Participation of Opiate and Serotonergic Systems in Brain Conditioning Stimulatory Inhibition of the Potentials Evoked by Tooth Pulp Stimulation in the Pars Caudalis of the Trigeminal Sensory Nucleus of the Rat

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Abstract—Effects of conditioning stimulations of the nucleus raphe magnus (NRM), nucleus reticularis paragigantocellularis (NRPG), mesencephalic periaqueductal central gray (PAG), nucleus dorsomedialis hypothalami (DMH), corpus striatum (CP), sensory cortex (SCT) and visual cortex (VCT) and actions of morphine, naloxone and metergoline on the potentials recorded from the pars caudalis of the trigeminal sensory nucleus evoked by the electrical stimulation of rat incisor pulps were examined. 1) The spinal potentials evoked by electrical stimulation of the pulp consisted of 3 components. Component 2 was mainly inhibited by morphine and antagonized by naloxone. 2) Conditioning stimulation of NRM, NRPG, PAG, CP, SCT and DMH strongly inhibited component 2. VCT did not show any inhibition. 3) 33–67% of antagonism was observed by naloxone in the NRM, NRPG, PAG, CP, SCT and DMH. On the other hand, 27–44% of antagonism was observed by metergoline, and the antagonism was not enhanced by the additional administration of naloxone. These results conclusively show that the endorphin system as well as the serotonergic system is in the series involved in the descending inhibition for nociception in the trigeminal sensory nucleus.

Reynolds (1) has shown that electrical stimulation of the mesencephalic periaqueductal central gray (PAG) in rats results in sufficient analgesia to perform a laparotomy. Since then, the stimulation-produced analgesia (SPA) due to stimulation of PAG has been observed in rats (2), cats (3) and humans (4). Wide distribution of the sites producing SPA was also found in the medial hypothalamus (MH) situated between the posterior part of the 3rd ventricle and the rostral part of the 4th ventricle and such brainstem nuclei as nucleus raphe magnus (NRM) and nucleus reticularis paragigantocellularis (NRPG) (5). Furthermore, strong analgesia was obtained by the injection of a very small amount of morphine to these parts of the brain (6–8).

Using immunohistochemical methods, on the other hand, methionine enkephalin (Met-enk) is predominantly found in the corpus striatum (CP), hypothalamus, PAG, NRM and I, II, V, VII layers of the dorsal horn of the spinal cord and in the marginal and substantia gelatinosa layers of the trigeminal sensory nucleus (9), where many neurones were activated by noxious stimulation of the tooth pulp (10).

There are descending projections from the PAG, NRM and NRPG to the medulla oblongata and the spinal cord. Stimulation of these brainstem nuclei inhibits activities of the dorsal horn neurones in the spinal cord (11–13). There is evidence that serotonergic systems are involved in SPA from the PAG and NRM (14) and adrenergic ones from the NRPG (15). Moreover, an opiate mechanism is also involved in SPA, because SPA from the PAG has also shown cross tolerance with morphine.
In this study, potentials evoked by electrical stimulation of the tooth pulp were recorded from the superficial layers in the pars caudalis of the trigeminal sensory nucleus, and the inhibitory or excitatory effects of the conditioning stimulation of some brain nuclei on the potentials were investigated to determine if endogenous opiates participate in the pain mechanism elicited by the brainstem on the pars caudalis of the trigeminal sensory nucleus.

**Materials and Methods**

1) **Animals and experimental procedure:** Male Sprague-Dawley rats weighing 250-300 g were cannulated for respiration under ether anesthesia and were fixed in a stereotaxic instrument. Surface anesthesia was made by 10% lidocaine on parts of the injured skin and both auricular holes. During the experiment, adequate doses of gallamine were also administered, and the animals were placed on the heating plate (37.5-39.0°C).

Morphine and naloxone were administered intravenously into the femoral vein via a catheter.

2) **Recording of the potentials:** For recording of the evoked potentials in the pars caudalis of the trigeminal sensory nucleus elicited by electrical stimulation of the pulp, bipolar stimulating stainless steel electrodes, measuring 100 µm in diameter and 3 mm in polar distance, which had been completely insulated except for the tip, were carefully inserted into the excavated incisor pulp of the mandible. Supramaximal stimulation of the pulp was always carried out with a single rectangle wave of 0.5 msec duration with a range of 10-30 Volts in each case.

To orient the pars caudalis of the trigeminal sensory nucleus, the occipital part of the cranium and the dorsal part of the 1st cervica vertebra were removed, and the dura mater and choroidal membrane were cut out to expose the spinal cord. To prevent drying, the exposed areas of the spinal cord were covered with liquid paraffin. Recording was made at the area of 0.1-2.0 mm caudal from the obex and 1.2-2.0 mm lateral from the sagittal line, using a multicombed glass electrode which contained 3 M KCl and lithium carmine. The area at the depth of 150 µm from the surface was designated as the outermost layer in this experiment; and each recording was made at the depth of 50, 100 and 150 µm. Furthermore, the deeper area containing the substantia gelatinosa and magnocellular layers to 300 µm in depth was designated as the SG-MC area; and recording was made at a depth of 200, 250 and 300 µm (Fig. 1).

Potentials evoked in the pars caudalis by electrical stimulation of the pulp were amplified through a preamplifier (DAM-6A, Nihon Kohden) and observed on a 2-beam oscilloscope (VC-10, Nihon Kohden) and recorded on tape. Twenty superimposed potentials recorded by an X-Y recorder (Type 3077, Yokogawa) were analyzed by a data analyzing calculator (ATAC 501-10, Nihon Kohden).

Recordings began at 5 min and 2 min after the administration of morphine (5 mg/kg, i.v.) and naloxone (2 mg/kg, i.v.), respectively. After the end of each experiment, the recording site in the spinal cord was stained with lithium carmine administered iontophoretically with direct currents of 10-15 µA for 10-15 min and fixed with 10% formalin, embedded in paraffin and consecutive sections were stained with cresylecht violet.

![Fig. 1. Recording site in the pars caudalis of the trigeminal sensory nucleus.](image)

- **COM:** The outermost layer (to 150 µm in depth from the surface of the spinal cord).
- **SG-MC area:** (150-300 µm in depth from the surface of the spinal cord).
- **Z:** subnucleus zonalis. **SG:** subnucleus gelatinosus. **MC:** subnucleus magnocellularis.
using Nissl's method to make sure of the recording sites.

3) Conditioning stimulation: Stimulating sites were the PAG, CP, nucleus dorsomedialis hypothalami (DMH), NRM, NRPG and sensory cortex (SCT) as regions having some relation to the pain mechanism and the visual cortex (VCT), as a region having no relation to pain mechanism.

According to the brain atlas (16), as shown in Table 1, stainless steel bipolar recording electrodes which were completely coated except for the tip (diameter 200 μm, interpolar distance 0.4 mm) were inserted into the PAG, CP, DMH, NRM and NRPG on the contralateral side of the pulp stimulation. For the stimulation of SCT and VCT, a bipolar stimulating electrode with polar distance of 0.5 mm was used.

Four pulses / train of rectangular waves with 0.5 msec duration, 250 Hz in frequency were used as the conditioning stimulus. Effect of the conditioning stimulation on the evoked potentials was judged from the inhibition of the maximal height of the evoked potentials induced by the supramaximal stimulation of the pulp. When the supramaximal conditioning stimulation was applied, a decrease of more than 30% in the control height was interpreted as an inhibition in this experiment. When an increase in the potential height was at least observed after the administration of antagonist or antagonistic procedure, it was regarded as recovering from the inhibition. Effects were expressed as the rate to the total recording number.

In the observations for the effects of naloxone, conditioning stimulations to each part described above were carried out 2 min after naloxone administration. In the observations for the effects of metergoline, conditioning stimulations of NRM and PAG were carried out 30 min after the administration of metergoline.

Results

1) Potentials evoked by electrical stimulation of the pulp in the pars caudalis of the trigeminal sensory nucleus

As shown in Fig. 2, potentials evoked by electrical stimulation of the incisor pulp were recorded as 3 peak components. Peaks of negative components followed by the artifact of the stimulation were designated as \( N_1, N_2, \) and \( N_3 \).

![Fig. 2](image)

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Table 1. Rat brain stereotaxic position in this experiment

|        | A.P. | Lat. | H.V. |
|--------|------|------|------|
| NRM    | 9.5  | 0.2  | 10.6 |
| NRPG   | 10.0 | 0.8  | 10.5 |
| PAG    | 2.0  | 0.2  | 4.8  |
| DMH    | 1.5  | 0.5  | 8.2  |
| CP     | -0.5 | 3.5  | 4.0  |

A.P.: anterior, Lat.: lateral, H.V.: vertical
and N3, consecutively, and those of positive components followed by N1, N2 and N3 were designated as P1, P2 and P3, respectively. Each constituent of N1-P1, N2-P2 and N3-P3 was designated as component 1, 2 and 3, respectively. Latency times of each component 1, 2 and 3 were 5.2-6.1, 9.4-10.4 and 13.1-18.0 msec, respectively.

When the dental pulp was stimulated at high frequencies, component 2 completely disappeared at 50 Hz; component 3 disappeared at 20 Hz, but component 1 did not disappear completely. Prolongation of the latency was also observed in components 2 and 3.

Morphine (5 mg/kg, i.v.) inhibited component 2 and scarcely inhibited components 1 and 3. The inhibitory effect of morphine on component 2 was dominantly observed in the SG-MC area. Naloxone (2 mg/kg, i.v.) reversed the reduced component 2 to the control level; that is, naloxone antagonized the morphine effect.

2) Effects of conditioning stimulation of each part of the brain on evoked potentials

Amplitude of potentials evoked by the stimulation of the dental pulp in the pars caudalis of the trigeminal sensory nucleus was inhibited by the conditioning stimulations of PAG, CP, DMH, NRM, NRPG and SCT but not VCT. Amplitudes of component 2s were measured when conditioning test intervals (C-T intervals) were variably changed. As shown in Fig. 3, the strongest inhibition of ca 65% of the control amplitude was observed during a C-T interval of 20–50 msec in NRM, ca 65% in 20–40 msec in NRPG, ca 55% in 20–30 msec in CP, ca 50% in 20–40 msec

![Fig. 3. Conditioning test interval in each part of the brain for component 2. Ordinate: Percent of control in amplitude of the potential. Abscissa: Conditioning-test interval (msec). In the figures, each amplitude used was correspondingly shown for each part of the brain. Vertical bars represent the S.D.](image-url)
in SCT and ca 45% in 20–30 msec in DMH, respectively. Prolongation in C-T interval generally resulted in a decrease of inhibition in every area. Therefore, a C-T interval of 25 msec was used in the experiments thereafter.

3) Descending inhibition by the conditioning stimulation of each part of the brain on the evoked potentials

As mentioned above, more than 30% of decrease in amplitude of the control evoked potential was regarded as an inhibition, which was expressed as the rate to the total recording number.

A slight inhibition of component 1 was observed for SCT (14%, n=15), less inhibition for PAG, NRM and NRPG, and none for CP and VCT (Fig. 4). In general, the strong inhibition of component 2 was observed for all parts except with VCT. As shown in Fig. 5, the inhibitions in the outermost layer and SG-MC area were respectively observed as follows: 100% and 100% for NRM (n=15) and NRPG (n=12), 83% and 91% for PAG (n=12), 89% and 67% for CP (n=9), 55% and 78% for DMH (n=9) and 73% and 73% for SCT (n=15). Weak inhibition of 11% and 11%, respectively, were observed for VCT (n=9). Stronger inhibition was also observed in the SG-MC area for PAG and DMH and in the outermost layer for CP.

As shown in Fig. 6, the inhibitions of component 3 in the outermost layer and SG-MC area were respectively observed as follows: 33% and 56% for NRM, 55% and
Fig. 6 Descending inhibition against component 3 by the conditioning stimulation in each part of the brain. Refer to the explanation in Fig. 4. (n=9–15).

Fig. 7. Effect of metergoline on the descending inhibition against component 2 by the conditioning stimulation of the NRM and PAG. □: Percent of recording numbers showing the inhibition (N) by the conditioning stimulation against total recording numbers (n), ◼◼◼: Percent of recording numbers showing the recording (n') by metergoline (50 mg/kg, i.p.) against total numbers showing the inhibition (N), ◼◼◼◼: Percent of recording numbers showing the inhibition more (n'') by metergoline (50 mg/kg, i.p.) against total numbers showing the inhibition (N). Left column: Recording from the outermost layer, Right column: recording from the SG-MC area. Total recording numbers in both columns were each 14–18.

66% for NRPG, 22% and 56% for PAG, 56% and 55% for CP, 44% and 55% for DMH and 46% and 53% for SCT. A strong inhibition in the SG-MC area was observed in all parts except for the VCT.

4) Effects of drugs on the descending inhibition by the conditioning stimulation of each part of the brain

1. Antagonism by naloxone: Naloxone in doses of 2 mg/kg (i.v.) partially antagonized the descending inhibitions. Component 2 was antagonized the most among the other components. Inhibition percentages for component 2 in the outermost layer and the SG-MC area were respectively shown as follows: 60% and 67% for NRM (n=15), 75% and 58% for NRPG (n=12). 42% and 50% for PAG (n=12), 56% and 45% for CP (n=9), 33% and 33% for DMH (n=9) and 40% and 53% for SCT (n=15). Stronger inhibition was observed in the outermost layer for NRPG and CP and in the SG-MC area for NRM, PAG and SCT (Fig. 5). Inhibition percentages for component 3 are also shown in Fig. 6. Antagonism for component 1 was scarcely observed (Fig. 4).

2. Augmentation by naloxone: Naloxone in doses of 2 mg/kg (i.v.) augmented the descending inhibition in some neurones. Component 2 for PAG (n=12) was augmented in 33% of the neurones tested in the outermost layer. The augmentation was more dominant in the outermost layer than in the SG-MC area for the NRM, NRPG and
PAG (Fig. 5). For component 3, no similar results as in component 2 were obtained.

5) Effects of metergoline on the descending inhibition from NRM and PAG

1. Antagonism by metergoline: Metergoline in doses of 50 mg/kg (i.p.) showed a partial antagonism to the descending inhibition from the NRM and PAG (Fig. 7). The inhibition of component 2 in the outermost layer and the SG-MC area decreased to 73% (n=15) and 71% (n=14) for NRM and 56% (n=18) and 65% (n=17) for PAG, respectively. Additional administration of naloxone in doses of 2 mg/kg (i.v.) neither antagonized nor augmented the inhibition due to metergoline.

2. Augmentation by metergoline: About 10% of the neurons in the outermost layer and the SG-MC area for NRM and PAG were augmented by metergoline in doses of 50 mg/kg (i.p.).

Discussion

Naloxone, an opiate antagonist, has a significant decreasing action on pain threshold in rats and mice due to its antagonistic action against endogenous opioid peptides (17, 18). By applying this action of naloxone to SPA, participation of the endorphin system on SPA can be investigated. Spinal evoked potentials obtained by electrical stimulation of dental pulp featured 3 peak potentials consisting of 3 components in this experiment. Polysynaptic neurone activity was affirmative for this evoked potential because of the disappearance of components 2 and 3 when frequent stimulations of 50 and 20 Hz were applied to the pulp.

On the other hand, from the results obtained in the present study that component 2 was depressed by morphine and the inhibited potential was antagonized by naloxone, it was suggested that opiate receptors were involved in the pain mechanism.

Engstrand et al. (19) have reported that electrical stimulation of the incisor pulp of the mandibula does not produce a pure stimulation strictly for pain sensation, but produces the firing of fibers other than the ones conducting pain impulses. Existence of the potentials which were not inhibited by morphine in this study affirmed Engstrand's idea.

Analgesia has taken place after the intrathecal administration of morphine and Met-enk, and it has been antagonized by naloxone (20). Only the C-fiber reflex induced by noxious stimulation was naloxone-reversibly inhibited by the intraspinal injection of a small amount of morphine and Met-enk (21). Furthermore, it has been demonstrated that many opioid receptors exist at the laminae I and II and the marginal layer of the trigeminal sensory nucleus in the spinal cord, and Met-enk containing neurones also exist at the laminae I and II (22) and substantia gelatinosa layer of the trigeminal sensory nucleus (23, 24).

Oliveras et al. (3) demonstrated that the inhibition of the nerve cells in lamina V of the spinal cord was involved in SPA due to the stimulation of the PAG and also demonstrated that mouth opening reflex due to the electrical stimulation of the pulp was inhibited by the stimulation of the PAG (25). Kuypers and Maisky (26) have demonstrated direct projection from the PAG to the dorsal horn in the spinal cord using the horseradish peroxidase method. On the other hand, it was reported (27, 28) that many fibers from the PAG projected to the NRM and the electrical stimulation of the PAG or microinjection of morphine into the PAG caused an excitation of NRM neurones. Yaksh and Tyce (29) also reported that microinjection of morphine into the PAG elevated the release of serotonin in the spinal cord. From these findings, it could be considered that the PAG is a superior center to the NRM. Although Yaksh et al. (2) reported that SPA due to the stimulation of the PAG was not antagonized by naloxone, Sessle and Hu (30) demonstrated that firing of a single neurone in the pars caudalis of the trigeminal sensory nucleus by the pulp stimulation was shown to be naloxone-reversibly inhibited by the electrical stimulation of the PAG. As the present study showed that the descending inhibition from the PAG was antagonized to 42% and 52% by naloxone and to 56% and 65% by metergoline, the descending inhibition from the PAG was evaluated to be weaker than
that from the NRM or NRPG.

It has been obvious that many serotonin-containing neurones exist in the NRM, from where serotonergic fibers project to the dorsal horn of the spinal cord. The descending pathway directly projects to I, II, V and VI laminae of the spinal cord through dorsal parts of the lateral funiculus (31, 32). Analgesia induced by morphine decreased after the administration of p-chlorophenylalanine (p-CPA), tryptophan hydroxylase inhibitor or cinanserin, serotonergic receptor antagonist (33, 34) and the electrical stimulation of NRM resulted in the increase of 5-hydroxytryptophan in the spinal cord due to activation of serotonin synthesis (35). Moreover, the electrical stimulation of the NRM depressed the nociceptive responses of dorsal horn cells (12, 33).

Rivot et al. (36) suggested from naloxone antagonism that the endogenous opioid system was involved in SPA induced by the stimulation of NRM. It has been demonstrated that not only opiate receptors but also much enkephalin contents are observed in NRM and immunoreactive enkephalin-positive nerve fibers projected from the lower medulla oblongata containing NRM to the spinal cord (37). Dickenson et al. (38) also demonstrated that the single neurone activity due to the stimulation of the pulp recorded from the superficial layer (marginal layer and magnocellular layer) of the sensory trigeminal nucleus consisted of 2 groups with short and long latencies, 1–14 msec and more than 14 msec, and conditioning stimulation of the NRM preferentially inhibited the long latency group. There have been few reports suggesting the involvement of the endorphin system in the descending inhibition from the NRM. In the present study both naloxone and metergoline antagonized to the same extent, 60–70%, the descending inhibition from the NRM.

Since no additional inhibition was observed when naloxone and metergoline were concomitantly administered, it might be suggested that neurones belonging to endorphin and serotonin systems connect together in the series.

Takagi et al. (13) demonstrated that the firing of lamina V cell in the dorsal horn induced by the intraarterial injection of bradykinin was inhibited by the conditioning stimulation of the nucleus reticularis gigantocellularis (NRG) or NRPG, and Basbaum et al. (39, 40) also demonstrated the descending path passing through the dorsolateral funiculus from the NRPG and terminating in the lamina I, II, III and V layers of the dorsal horn of the spinal cord. Azami et al. (6) reported that the most sensitive neurone to morphine existed in the NRPG, because the earliest appearance of analgesia was obtained when a very small amount of morphine was injected into the NRPG and also reported that the analgesia was also antagonized by phenoxybenzamine and NRM destruction (41). Satoh et al. (42) stressed the involvement of the α-adrenergic mechanism more important than the opiate mechanism in SPA due to the stimulation of NRPG, because the SPA was much more antagonized by phenoxybenzamine than naloxone. In the present study, we have demonstrated that almost the same degree of descending inhibition and naloxone antagonism were observed in the SPAs of the NRPG and NRM.

Involvement of the opiate mechanism was also suggested in MH, because injection into the MH with a small amount of morphine caused analgesia (8). Destruction of the MH caused hypersensitivity with hyperalgesia (43) and nerve activities due to the noxious stimulation in the dorsal horn were inhibited by electrical stimulation of MH (44). In the present study, the evoked potential induced by the electrical stimulation of pulp in the trigeminal sensory nucleus was inhibited by electrical stimulation of DMH, but involvement of the endorphin system in SPA due to the stimulation of DMH was considered to be less important because of little naloxone antagonism. Moreover, SPA due to stimulation of DMH was considered to be more multisynaptic than other SPAs, for in even more than 90 msec of C-T interval the inhibition was still observed.

Many opiate receptors and much more enkephalin were found in CP, where the extrapyramidal system was known to be much more relevant, but SPA due to the electrical stimulation of CP has never been
reported. In this study, however, the involvement of the endorphin system in SPA due to the electrical stimulation of CP was observed, for relatively strong inhibition of 89% and 67% was observed by the electrical stimulation of CP and inhibition of 56% and 45% as a result of naloxone antagonism was observed.

Although the pain mechanism in the cerebral cortex is not yet clear since there have been few reports about it, some neurones responding to noxious stimulation to the pulp were found. The stimulation of the sensory area I and II (SI and SII) increased the content of endorphin in the part of the spinal cord containing the trigeminal sensory nucleus with a few aminergic mechanisms (45). On the other hand, it has been clear that centrifugal projections from SI and SII extended to the spinal cord (46). Stimulation of SCT was carried out at SI on both sides in this study; and in the comparison with the cases of CP or DMH, similar levels of inhibition of component 2 due to the stimulation of SCT and antagonism with naloxone were obtained, suggesting the involvement of the opiate mechanism.

As seen in Fig. 1, marginal and substantia gelatinosa layers of the trigeminal sensory nucleus in the spinal cord were topographically involved in the outermost layer, many pure nociceptive neurones exist as seen in the lamina I of the dorsal horn; and these neurones are mainly engaged in the recognition of orientation for pain (47), while in the layer of substantia gelatinosa, a lot of small neurones exist, and these small neurones worked in the control of intensity or change in character of pain as substantia gelatinosa neurones in the gate control theory (48). In the present study, no great difference was observed between both layers for NRM, NRPG and SCT, but greater inhibition was observed in the outermost layer for CP and in the SG-MC area for PAG and DMH. Naloxone showed not only antagonistic action, but also that of enhancement of inhibition in some neurones. It is reasonable to consider from the result that the endorphin system may not only be involved in the inhibition of nociceptive modality. However, naloxone as well as metergoline generally showed an antagonism of 30–70% for the inhibitions due to the conditioning stimulation of either PAG, an endorphin-rich region, or NRM, a serotonin-rich region, so it can be concluded that the endorphin system as well as the serotonergic system is involved in the descending inhibitory mechanism of pain.

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