Inhibitory Effects of Acupuncture Manipulation and Focal Electrical Stimulation of the Nucleus Submedius on a Viscerosomatic Reflex in Anesthetized Rats

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Abstract: To examine the participation of nucleus submedius (Sm) in the medial thalamus of pain inhibitory systems, we investigated the effects of acupuncture and focal electrical stimulation of the Sm and adjacent brain sites (0.3 ms, 50 Hz, 50–100 μA, 10 s) on the EMG activity of the external oblique muscle evoked by colorectal distension in urethane-anesthetized Wistar rats. The viscerosomatic reflex (VSR) activity was suppressed after the administration of morphine (1.0 mg/kg, i.v.) and the effect was reversed by naloxone (0.5 mg/kg, i.v.). Transection of the spinal cord at the Th2 level also eliminated the VSR. Acupuncture manipulation applied to the cheek (manual rotation at 1 Hz) suppressed the VSR, and this inhibition was eliminated by microinjections of lidocaine into the bilateral Sm nuclei (0.5 μl of 1.0% solution). Electrical stimulation in the ventral part but not the dorsal part of the Sm suppressed the VSR. The inhibition of the VSR induced by electrical stimulation of the Sm was not reversed by the administration of naloxone (1.0 mg/kg, i.v.). Electrical stimulation of the adjacent medial thalamic nuclei (mediodorsal nucleus (MD) or centromedial nucleus (CM)) and ventrobasal complex (VB) of the thalamus had very little effect on the VSR. These results suggest that the Sm is not only involved in the relay of nociceptive information to the cortex, but may also be involved in a non-opioid mediated pain inhibitory system and may participate, at least in part, in the suppressive effects of intense acupuncture manipulation on VSR activity. [Japanese Journal of Physiology, 47, 121–130, 1997]

Key words: nucleus submedius, viscerosomatic reflex, acupuncture, endogenous pain inhibitory systems, rat.

The analgesic effects of acupuncture have been well established by several lines of evidence [1, 2]. It has been suggested that these effects are the result of activating endogenous opioid or non-opioid mediated pain inhibitory systems [3]. Polymodal receptors are suggested as one of the possible candidates for the peripheral input in such analgesic mechanisms [4]. Some studies have implicated the activation of descending pain inhibitory systems from the brainstem by acupuncture analgesia [1, 3], however, the details are unclear and controversial [5].

It is well known that acupuncture manipulation and electroacupuncture stimulation can activate various types of sensory receptors and afferent fibers, and these various afferent inputs may induce various types of analgesic effects [6, 7]. Therefore, it seems to be very important to define acupuncture stimulation to analyze its central mechanism in detail.

In this study, we used acupuncture manipulation as noxious mechanical stimulation which may activate the polymodal receptors. Previously, we demonstrated that selective activation of the polymodal receptors can induce analgesic effects [8] and capsaicin treatment can eliminate the effects induced by manual acupuncture [9]. These analgesic effects are quite similar to those known as diffuse noxious inhibitory controls (DNIC). Le Bars et al. [10], and several recent reports suggest close relationships between DNIC and acupuncture analgesia [9, 11].

The central mechanisms of DNIC have not been
clarified, but the lesions of periaqueductal gray matter (PAG) and rostral ventromedial medulla (RVM), which are known as the central origins of descending pain inhibitory pathways, did not affect the DNIC produced by the noxious thermal stimulation of rats [12, 13]. Therefore, the medial thalamus has been proposed as a possible site for the central mechanism of DNIC [14].

The nucleus submedius (Sm) of the medial thalamus has been proposed to play an important role in mediating pain at the thalamic level [15]. Several studies have reported that the Sm nucleus contains many nociceptive specific neurons, that their receptive fields are large, and that they respond to noxious cutaneous thermal and chemical stimuli [16] and noxious visceral stimulation [17].

Recently, Roberts et al. [18] reported that the intensity of electrical shock required to elicit vocalization responses was decreased (hyperalgesia) after bilateral lesions on the Sm nuclei in awake rats, and Zhang et al. [19] reported that electrical stimulation of the Sm nucleus inhibited the tail flick reflex. These observations suggest the possibility that the Sm nucleus is not only a thalamic relay nucleus for pain, but also may participate in endogenous pain inhibitory mechanisms.

The aim of this study was to examine the participation of the Sm nucleus in the inhibitory effects of acupuncture and DNIC on visceral noxious inputs. We used the EMG activity of the external oblique muscle evoked by colorectal distension (CRD) as an index, because the CRD has been widely used as an acute visceral pain model for rats [20, 21] and similar CRD evoked unpleasant pain sensation in human subjects [22].

MATERIALS AND METHODS

Experiments were performed on 24 male Wistar rats (220–590 g). The animals were anesthetized with urethane (1.2 g/kg, i.p.) after having been food-deprived for 24 h. A tracheotomy was performed and blood pressure monitored from the carotid artery. A polyethylene catheter was inserted into the jugular vein for the administration of test drugs. Body temperature was maintained at about 37–38°C by a heating pad. The animal's head was positioned in a stereotactic frame with the incisor bar 3.0 mm below the horizontal zero. A small craniotomy was performed, and the dura removed carefully.

Extracellular single-unit recording in the Sm nucleus. Glass micropipettes filled with 2% Pontamine Sky Blue in 0.5 M sodium acetate and having impedances of 10–13 MΩ were used. The coordinates used for recording in the Sm nucleus were 2.3–3.0 mm caudal to the bregma, 0.5–0.9 mm lateral and 6.0–6.9 mm below the cortical surface [23]. Neural activities were amplified, filtered (DAM-80, WPI), and displayed on an oscilloscope (VC-11, Nihon Kohden), and also fed into an audio monitor. The shape of the discriminated action potential was continuously monitored to ensure that a single unit was under study. The response to tactile stimulation with a soft paint brush, noxious pinching with toothed forceps, noxious radiant heat with an incense stick, acupuncture manipulation (0.25 mm in diameter, manual rotation of 1 Hz) and moxibustion stimulation (10 mg of moxa burnt on the skin) applied to the tail, all 4 paws and cheek were examined.

Recording of the viscerosomatic reflex (VSR). The skin of the left abdominal side was cut longitudinally, and electromyographic responses evoked by CRD were recorded from the left external oblique muscle via a pair of metal surface electrodes (Ag-AgCl round plate, 10 mm in diameter). Electrodes were placed on the muscle at intervals of 20 mm, and covered with a thin clear wrapping sheet to prevent tissue aridity and to keep their position. A 50–60 mm flexible latex balloon was inserted intranally. The balloon assembly was kept in position (end of balloon in 10 mm from the anus) by taping the balloon catheter to the base of the tail. Pressure in the balloon was monitored continuously by using a transducer and saline solution was injected by syringe pump at a rate of 0.1 ml/s. In order to avoid tissue damage in the colon and rectum, maximum pressure was adjusted to 120 mmHg, and the duration of distension was limited to 30 s and given at intervals of no less than 5 min. The external oblique muscle EMG activity was amplified, filtered, and fed to an integrator (time constant of 0.3 s). The raw and integrated EMG activity were recorded on a data recorder (RD-135T, TEAC) and thermal array recorder (RTA-1200M, Nihon Kohden), and monitored on an oscilloscope.

For the quantitative analysis of the suppression of VSR activities, the decrement of integrated VSR response magnitude during conditioning stimulation was used. When the integrated VSR magnitudes were reduced to less than 50% of the baseline during conditioning stimulation, we evaluated the brain site as effective (see Table 1 and Fig. 5).

Morphine and naloxone administration. In order to examine the characteristics of the VSR activity, the effects of morphine hydrochloride (1.0 mg/kg, i.v.) and naloxone hydrochloride (0.5 mg/kg, i.v.), an antagonist of morphine, were examined. The CRD
was applied repetitively at intervals of 5 min after drug administration.

**Spinal transection.** To determine whether supraspinal pathways were involved in mediating the CRD-evoked reflex, a spinal transection was performed at the Th2 level and its influence on the CRD-evoked reflex activity examined. Following spinalization, the rats were artificially resired via the tracheal cannula and the systolic blood pressure was kept above 70 mmHg by the administration of fiool (4.0%, i.v.).

**Acupuncture manipulation.** In order to examine the effects of acupuncture manipulation on the VSR, an acupuncture needle (0.25 mm in diameter) was inserted vertically into the skin of the cheek or hindpaw and rotated at 1 Hz for 10 s. Acupuncture manipulation was applied 10 s after beginning CRD.

**Microinjection of lidocaine into the bilateral Sm nuclei.** A microscope was attached to a micro-manipulator and the needle was inserted stereotaxically into the Sm nucleus. Lidocaine (1.0%, 0.5 μl) or saline (1.0 μl) was slowly injected (more than 1 min) into the bilateral Sm nuclei.

**Electrical stimulation of the brain.** Monopolar metal stimulating electrodes (0.18 mm in diameter) were inserted from the cortical surface. Their impedances were about 10 MΩ. Ten seconds after the appearance of VSR activity, electrical stimulation of the brain site (0.3 ms, 50 Hz, 50–100 μA) was performed, and CRD terminated 10 s later. The stimulation sites were in the Sm nucleus, adjacent sites and the ventrobasal complex (VB). In order to examine the participation of endogenous opioids in the effect of electrical stimulation of the Sm nucleus on VSR activity, naloxone (1.0 mg/kg, i.v.) was administered 10 min before the conditioning Sm stimulation.

**Effect of microinjection of glutamate in the Sm nucleus.** In order to examine whether the effects of electrical stimulation of the Sm nucleus were due to activating Sm cell bodies, the microinjection of glutamate (0.2 μl, 1.0 μl) was performed using a microsyringe. As a control, the microinjection of saline was also done.

**Histology.** Most of the recording sites were marked by the ejection of Pontamine Sky Blue from the electrode (2 μA, 30 min), and electrical stimulation sites by making a small lesion (20 μA, 10 s, DC). At the end of the experiment, the animal was perfused transcardially with saline followed by 10% buffered formalin. The brain was then removed and fixed in

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**Fig. 1. Response of a Sm neuron to pinch, heat, acupuncture, and moxibustion stimuli.** Top row shows oscilloscope traces of the responses of the neuron to each of the stimuli. The middle row shows the peristimulus firing rate histograms of the activity shown in the top row (bin width = 1 s). The bottom row shows the blood pressure changes induced by the stimuli. Pinch, heat, and acupuncture (acp) were applied to the left hindpaw, and moxibustion (mox) to the right hindpaw. The horizontal bars above the top line indicate the period of application of each stimulus. The recording site in the Sm is indicated on the medial thalamus drawing at the bottom of the figure.
fresh formalin for over 24 h. Either 50 or 100 μm sections were cut with a vibratome, mounted, and stained with Cresyl Violet. Recording and stimulation sites were identified and marked on drawings of the thalamus. The locations of recording and stimulation sites not marked were determined indirectly by comparing the coordinates recorded from microdrive readings to the histologically determined coordinates of electrode tracks and marks.

RESULTS

Response of the Sm neurons to pinch, heat, and acupuncture manipulation and moxibustion

Figure 1 shows an extracellular single-unit recording in the Sm nucleus obtained with a glass micropipette. The neuron was excited by noxious pinching with toothed forceps (this stimulus was highly painful when applied to the experimenter’s hand), noxious radiant heat applied with an incense stick (this stimulus was very hot and painful when applied to the experimenter’s hand), acupuncture manipulation of the left hindpaw and moxibustion (10 mg) of the right hindpaw. This unit also responded to similar stimuli applied to most regions of the body, but did not respond to tactile stimulation with a soft paint brush. All neurons recorded from the Sm were nociceptive specific neurons and their receptive fields were usually very large and bilateral. Neither wide dynamic range neurons (WDR) nor low-threshold neurons (LTM) were found.

Characteristics of the VSR

When the colon-rectum was distented by inflating the balloon inserted intra-anally, tonic EMG activity was recorded from the external oblique muscle. This VSR activity usually ceased immediately after the cessation of CRD. On some occasions, a brief after-discharge of external oblique muscle was observed. The threshold of the reflex activity evoked by CRD was about 40 mmHg. VSR activity tended to increase with the pressure intensity of CRD, and reproducible responses to CRD were obtained at 80 and 100 mmHg. When pressures over 120 mmHg were applied repetitively, the reproducibility deteriorated.

Fig. 2. Effects of morphine and spinal transection on VSR activity. A shows the effects of morphine and reversal by naloxone administration, and B the effect of transection at Th2. In each part, the top rows show the EMG activity of the external oblique muscle, middle rows show the CRD pressure, and bottom rows show the blood pressure. On the left, the control responses are shown; middle column of A shows the responses 1 min after the administration of morphine (1.0 mg/kg, i.v.), and right column shows responses 1 min after the administration of naloxone (0.5 mg/kg, i.v.). In B, the right column shows the lack of effect of CRD 60 min of transection.
Effects of morphine and naloxone on VSR activity

Systemic administration of morphine completely inhibited VSR activity, and naloxone completely reversed the inhibitory effect of morphine (Fig. 2A). Similar results were observed in all 5 cases tested.

Spinal transection

Spinal transection at the Th2 level eliminated VSR activity in all 5 cases tested. Figure 2B shows an example. The spinal flexion reflex evoked by thermal stimulation of the hindpaw was evoked both before and after transection.

Effect of acupuncture on VSR activity

Acupuncture manipulation applied to the contralateral cheek was found to cause almost complete depression of the CRD-evoked VSR activity in all 8 cases tested. Figure 3 shows an example of acupuncture-induced reduction in the VSR. Acupuncture applied to the ipsilateral cheek, forepaws, and hindpaws (data not shown) was also effective in depressing the VSR. These inhibitory effects were eliminated after the microinjection of lidocaine into the bilateral Sm nuclei (Fig. 3A). Control injections of saline had little effect on the acupuncture-induced inhibition of VSR activity (Fig. 3B). Similar results were obtained in all 3 cases where lidocaine and saline injections were made into the Sm nucleus. It is shown that acupuncture manipulation which suppressed VSR activity usually produced a pressor response during CRD (see Fig. 3), whereas it normally produced a depressor response (see Fig. 1). Relatively intense acupuncture manipulation was required for the suppression of VSR activity.

Effects of electrical stimulation of the Sm and other brain sites on VSR activity

VSR activity was inhibited by electrical stimulation at several of the brain sites tested. Usually, the inhibitory effects on VSR were very clear and seemed to be all or none under the experimental conditions reported here. Figure 4 shows an example from one experiment where electrical stimulation was performed at successively deeper sites as the electrode was driven through the thalamus. It can be seen that stimulation of the ventral part of the Sm nucleus at 50 μA produced marked suppression of the VSR. Figure 5

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Fig. 3. The inhibition of VSR activity by acupuncture and the effect of microinjecting lidocaine into the bilateral Sm on the inhibition of VSR activity by acupuncture. A shows the effect of lidocaine microinjection, and B shows that of saline. Records show the control responses, effect of acupuncture manipulation to the cheek, acupuncture manipulation applied 10 min after the microinjection of lidocaine (1.0%, 0.5 μl in the Sm bilaterally), and 60 min after the microinjection of saline (1.0 μl in the Sm bilaterally), respectively. The injection sites of lidocaine and saline in the Sm are shown at the bottom right.
shows a reconstruction of the effective and ineffective sites for depressing the VSR. The data were obtained from 7 electrode tracks through the thalamus and hypothalamus of 5 rats.

Table 1 shows the incidence of stimulation sites of the CM, Sm, VB, and hypothalamus that were found to be effective in depressing the VSR at 50 µA. In particular, the data show that stimulation sites in the ventral part of the Sm were much more effective in inhibiting the VSR than those in the dorsal part of the Sm. VSR activity of the left external oblique muscle was inhibited by electrical stimulation of both the ipsilateral and contralateral Sm nucleus. Additionally, electrical stimulation of the habenular complex and hypothalamus was usually effective, whereas stimulation of the VB had little inhibitory effect even when more intense electrical stimulation (100 µA) was applied.

**Effects of naloxone on the inhibition of VSR activity by electrical stimulation of the Sm nucleus**

The effects of naloxone on the Sm-evoked inhibition of VSR activity were tested in 4 cases. An example showing the lack of effect of naloxone is shown in Fig. 6.

**Effects of the microinjection of glutamate in the Sm nucleus on VSR activity**

The effects of the microinjection of glutamate on VSR activity were examined in 2 cases. In both cases, the unilateral injection of glutamate in the Sm nucleus totally eliminated VSR activity (see Fig. 7A). Control injections of saline had no or very little effect (see Fig. 7B).

**DISCUSSION**

In this study, direct electrical stimulation or chemical activation of the neurons in the Sm nucleus, or excitation of the Sm nucleus by intense acupuncture manipulation caused a naloxone-insensitive inhibition of supraspinal CRD-evoked reflex activity. The inhibitory effect of acupuncture manipulation on VSR activity was completely eliminated after the microinjection of

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**Fig. 4.** This figure shows the effects of electrical stimulation at 50 µA at various brain sites on VSR activity during insertion of the stimulating electrode. Solid circles in the brain map indicate the sites of inhibition and solid triangles indicate the sites where no inhibition of VSR activity was elicited. The right column shows a recording of the EMG activity of the external oblique muscle when electrical stimulation was applied to each site. The vertical calibration bar at the bottom right is 200 µV. The horizontal bar at the bottom indicates the length of electrical stimulation. MD, mediodorsal nucleus; CM, centrolateral nucleus; CM, centromedial nucleus; Re, reuniens nucleus; Sm, dorsal Sm; Smv, ventral Sm; DA, hypothalamic dorsal area.

**Fig. 5.** A diagram showing the locations of the effective and ineffective stimulation sites. Symbols are the same as in Fig. 4. Re, rhomboid nucleus; mt, mammillothalamic tract.
local anesthetics in the Sm. These results demonstrate that the Sm neurons may participate in the suppressive effects of acupuncture manipulation on CRD-evoked VSR activity.

**CRD-evoked VSR activity**

Previous studies have demonstrated that CRD is an useful method for evoking reproducible acute visceral pain in anesthetized animals [20, 21]. In awake rats, it was shown that avoidance behavior, cardiovascular response (pressor) and visceromotor response (contraction of the abdominal and hindpaw musculature) are evoked by CRD [20]. It was also reported that similar CRD in human subjects provokes cardiovascular, respiratory, and visceromotor responses as well as unpleasant visceral pain sensations [22]. Our present results clearly demonstrate that VSR activity was completely inhibited by morphine and reversed by naloxone. These observations further support CRD as an effective method to provoke visceral pain and indicate that the VSR is also useful for assessing visceral nociception in experimental animals.

The reflex pathways of the VSR have not been clearly established. However, the CRD-evoked external oblique muscle reflex must involve a brainstem loop since it has been shown to persist after mid-collicular decerebration and eliminated after spinal transection at the C1 level [20]. Our present results are consistent with the existence of such a supraspinal loop.

**Table 1. Summary of effects of focal brain stimulation on the VSR activity.**

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<th>Inhibited</th>
<th>No change</th>
<th>Total</th>
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<tr>
<td>Thalamus (CM)</td>
<td>0/6</td>
<td>6/6</td>
<td>6</td>
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<tr>
<td>Thalamus (Sm)</td>
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<tr>
<td>Dorsal</td>
<td>2/10</td>
<td>8/10</td>
<td>10</td>
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<tr>
<td>Ventral</td>
<td>12/13</td>
<td>1/13</td>
<td>13</td>
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<tr>
<td>Thalamus (VB)</td>
<td>0/6</td>
<td>6/6</td>
<td>6</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>6/7</td>
<td>1/7</td>
<td>7</td>
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<tr>
<td>DA, VM</td>
<td>7/29</td>
<td>22/29</td>
<td>29</td>
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<tr>
<td>Other sites</td>
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**Inhibition of VSR activity by acupuncture manipulation**

VSR activity was inhibited by acupuncture manipulation. This inhibition was observed by acupuncture applied to various parts of the body (cheek, forepaws, and hindpaws). In most cases, this inhibition appeared immediately after the onset of rotation of the acupuncture needle and continued until the termination of CRD. Since the acupuncture stimulus apparently activates nociceptive afferents, as confirmed by our observations that it excites Sm neurons, this inhibition may be similar to the phenomenon termed “diffuse noxious inhibitory control” (DNIC). It has been shown that nociceptive stimuli applied over widespread regions

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Fig. 6. The effects of naloxone on the inhibition of VSR activity produced by electrical stimulation of the Sm. A shows control responses, B shows the inhibitory responses to electrical stimulation of the Sm at 100 μA before the administration of naloxone, and C shows the suppressive responses to electrical stimulation of the Sm 10 min after the administration of naloxone (1.0 mg/kg, i.v.). The horizontal bars at the top indicate the length of electrical stimulation of the Sm. Stimulation sites in the Sm are shown at the bottom. ES, electrical stimulation.
of the body inhibit the nociceptive responses of wide dynamic-range dorsal horn neurons [10]. A DNIC-like phenomenon has also been reported to occur in conscious human subjects using the evoked EMG activity of hind leg as an index [24]. Bing et al. suggested that the analgesic effects of acupuncture manipulation might be, at least in part, mediated by a mechanism similar to DNIC [11].

Our data show that the inhibitory effect of acupuncture manipulation on VSR activity is eliminated after the microinjection of lidocaine into the Sm. This suggests that the Sm might participate in the analgesic action of acupuncture manipulation. In this respect, our present data are consistent with the report that the medial thalamic nuclei, including the Sm nuclei, are involved in mediating DNIC [14]. The fact that relatively intense acupuncture stimulation, which induces the pressor response, is required for the inhibition of the CRD-evoked reflex also supports the idea that acupuncture analgesia is the result of the activation of thin afferent fibers such as the polymodal receptor afferents [4, 9].

**Effects of the electrical stimulation of various brain sites on the VSR**

The fact that electrical stimulation within the brain can produce analgesia was first reported by Reynolds [25]. Today, it is termed "stimulation produced analgesia" (SPA). In this study, VSR activity was inhibited by the electrical stimulation of several brain sites. The habenular complex, the ventral part of the Sm nucleus and hypothalamus were found to be effective sites, whereas the VB had little inhibitory effect on VSR activity.

Previous studies have reported that focal electrical stimulation of the habenular and hypothalamus can produce analgesia [26, 27], but the VB has no such analgesic effect [28]. This study confirms these reports. The VB is well known as a thalamic relay center for the sensory-discriminative aspects of pain [29]. In this respect, the fact that electrical stimulation of the VB had no inhibitory effect on the CRD-evoked reflex is consistent with this function.

**Naloxone reversibility**

Our findings that inhibition of the VSR by electrical
stimulation of the Sm was not reversed by naloxone administration indicate that the non-opioid-mediated endogenous inhibitory pathways are involved in mediating this inhibitory effect. Earlier studies on acupuncture analgesia maintained that the analgesia could be reversed by naloxone [30, 31]. However, a recent report showed that naloxone-reversible analgesia could not be demonstrated in acupuncture-naïve rats [32]. It has also been demonstrated that naloxone-reversible analgesic effects are induced by a certain form of footshock applied to the rat [33] as well as by an intermittent cold-water swim at 2°C [34]. Moreover, the methods of pain measurement were shown to be critical for morphine analgesia in the PAG-lesioned rats [35].

Thus, naloxone antagonism seems to be inadequate criterion for acupuncture analgesia, and our findings are not necessarily inconsistent with possible Sm involvement in acupuncture analgesia.

### Participation of the Sm in endogenous pain inhibitory mechanisms

Electrical stimulation of the Sm, especially of its ventral part, induced rapid and prolonged inhibition of VSR activity, and the microinjection of glutamate also produced similar inhibitory effects. These results show that the neurons in the ventral part of the Sm play a role in the inhibitory mechanism of VSR activity. Zhang et al. also reported that tail flick latency was increased by electrical stimulation of the ventral part of the Sm. Their study also demonstrated that this inhibitory effect was attenuated or eliminated after the lesioning of the ventral part of the VLO or the ventrolateral part of the PAG [19]. They also demonstrated that the analgesic effects of intense conditioning stimulation of the peripheral nerve were eliminated after electrical lesioning of the Sm [36]. Based on these findings, and anatomical evidence that the ventral part of the Sm has a dense reciprocal projection to the VLO [37] and the VLO and PAG have reciprocal connections [38], the proposed analgesic effect of conditioning stimulation of small-diameter afferents as well as of electrical stimulation in the Sm might be the result of descending modulation at the spinal level via an Sm-VLO-PAG system [36].

On the contrary, Roberts et al. reported that the intensity of electrical shock required for the vocalization response decreased after bilateral lesioning of the Sm in awake rats, but that tail flick latency was little affected [18]. It has also been reported that electrical stimulation of the VLO induces the facilitation of tail flick latency [39]. These contradictory results might be, at least in part, dependent on the different experimental methods used in the studies.

The neural mechanism underlying the inhibitory effect on CRD-evoked VSR activity produced by intense acupuncture manipulation as well as electrical stimulation of the Sm in this study has not been clarified yet. It could be suppression of the brainstem and/or spinal cord regions involved in mediating the VSR by direct excitation of the neurons in the Sm or via the VLO and PAG indirectly. In this respect, further investigations are needed.

In conclusion, Sm neurons may participate, at least in part, in the endogenous pain inhibitory systems which are activated by DNIC-like intense acupuncture manipulation.

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### REFERENCES


34. Girardot MN and Holloway FA: Cold-water stress analgesia in rats: different effects of naloxone. Physiol Behav 32: 547–555, 1984


