Korean J Physiol Pharmacol
Vol 5: 213-221, June, 2001

Dual Effect of Dynorphin A on Single-Unit Spike Potentials in Rat Trigeminal Nucleus

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The amygdala is known as a site for inducing analgesia, but its action on the trigeminal nucleus has not been known well. Little information is available on the effect of dynorphin on NMDA receptor-mediated electrophysiological events in the trigeminal nucleus. The purpose of this study was to investigate the changes in the single neuron spikes at the trigeminal nucleus caused by the amygdala and the action of dynorphin on the trigeminal nucleus. In the present study, extracellular single unit recordings were made in the dorsal horn of the medulla (trigeminal nucleus caudalis) and the effects of microiontophoretically applied compounds were examined. When [D-Ala2, N-Me-Phe4, Glys5-ol]enkephalin (DAMGO, 10-25 mM), a μ-opioid receptor agonist, was infused into the amygdala, the number of NMDA-evoked spikes at the trigeminal nucleus decreased. However, the application of naloxone into the trigeminal nucleus while DAMGO being infused into the amygdala increased the number of spikes. Low dose (1 mM) of dynorphin in the trigeminal nucleus produced a significant decrease in NMDA-evoked spikes of the trigeminal nucleus but the NMDA-evoked responses were facilitated by a high dose (5 mM) of dynorphin. After the κ receptors were blocked with naloxone, dynorphin induced hyperalgesia. After the NMDA receptors were blocked with AP5, dynorphin induced analgesia. In conclusion, dynorphin A exerted dose-dependent dual effects (increased & decreased spike activity) on NMDA-evoked spikes in the trigeminal nucleus. The inhibitory effect of the dynorphin at a low concentration was due to the activation of κ receptors and the excitatory effect at a high concentration was due to activation of NMDA receptors in the trigeminal neurons.

Key Words: Dynorphin, Trigeminal nucleus, Pain, Extracellular recording

INTRODUCTION

It is generally accepted that neurons in the dorsal horn of the medulla (trigeminal nucleus caudalis) are involved in processing nociceptive and thermosensory information originated from the orofacial region (Dubner & Bennett, 1983; Sessle, 1987). Excitatory amino acid (EAA) receptors may be involved in nociceptive information in the dorsal horn of the medulla since a broad spectrum EAA receptor antagonist reduced the noxious mechanical stimuli-evoked response of neurons in the trigeminal nucleus caudalis (Salt & Hill, 1983). Furthermore, EAA binding sites have been demonstrated in the medullary dorsal horn (Tallaksen-Creene et al, 1992).

Among them, glutamate is a putative excitatory neurotransmitter, which mediates synaptic transmission between primary afferent fibers and dorsal horn neurons (Watkins & Evans, 1981). It produces its action by acting on N-methyl-D-aspartic acid (NMDA), non-NMDA ionotropic such as α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainate, and metabotropic receptors in the spinal cord (Watkins et al, 1990).

It seems that other neuronal systems have a control over the noxious information of the trigeminal nucleus. The amygdala, periaqueductal gray (PAG), and

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rostral ventromedial medulla are responsible for the expression of several forms of hypoalgesia in rats (Helmstetter et al, 1998). As a site for inducing analgesia, the amygdala is a complex of nuclei located immediately beneath the medial surface of the cerebral cortex in the anterior pole of each temporal lobe, and it has abundant bidirectional connections with the hypothalamus. Oliveira & Prado (1998) evaluated the antinociceptive effects of stimulating the medial and central nuclei of the amygdala in rats. And the antinociceptive effects of stimulating the medial and central amygdala involve at least opioid, serotonergic, adrenergic, and muscarinic cholinergic descending mechanisms. However, its action on the trigeminal nucleus has not been elucidated. Therefore it is necessary to investigate the role of the amygdala, which is supposed to act on the nociceptive perception in the trigeminal nucleus.

The opioid is one of the important substances inducing analgesia. The effects of opiates and opioid peptides have been extensively investigated (Basbaum & Fields, 1984), with a major action being an inhibition of nociceptive transmission in the dorsal horn. It also contains high densities of μ-, δ- and κ-opioid receptors (Arvidsson et al, 1995). As one of the opioids, endogenous dynorphin A(1-17) and synthetic dynorphin A(1-13), which have a high degree of selectivity for the κ subtype of opioid receptors (Chavkin et al, 1982) modulates the excitability of nociceptive neurons. It was demonstrated that activation of κ opioid receptors by dynorphin inhibited voltage activated calcium channels (Werz & MacDonald, 1985) and produced moderate analgesic effects with restricted types of noxious stimuli that were often accompanied by a large variety of naloxone-insensitive biochemical and behavioral effects. Kato et al (1995) investigated the changes in the expression of dynorphin immunohistochemically after pain related injuries.

Since the level of dynorphin is changed according to the noxious stimuli and dynorphin has dual actions (Laughlin et al, 1997), it would be important to investigate the action of dynorphin. Consequently it seems especially important to investigate the action of dynorphin at the trigeminal nucleus where maxillofacial noxious stimuli are transmitted. However, little has been known about the functional significance of dynorphin and electrophysiological evidence regarding the modulation of dynorphin receptor-evoked responses in the medullary dorsal horn. Also, opioid agonists have been reported to modulate the NMDA and non-NMDA receptor-mediated responses of neurons in the dorsal horn of the spinal cord in vivo, as well as in vitro (Chen & Huang, 1991; Kolaj et al, 1995). Therefore it is certainly necessary to inject dynorphin into the trigeminal nucleus, and, considering the relationship with NMDA receptors, to record electrical events of the nerve activity directly.

The present study investigated, therefore, the action of the amygdala to the trigeminal nucleus and the modulation of NMDA-evoked responses by action of dynorphin in the trigeminal nucleus caudalis.

METHODS

Animal preparation

Experiments were carried out on male Sprague-Dawley rats (300~350 g). They had free access to water and rat chow. The rats were anesthetized with urethan (0.1 g/100 g i.p.), which was administered in small quantity at a time, titrating the dose necessary to suppress withdrawal reflexes to noxious pinching (final dose 1~1.4 g/kg). The right femoral artery and vein were cannulated for measurement of arterial pressure and administration of drugs and the femoral artery was coupled to a Statham physiological pressure transducer.

After ensuring that the level of anesthesia had stabilized under urethan, the distal trachea was cannulated for artificial ventilation (model 683, Harvard Apparatus) and ventilated with oxygen-enriched room air after the animal was paralyzed with pancuronium bromide (1 mg/kg i.v.). To check the rat's condition during the experiment, electrocardiogram was monitored continuously through the oscilloscope (Narco Bio-System Inc.). Body temperature was maintained at 38°C by means of a feedback control unit (Harvard Apparatus).

Operation for the amygdala

For drug injections to amygdala, the animal was placed on a stereotaxic frame (model 1404, David Kopf Instruments) and the skull was exposed. A hole was drilled through the skull overlying the amygdala. A 22 gauge stainless steel guide cannula was implanted into the amygdala (using the coordinates: 2.6 mm caudal, 4.2 mm lateral to bregma and 7 mm deep

from the bone) with acrylic dental cement. A stainless steel obturator was used to seal the cannula. For injection of [D-Ala^2, N-Me-Phe^4, Glys^5-ol]enkephalin (DAMGO, 10~25 mM, pH 4.5 in double distilled water), a 27 gauge injection cannula connected to a PE-20 tubing (Clay Adams) was inserted through the guide cannula. The other end of the tubing was connected to a 10 μl Hamilton syringe, which was mounted on an infusion pump (Harvard Apparatus).

Preparation of seven-barrel microelectrode and operation for the trigeminal nucleus

Before the animal surgery, the tip of recording seven-barreled glass micropipettes (glass tubing purchased from A-M Systems) was bumped to tip sizes of 5 μm. The impedances of the recording electrodes in vitro were between 3 and 6.5 MΩ when measured at 165 Hz with a microelectrode tester (Winston Electronics). The central barrel of the seven-barreled microelectrode was filled with 2 M NaCl or 2% pontamine sky blue in 0.5 M sodium acetate and served as the recording microelectrode. One of the side-barrels of the seven-barreled glass microelectrodes contained 2 M NaCl for automatic current balancing and the remaining barrels were filled with solutions for microiontophoretic application. Solutions in the drug barrels are: NMDA (50 mM in 140 mM NaCl, pH 8.0), dynorphin A(1-13) (1, 5 mM, pH 5.5), naloxone hydrochloride (10 mM in double distilled water, pH 5.0), D,L-2-amino-5-Phosphonovaleric acid (AP5, NMDA receptor antagonist, 50 mM in 150 mM NaCl, pH 8.0) and AMPA (5 mM in 140 mM NaCl, pH 8.0). All chemicals were obtained from Sigma Chemical Company.

Anesthetized rats were placed again on a stereotaxic frame with the head flexed downward at 45°C in a prone position. The medulla was exposed by cleaning the overlying musculature, cutting through and deflecting the dura and arachnoid mater. After removal of the membrane, the multibarrel microelectrode assembly was lowered to the medullary surface with a stepping driven micromanipulator (model 660, David Kopf Instruments). Single unit extracellular recordings were made caudal to obex (−0.5 to −2.0 mm) with particular attention paid to the medial two-thirds of the trigeminal nucleus caudalis, a region that receives terminations of the maxillary and mandibular divisions. The exposed surface of the medulla was covered with agar (4% agar in normal saline at about 40°C) in order to improve the stability of recording from neurons in the superficial dorsal horn of the medulla. Iontophoretic glutamate and mechanical stimulation were used to test and search for neuronal activity in the course of making an electrode track.

After searching the neuron by iontophoretic AMPA or NMDA, types of neurons were classified by mechanical stimulation of the receptive field. After shaving the face, their receptive fields on the same site were stimulated by brushing the skin with an artist's paint brush, applicating a large arterial clip with pressure of 144 g/mm^2 to a skin fold, grasping a skin fold with a pair of serrated forceps, and squeezing with maximal force. Neurons were classified as non-nociceptive (low threshold, LT), nociceptive (nociceptive-specific, NS) and multireceptive (wide dynamic range, WDR) neurons according to their responses to mechanical stimuli applied to the most sensitive portion of trigeminal receptive field (face) for 10 s. Neurons were considered to be LT if the maximum response was evoked by either brush or pressure. WDR cells responded maximally to squeeze and the response to brush was greater than 10% of the response to squeeze. NS cells responded maximally to squeeze, but the response to brush or pressure was minimal (Chung et al, 1986).

Extracellular recording of a single neuronal action potential was made with the central recording barrel of seven-barreled glass micropipettes. Drug retention, current balancing, and injection procedures were controlled by using the Neurophore BH2 system (Medical Systems Corp.). Retaining currents were adjusted between 5 and 10 nA to prevent drug diffusion. Application of microiontophoretic currents up to 140 nA through a saline containing barrel did not alter the responses of neurons significantly and was used as a control for current injection. Using a six-channel current generator, all drugs were ejected with positive current except NMDA and AP5, which were ejected with negative current. Time period of drug ejection was 5~20 s ejection/30 s retention epochs, and a minimum of 1 min ejection periods for the remaining drugs.

Signals from the pipette were preamplified and then amplified (×10,000), filtered (high pass 100~500 Hz, low pass 3 kHz, model CyberAmp 380, Axon), and displayed on an oscilloscope (model TDS430A, Tektronix). The spontaneous and evoked activities were fed to a window discriminator, which isolated from the background with an amplifier/voltage dis-

criminator (model 121, WPI) to allow sampling and analysis of the neuronal spike and were connected to a CED 1401 *plus* interface (Cambridge Electronic Design) and a PC computer (Spike 2.01 software). The Spike2 program generated histograms of the spike count of the neurons in 1 s time bins and quantified the data.

Spike numbers were counted during drug ejection. Drug-induced responses were evaluated by comparing neuronal firing at the end of the drug application period, with firing rate that immediately preceded the ejection of the drug. This assessment was aided by a visual inspection of the real time-rate histograms.

In addition, selected single unit sites were marked by passing 5~10 nA of negative current for 5~10 min from the central recording barrel or one of the outer barrels to deposit pontamine sky blue dye for subsequent identification.

Histological analysis

At the end of the experiment, the animal was given a lethal dose of pentobarbital sodium (100~200 mg i.v.) and perfused with formalin. The brain stem was then removed and stored in a 10% formaldehyde solution for late placement verification by histologic examination. Transverse sections (40 μm thick) through the brain were made on a cryostat and mounted on microscope slides. Data from animals with incorrectly placed electrodes were discarded from the experiments.

Statistical analysis

Data were expressed as mean±S.E.M. Difference was considered significant at $p<0.05$ using the paired and unpaired t-test.

RESULTS

When application of the selective μ-opioid receptor agonist, DAMGO, was done into the amygdala, the number of spikes at the trigeminal nucleus decreased to 62±2%. When naloxone hydrochloride was microiontophoretically administered into the trigeminal nucleus while DAMGO being infused into the amygdala, the number of spikes increased to 231 ±55% (Fig. 1).

A seven-barreled microelectrode assembly was used for the microiontophoretic application of ligands and the extracellular recordings of the individual action potentials in the trigeminal nucleus. Fig. 2 illustrated a typical example of rate histograms showing the inhibitory (A) and excitatory (B) effects of dynorphin on the NMDA-evoked responses of single neurons in the rat trigeminal nucleus. The results were statistically analyzed and represented as a histogram in Fig. 3. Dynorphin produced dose-dependent dual effects on nociception. That is, the low dose (1 mM) of dynorphin produced a significant decrease in NMDA-evoked spikes of the trigeminal nucleus to 30 ±6%, but the NMDA-evoked responses were facilitated to 251±24% by a high dose (5 mM) of dynorphin.

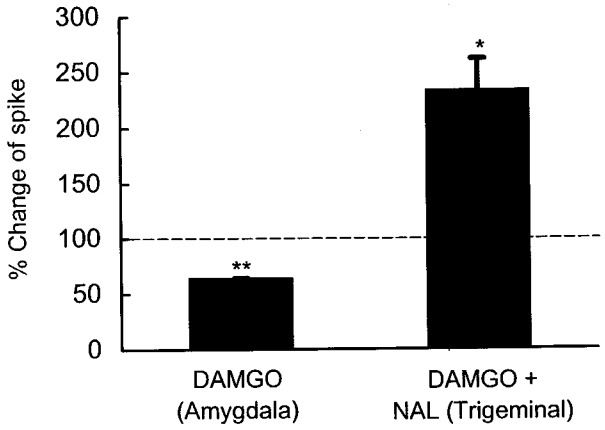

Fig. 1. Left: Inhibitory effect of DAMGO in the amygdala on the NMDA-evoked responses of neurons in the rat trigeminal nucleus (n=7). Dashed horizontal line indicates basal level of NMDA activity. Right: Excitatory effect of DAMGO in the amygdala when naloxone(NAL) was injected into the trigeminal nucleus (n=6). *$p<0.05$, **$p<0.01$, vs. basal level.

Fig. 4 shows whether the response to NMDA is proportional to the response to noxious stimuli. Pain was induced by serrated forceps. The result was a decreased number of spikes at a lower concentration of dynorphin and an increased number of spikes at a higher concentration.

We assumed that dynorphin acted on the κ receptors at a low concentration and on NMDA receptors at a high concentration. Under such hypothesis, when naloxone was infused alone into the trigeminal nucleus to block the κ receptor there was no difference. But, after naloxone was injected into the trigeminal nucleus, the subsequently injected dynorphin increased the number of spikes (Fig. 5). Since the

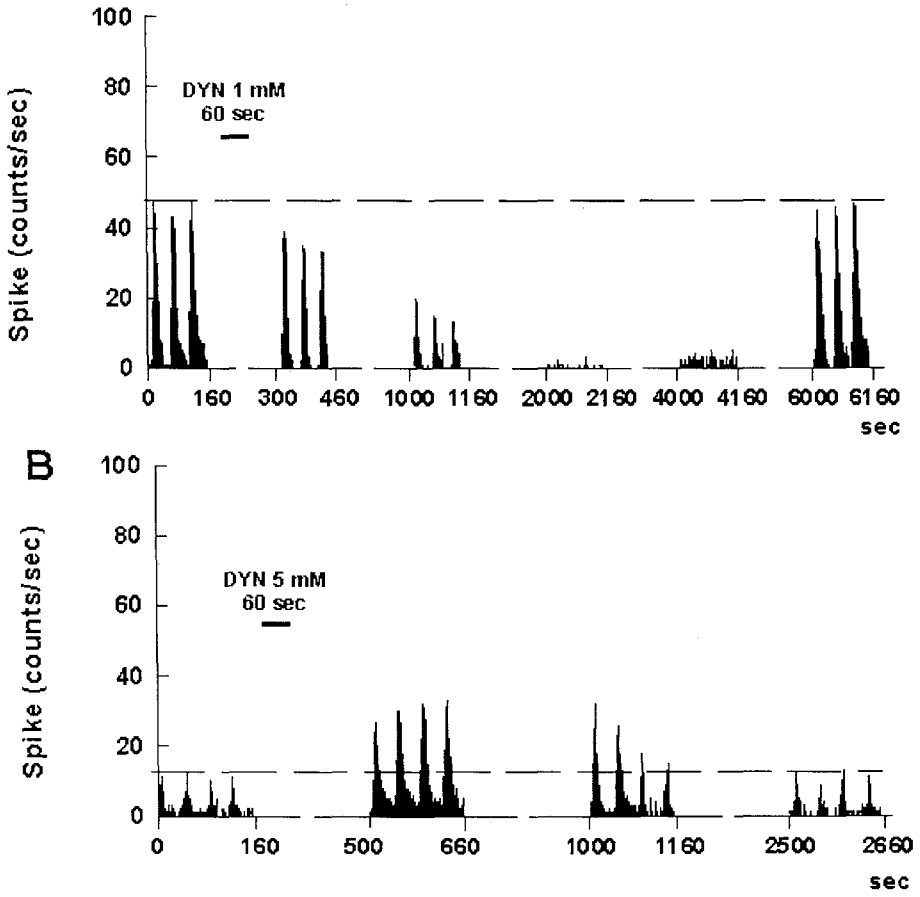

Fig. 2. Illustration of spike histograms showing the inhibitory (A) and excitatory (B) effects of dynrophin(DYN) on the NMDA-evoked responses of single neuron in the rat trigeminal nucleus. Dashed horixontal lines indicate basal levels of NMDA activity.

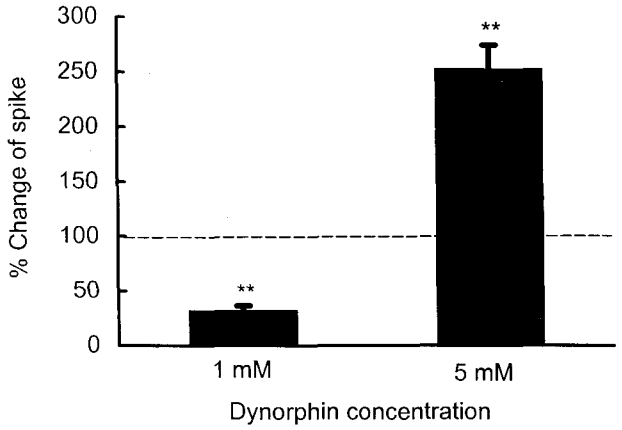

Fig. 3. Inhibitory (1 mM) or excitatory (5 mM) effects of dynorphin on the NMDA-evoked responses of neurons in the trigeminal nucleus (n=8). Dashed horizontal line indicates basal level of NMDA activity. *p<0.01, vs. basal level.

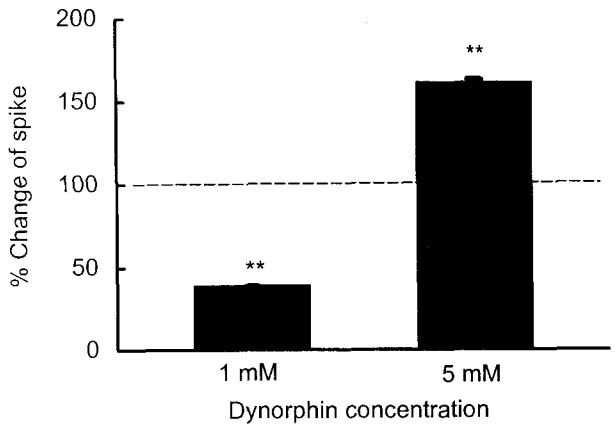

Fig. 4. Effects of facial pain induced by serrated forceps on trigeminal neuron activity (n=6). Dashed horizontal line indicates basal level of mechanical stimulation. Number of experiment was 6 (left) and 6 (right). **p< 0.01, vs. basal level.

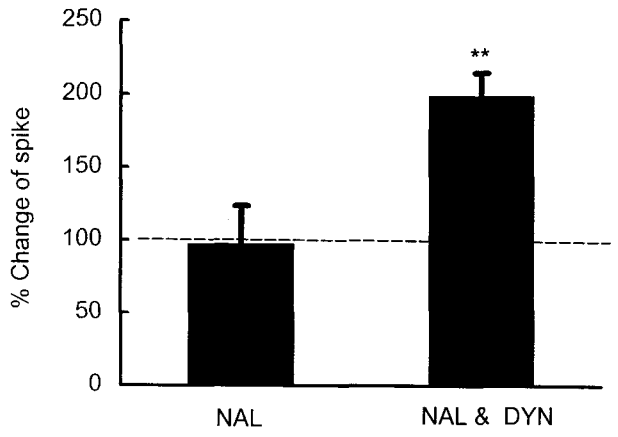

Fig. 5. Effects of dynorphin (DYN, 1 mM) after naloxone (NAL) injection on the NMDA-evoked responses of neurons in the rat trigeminal nucleus. Dashed horizontal line indicates basal level of NMDA activity. Number of experiment was 6 (left) and 8 (right). **p<0.01, vs. basal level.

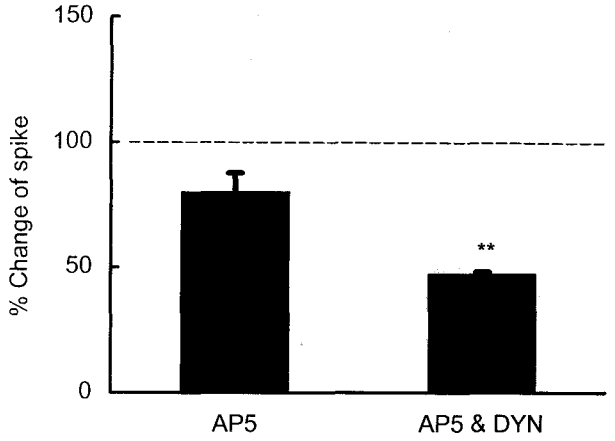

Fig. 6. Effects of dynorphin (DYN, 5 mM) after AP5 injection on the AMPA-evoked responses of neurons in the rat trigeminal nucleus (n=6). Dashed horizontal line indicates basal level of AMPA activity. **p<0.01, vs. basal level.

spikes due to NMDA could not be observed when NMDA receptor was blocked by AP5, the spikes due to AMPA were observed. AMPA spikes were not significantly altered by AP5 itself that was injected to block the NMDA receptors. After the NMDA receptors were blocked with AP5, dynorphin decreased the number of spikes (Fig. 6).

DISCUSSION

DAMGO infused into the amygdala decreased the number of spikes, which was consistent with previous studies (Pavlovic & Bodnar, 1998). It is generally accepted that the spinoponto-amygdaloid pathway appears to play a role in the cognitive and affective components of pain. Although PAG system is the main system to modulate ascending nociceptive and/or visceral information (Krout et al, 1998), a neural circuit including the amygdala is involved in hypoalgesia in the rat (Helmstetter et al, 1998). The analgesic action of amygdala was also demonstrated at the trigeminal nucleus in the present study.

Oliveira & Prado (1998) suggested that opioid receptors are involved in the antinociceptive effects of the amygdala. When naloxone, an opioid antagonist, was injected microiontophoretically into the trigeminal nucleus, NMDA evoked response was not altered. However, when naloxone was injected into the trigeminal nucleus while DAMGO being infused into the amygdala, it showed hyperalgesia in this experiment. Zhang et al (1996) demonstrated that microiontophoretic application of a selective μ-opioid receptor agonist, DAMGO, and a selective δ1-opioid receptor agonist, DPDPE in the dorsal horn of the medulla reduced the NMDA-evoked responses of neurons. They also observed that naloxone reversed the inhibitory actions of DAMGO and DPDPE. In the present study, the number of spikes was increased due to the other mechanism. It was postulated that, because of the alteration caused by naloxone, opioid neurotransmitter may be released in response to stimulation of the amygdala. The result of increased number of spikes instead of decreased indicates that a type of opioid with special actions might have been involved. Other studies in the dorsal horns of the spinal cord (Jones et al, 1990; Chen & Huang, 1991) and the medulla (Mokha, 1993) also showed the excitatory effects of opiates and opioid peptides. These results suggest that dynorphin may act on the κ receptors and produces a dual effect.

When dynorphin was injected into the trigeminal nucleus, it produced different responses of NMDA spikes at different doses: low concentration of dynorphin produced an inhibition of NMDA-evoked responses of medullary dorsal horn neurons, and high concentration made an excitaton. This experimental result indicated that NMDA receptor mediated electrophysiological events had been either inhibited or enhanced by dynorphin depending on preparations used in this experiment. The reason was that dynorphin may have a nonopioid action as well as an

opioid action. Over the last several years, significant progress has been made in understanding of interactions between NMDA and opioid receptors. Importantly, an emerging evidence indicated that opioids activate NMDA receptor (Mao, 1999), and vice versa. A considerable number of studies clearly indicated that opioids can directly modulate NMDA receptor-mediated electrophysiological events within the CNS. Likewise, dynorphin also may modulate the NMDA actions.

Dynorphin exerts an analgesic action like other opioids. Brauneis et al (1996) reported that dynorphin inhibited the NMDA-activated currents of all heteromeric NMDA receptor subunits tested. They suggested that inhibitory effect of dynorphin on NMDA receptor function was mediated by an interaction of dynorphin with NMDA receptors, rather than an action involving κ-opioid receptors. Chen et al (1995) also showed that in both whole-cell and single-channel preparations taken from trigeminal neurons, dynorphins 1-13, 1-17, and 1-32 reduced NMDA receptor-mediated currents independent of the κ-receptor, since neither naloxone nor norbinaltorphimine (nor-BNI, a κ-antagonist) blocked the inhibition produced by dynorphins.

On the contrary, an excitatory action of dynorphin could be observed. Dubner & Ruda (1992) proposed that dynorphin A may contribute to spinal hyperexcitability and excitotoxicity by producing specific facilitation of NMDA receptor activity in rats subjected to peripheral inflammation. The involvement of the NMDA receptor in inflammatory processes was also supported by the study of Ren & co-workers (1992) who demonstrated that the hyperalgesic response to inflammation was attenuated by the NMDA receptor antagonist, dizocilpine. In this regard, dynorphin has potent stimulatory effects on the C-fiber reflex by non-opioid NMDA receptor-mediated polysynaptic mechanisms (Knox & Dickenson, 1987; Caudle & Isaac, 1988). In studies of endogenous dynorphin it was demonstrated that peripheral injury produced a large increase in spinal dynorphin concentration (Iadarola et al, 1986; Ruda et al, 1988; Pohl et al, 1997). This increase closely matched the progressive increase in hyperalgesia and allodynia produced by the injury. On the basis of the model of neuronal injury induced by dynorphin and related peptides, it had been proposed that dynorphin-related peptides stimulate the NMDA receptor indirectly by a non-opioid mechanism (Long et al, 1988). Contrary to this, a recent study demonstrated that the motor dysfunction induced by dynorphin appeared before any change in cerebrospinal fluid levels of EAA, suggesting a possible direct interaction between the opioid and the NMDA receptor complex (Skilling et al, 1992).

In light of these facts, different results might be obtained with different protocols of experiment as demonstrated in the present study. Low concentration of dynorphin seems to induce analgesia by acting on the κ-receptors, whereas high concentration of dynorphin to induce hyperalgesia by acting on the NMDA receptors. Both excitatory (Henriksen et al, 1982) and inhibitory (Walker et al, 1982) effects of dynorphin and related peptides also have been described in rat hippocampus. These electrophysiological findings were consistent with the in vitro receptor binding studies that demonstrate a direct interaction between κ-opioid and NMDA receptors (Bhargava et al, 1995). Although exact mechanisms of such dual functions remained to be elucidated, this study provided valuable information concerning the two most important systems in nociception and antinociception: the NMDA and opioid receptor systems in the dynorphin.

The response to NMDA was compared with the noxious response to reveal that the two responses were identical as shown in Fig. 4. We suggested the NMDA receptor activation plays an important role in mediating nociceptive information in the medullary dorsal horn considering that a painful stimulation excited NMDA spikes in the present investigation. The superficial dorsal horn of the medulla contains the terminations of nociceptive primary afferent fibers (Light, 1992), glutamate immunoreactive nerve terminals (Clements et al, 1991) and binding sites for NMDA and non-NMDA receptors (Tallaksen-Creene et al, 1992). Wilcox (1993) suggested that NMDA receptor activation enhances responses to noxious stimuli to a greater extent than to non-noxious stimuli, produces hyperalgesia in the dorsal horn of the spinal cord.

In order to clarify the different actions of dynorphin according to different protocols, naloxone was infused alone into the trigeminal nucleus and then dynorphin was also injected following naloxone. With naloxone injection alone to block the κ-receptors, there was no change. Hyperalgesia, however, was induced by dynorphin injection following naloxone. When dynorphin cannot act on the κ receptors, it seems to act on the NMDA receptors.

On the other hand, when NMDA receptors were

blocked with AP5, dynorphin may act on the κ-receptors to manifest an analgesic effect. However, when NMDA receptors were blocked, spikes due to NMDA cannot be observed. While some researchers reported that NMDA receptor activity was not related with AMPA receptor activity (Fedorov & Reymann, 1993), others reported that NMDA receptor activity did influence the AMPA receptor activity (Fukunaga et al, 1996; Malenka & Nicoll, 1999). Considering a report that dynorphin-induced activity of κ receptors was related to AMPA (Kolaj et al, 1995) and in a viewpoint that pain can also be induced through AMPA receptors (Cumberbatch et al, 1994; Zhou et al, 1996), we blocked the NMDA receptors by AP5 and observed the activity of AMPA instead of that of NMDA receptors. When NMDA receptors were blocked with AP5 and κ receptors were excited by dynorphin, an analgesic action was observed.

In conclusion, the amygdala exerts an analgesic effect on the trigeminal nucleus, where opioids are involved. Dynorphin can produce dose-dependent dual effects of hyperalgesia and analgesia in the trigeminal nucleus. It appears that NMDA receptors were associated with hyperalgesia and κ receptors were associated with analgesia. And with blockade of the NMDA receptors, analgesia was produced by dynorphin. On the other hand, with blockade of the κ receptors, hyperalgesia was produced.

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