammation stimulates the excitatory amino acid receptors in the peripheral tissues followed by sensitization of the afferents by inflammatory mediators. Glutamate also activates phospholipase A_2 and phospholipase C, platelet-activating factor, arachidonic acid, and diacylglycerol, lipids which are responsible for the inflammatory responses (Cole-Edwards and Bazan, 2005; Bazan, 2000; Miller et al., 2011). One of the numerous mechanisms supporting the inflammatory responses is the recruitment of leukocytes from vessels to an area of inflammation (Garcia-Ramallo et al., 2002). Extravasated PMNs become activated once in the inflammatory sites, secreting a variety of substances, such as growth factors, chemokines and cytokines, complement components, proteases, NO, reactive oxygen metabolites, and peroxynitrite, all impor-tant mediators of inflammation (Capuozzo et al., 2011; Salvemini et al., 1996). Carrageenan causes neutrophil infiltration that is followed by NADPH oxidase activation and an oxygen-respiratory burst. Then, oxygen-derived free radicals are generated, which cause lipid peroxidation, increase vascular permeability, elicit cellular recruitment and produce tissue damage (Salvemini et al., 1996). In order to explore the effects of anti-oxidant defenses on the acute inflammation process, in all paw tissues, the anti-oxidant enzyme levels (SOD and GPx), MPO activity and the MDA levels were evaluated. In accordance to our data, intraperitoneal NAAG (10 mg/kg) was an effective *in vivo* inhibitor of the neutrophil infiltration; as determined by the MPO levels in the inflammatory paw tissue. MPO is an enzyme found primarily in azurophilic granules of neutrophils, which is used as a marker for tissue neutrophil content and its inhibition implies the presence of anti-inflammatory activity. In the present study, we established that administration of the NAAG significantly decreased the paw tissue concentrations of the MDA, an index of ROS-mediated injury as well as an end product of the lipid peroxidation and free radical formation. MDA reveals lipid peroxidation, whereas SOD and GPx are important antioxidant enzymes involved in the clearance of superoxide and H_2O_2 to preserve the structure and function of biological membranes (Somi et al., 2009; Chandrasena et al., 2006). Also, we established that NAAG increased the SOD activity, which had been inhibited by carrageenan in the rat paw tissues (Wu et al., 2006). SOD destroys the highly reactive radical superoxide ($O_2^{-\bullet}$) by converting it into the less reactive

peroxide (H_2O_2) that can be destroyed by catalase reaction. The observed reduction in carrageenan-induced increase in the paw MDA levels and the increase in SOD activity indicated the ability of NAAG to attenuate the oxidative stress. NAAG may stimulate some important antioxidative enzymes like superoxide dismutase. These findings are in consistent with an emerging concept that the mGluRs may protect against the cellular injury by regulating the oxidative stress in the neuron (Cuzzocrea et al., 1999; Salvemini et al., 1996). During the inflammation, prostaglandins and interleukins are produced and interact with their respective receptors located on the peripheral terminals of primary afferents (Woolf and Ma, 2007). One of the most important metabolites of the arachidonic acid is PGE_2 generated through an enzymatic cascade controlled by the COX enzymes. PGE_2 elicits a wide range of inflammatory responses, including increase of vascular permeability. The significance of this role is emphasized by the broad clinical administration of the COX inhibitors to alleviate the inflammation in a variety of inflammatory disorders. It was found out that the amount of PGE_2 measured in the inflamed paw tissue was significantly decreased by NAAG and seemed to be correlated with the number of migrating cells, as shown by the MPO level. NAAG is positioned to activate the presynaptic mGlu3 receptors on sensory neurons, resulting in inhibition of the glutamate release. NAAG also might be released from the sensory neuron endings at the site of injury or inflammation in the peripheral tissue. PGE_2 is also released at sites of the injury and enhances the inflammatory responses by activating a PGE receptor to increase cAMP levels in the sensory neurons. NAAG activation of the mGlu3 receptors decreases the levels of cAMP (Neale et al., 2005).

In this model, NAAG activation of mGlu3 receptors on sensory neurons alleviates the effects of PGE_2 and reduces sensory neuron communication. Agents derived from the inflamed tissue, such as bradykinin, stimulate the release of TNF-α which in turns stimulates the release of IL-1β. IL-1β promotes the release of COX enzymes, which converts arachidonic acid to prostaglandins (Loram et al., 2007). We measured the release profile of proinflammatory cytokines (TNF-α and IL-1β) at the site of inflammation and compared these profiles with equivalent profiles after the carrageenan injection. Herein, we report that injection of the NAAG reduces TNF-α and IL-1β levels in the paw tissue. Four hours after the challenge, the level of TNF-α was reduced coincident with the significant reduction of the IL-1β and PGE_2 levels. At the 4^{th} hour time point, when TNF-α, IL-1β, and PGE_2 were inhibited significantly, MPO level reduction was significant.

Conclusively, this experiment showed that the carrageenan successfully induced edema in the paw. NAAG reduced the paw volume; the responses were great at both phases of the inflammation. The level of anti-oxidant system enzyme (SOD) and MDA level were affected by edema induction. NAAG alleviated the effects of edema on these agents. We found out that the pattern of proinflammatory cytokine concentration in the paw tissue that was changed by NAAG, was correlated with the number of infiltrating cells. The anti-inflammatory properties of NAAG could be related to its positive effect on the mGluR3 resulting in reduction of cAMP level and inhibition of glutamate release.

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