Ascending pathways of skin and deep information projecting to the ventral posterior lateral nucleus of thalamus in the cat

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Abstract

In acupuncture, the typical sensation induced by insertion of a needle into the muscle that called "De Qi" is essential to induce acupuncture analgesia. The effect of acupuncture analgesia may depend on the deep afferents rather than to the cutaneous ones at the acupuncture point. The ascending pathways conveying these skin and deep afferents project to the ventral posterior lateral nucleus (VPL) of thalamus, one of the thalamic relay nuclei projected to the cerebral cortex, were examined by spinal cord lesion at the cervical level with observation of the responses evoked by electrical stimulation applied selectively to the skin and deep tissues.

Neuronal discharges were recorded extracellularly from the VPL using the multimicroelectrode technique. After spontaneous neuronal activities were recorded, the receptive fields and characteristics were determined by applying some kinds of mechanical stimulation to the receptive field. The skin and deep structures were then selectively stimulated using a pair of stainless steel needles and insulated needles except for tips. Units were classified for skin units, deep units, and skin-deep units according to the responses to the electrical stimulation. Responses were elicited in some neurons following to both skin and deep stimuli. The latency of response elicited by electrical stimulation applied to the deep structure was slightly shorter than that of the skin structure. The recording sites for deep units were located more rostrally in the dorsal region of the VPL than that for the skin units.

The afferents of some skin-deep neurons from both skin and deep structures were ascended through different pathways in the spinal cord. The skin and deep afferents were found to converge at the thalamic level.

Key words : Thalamic VPL, Skin inputs, Deep inputs, Spinal lesion, Ascending pathways

Introduction

Analgesic effects of acupuncture has been concerned with the modification in transmission of pain signal. The effective acupuncture analgesia requires a subjective sensation of the patient produced locally at the point of needling¹⁾. When

the needle is inserted to a given depth under the skin, the patient may have a feeling of soreness, distention, heaviness or numbness, called "De Qi"²⁾. This typical sensation by acupuncture is mediated afferently through the nervous system and the analgesic effect of acupuncture is related

to the deep afferents rather than to the cutaneous ones¹⁾. Chang³⁾ indicated that pinching of the Achilles tendon significantly inhibited the pain responses of neurons in the central lateral nucleus (CL) by electrical noxious stimulation applying to the peroneal nerve.

Various stimulating techniques have been utilized for activating various types of afferent fibers. Hashimoto and Aikawa⁴⁾ demonstrated that the inhibitory effect of manual acupuncture varied depending on the stimulated tissue at the acupuncture point, and they suggested the importance of increased activity of the muscle afferents. Afferents are activated by needle manipulation and by electrical stimulation. The needle manipulation effects on receptors and afferents only in a limited region at the site of needling whereas electrical stimulation may activate receptors in much larger area⁵⁾.

The effect of acupuncture analgesia varied depending on the peripheral structure and receptors stimulated, whereas the ascending pathways through the spinal cord remains unclear. The present study was performed to confirm the ascending pathways mediating information from skin and deep tissues to one of the thalamic relay nuclei, ventral posterior lateral nucleus (VPL), with the electrical stimulation technique applying selectively to the skin and deep structures.

Methods

Experiments were performed on 13 adult cats (2.4-4.6kg) anesthetized with sodium pentobarbital (30-35 mg/kg, i.p.), immobilized with pancuronium bromide (0.3 mg/kg/h, i.v.) and artificially ventilated. Unitary discharges in the VPL were recorded extracellularly using the multimicroelectrode system⁶⁾ that consists of four platinum-cobalt (Pt-Co) alloy microelectrodes. Multimicroelectrodes were inserted vertically through intact cortex to thalamus⁷⁾ using the modified "closed head"

technique⁸⁾. The site and size of receptive fields (Rfs) and the response properties of neurons were determined by mechanical stimulation such as bending hair with an air-puff, tapping the skin and/or pressing with a small rod, pinching the skin by forceps and joint movement.

Electrical stimulation was applied to the Rf through a pair of stainless steel needles (Φ 0.30 mm, 25 mm) or insulated needles with lacquer except for tips. To stimulate only the skin, uninsulated needles were used to insert only into the cutaneous surface. Deep structure such as muscle was stimulated by insulated needles that inserted into the muscle. Rectangular pulses of 0.1 ms duration with at twice the response threshold were used. Recordings of unitary discharges were amplified and continuously monitored on an oscilloscope, stored on magnetic tape and analyzed using a medical computer (ATAC-450, Nihon Kohden) for constructing post-stimulus time histograms (PSTHs).

After laminectomy was performed to expose the C3-C5 segments of the spinal cord, the cervical cord was sectioned with a scalpel in order to determine the ascending pathways of both from skin and deep structure. First, a dorsal column lesion was performed with a scalpel and a dorsal quadrant ipsilateral to the Rf was severed above the first lesion. Then, hemisection ipsilateral and contralateral to the Rf were performed successively above the prior sectioning.

At the end of the experiment, the recording sites were marked by passing currents through the recording microelectrodes. The animals were sacrificed by an overdose intravenous injection of sodium pentobarbital and perfused by 10% formalin. The brain and cervical spinal cord were removed and placed in formalin. After at least one week, the brain was frozen and cut into serial 40 μ m thick slices in frontal plane with a freezing microtome and stained with cresyl

violet.

Spinal cord blocks containing lesions were embedded in paraffin, serially sectioned in 10 μ m thick slices with a microtome and stained with cresyl violet. Recording sites and extension of spinal lesion were verified histologically.

Results

The present results are obtained from the 30

VPL units. Each unit was classified according to a response type. Units were divided into three categories; low-threshold mechanoreceptive (LTM) units were maximally driven by innocuous cutaneous stimuli (bending hair and/or tapping); wide dynamic range (WDR) units responded to both innocuous and noxious cutaneous stimuli, but with an increase in firing rate to higher-intensity stimuli; deep (DP) units responded to

Fig.1 Responses of VPL neurons to mechanical stimulation applied to the receptive fields

The sequential histograms on the left side represent responses of the units to mechanical stimulation of the skin and deep structures in the receptive fields. Stimuli used included bending hair with an air puff (Hair), tapping with a small rod (Tap), pinching the skin by forceps (Pinch), and joint movement (Joint). Shaded areas in drawings on the right side represent the receptive fields (a, b and c). Arrow indicates effective direction. Horizontal bars under histograms indicate the period of application of each stimulation. A : low-threshold mechanoreceptive (LTM) neuron. B : wide dynamic range (WDR) neuron. C : deep (DP) neuron.

Fig.2 Responses of VPL units to selective electrical stimulation of skin and deep structures

The responses evoked by electrical stimulation applied only to the skin structure are represented in the upper traces. The responses evoked by the stimulation to the deep structure such as muscle are represented in the lower traces. Triangles indicate the point of the stimulus artifact. D : shaded areas indicate the receptive fields (a, b and c).

Table 1 The latency of response of skin-deep unit evoked by restricted electrical stimulation independently applied to the skin and deep structure

unit	response type	Rf location	latency for	
			skin stim.(ms)	deep stim.(ms)
1	LTM	forepaw	8.5	7.7
2	WDR	forelimb	8.8	8.8
3	DP	upper arm	7.2	5.2
4	LTM	hindlimb	14.0	13.7

pressing and joint movement. Classification of the 30 units with responses characteristics was 15 LTM, 5 WDR, and 10 DP units. Sequential histograms for each response type are shown in Figure 1.

When restricted electrical stimulation was independently applied to the skin and deep structures, units were also divided into three

groups by appearance of responses to electrical stimulation applied to either skin or deep structure; skin units responded only to skin stimulation; deep units responded only to stimulation applied to the deep structure as muscle; skin-deep units exhibited responses to stimulation to both the skin and deep structures. Four units (2 LTM, 1 WDR, and 1 DP unit) were

classified as skin-deep units (Fig. 2). The latency of the response to electrical stimulation of the skin was 2.0 ms longer than the deep response in the same cell (Fig. 2C). The latency of the response to deep stimulation of 4 skin-deep units had a same or slightly shorter than that of the responses to skin stimulation (Table 1).

By selectively severing the spinal cord at the cervical level, the pathways conveying inputs

onto the VPL neurons were examined. Initially, the Rf was stimulated by an electrical pulse. After the dorsal column ipsilateral to the Rf was severed, the deep response disappeared (Fig. 3 Lesion 4). The skin response ceased after severing the ventral quadrant (Fig. 3 Lesion 6). The responses evoked by skin and deep signals appear to ascend through different regions of the cervical cord.

Fig.3 Effects of spinal cord lesion at C3 level on responses of VPL units to selective stimulation of skin and deep structures

A : cord sections show lesions made prior to post stimulus time histograms (PSTHs). Extent of new lesion is indicated by cross-hatched area. Darkened area show previous lesion. Responses to electrical stimulation applied to the skin (B) or deep structure (C) are shown in PSTHs. Triangles indicate point at which stimulation was applied to the skin and deep structure. Each PSTH represents average of 50 trials. Analysis time : 102.4 ms. Bin width : 0.8 ms. Vertical bar : 20 spikes. contra. : contralateral to the Rf. ipsi. : ipsilateral to the Rf.

Fig.4 Schematic diagrams of recording sites

Location of recording sites of skin units (A) and deep units (B) recorded and the lesion site the responses disappeared. ● : black circles indicate skin-deep units. ⊗ : recording sites of units in which evoked discharges disappeared after dorsal column was severed. ⊕ : recording sites of units in which evoked discharges disappeared after dorsal quadrant lesion of the spinal cord ipsilateral to Rfs. ⊗ : sites of units in which discharges disappeared after hemisection of the spinal cord contralateral to Rfs was severed.

The location of recording sites of 21 out of 30 units and the lesion area was represented in Figure 4. In the remaining 9 units, the unitary discharges were lost before a second lesion, and so the data of spinal lesion from these neurons were not included. Deep units were located more rostrally in the dorsal region of the VPL than the skin units (Fig. 4 A, B). In 2 out of 4 skin-deep units, the skin responses ceased after hemisection contralateral to the Rf and the deep responses disappeared after the dorsal column was severed (Fig. 4 A, B). In the remaining 2 skin-deep units, both responses were abolished after the dorsal column sectioned. Thus, a part of the skin and deep signals appear to ascend through different pathways at the cervical level.

Discussion

The effect of acupuncture analgesia had been reported to depend on a typical sensation by insertion of a needle into muscle. Andersson⁵⁾ described for the induction of analgesic effect by electroacupuncture that the activities in deep afferents were important in the pain-blocking mechanisms during sensory stimulation in human subjects. It has been suggested that the analgesic effect of acupuncture is related to the deep afferents rather than to the cutaneous ones¹⁾.

In the present study, unitary discharges were recorded in VPL neurons that received information from skin and deep structures. VPL neurons were usually classified into LTM, WDR, and NS (nociceptive specific) neurons. The NS neurons responded only to noxious stimulation

applied to peripheral Rfs were not observed in this study. To determine the Rf of a NS neuron, it is necessary to apply a strong stimulation such as pinching of the skin that left evidence of damage (cutaneous welt, or inflammation of the skin). We made an effort to keep minimal damage on the skin of animals. Innocuous stimulus was always tried before noxious stimulation was used. Then electrical stimulation was applied independently to the skin or deep structure. The electrical stimulation activates many receptors in the tissues stimulated.

In the case of skin-deep units, the latency of the skin response was slightly longer than that of the deep response (Fig. 2C). This suggests that the afferent fibers and the ascending pathways to the thalamic VPL differ between skin and deep structures, probably depending on the characteristics of the stimulated tissue. Andersson et al.⁹⁾ reported that the latency of the thalamic potential in the cat VPL evoked by stimulation of group I muscle afferents was shorter than that of potentials evoked by skin afferents. Furthermore, Jones¹⁰⁾ suggested that the latency of the superficial radial nerve response was slightly longer than that of the deep radial nerve although afferent volleys in the deep and superficial radial nerves of the monkey entered the nervous system at approximately the same time in the same thalamic cell. Similar findings were also observed for the cat thalamus.

In the present study, the response of VPL units to both skin and deep stimulation were observed in 4 units of 30 units. The convergence of skin and deep information on the cells has been previously reported in the central nervous system including the spinal cord¹¹⁾, dorsal column nuclei¹²⁾, thalamus¹³⁾, and cerebral cortex¹⁴⁾. Ma et al.¹⁵⁾ demonstrated in the electron microscopic study that the medial lemniscal from dorsal column nuclei and spinothalamic tract (STT) from spinal cord sometimes contact single

ventrobasal neurons of the rat and thus converge at the thalamic level.

Following cordotomy at the cervical level, the deep responses may ascend through the dorsal column tract as well as the spinocervicothalamic tract passing through the dorsal quadrant of the spinal cord. The spinocervicothalamic tract transmits information from the skin¹⁶⁾, muscle and joint afferents.

In four skin-deep units, deep responses disappeared after dorsal column lesion. Recently, it was suggested that the postsynaptic dorsal column pathways play an important role in visceral nociception¹⁷⁾ and that both the postsynaptic dorsal column and spinothalamic pathways are converged at the thalamic level and that both pathways are related to the referred pain¹⁷⁾. Although not all signals through the dorsal column are related to visceral nociception in present study, some may have a role concerned to referred sensation.

The deep responses disappeared after lesion of dorsal column, whereas the skin responses of the two skin-deep units disappeared by lesion of hemisection contralateral to the Rf (Fig. 4) and the latencies of deep responses of those units were shorter than that of skin responses (Table 1). It suggests that signals from skin may ascend through more complex pathways than that from deep structures. It is also suggested that both the dorsal column and spinothalamic pathways appear to converge on the same neuron at the thalamic level.

In contrast to deep input, skin input appears to pass through both sides of the ventrolateral region of the spinal cord. In general, the axon of a given STT neuron decussates and ascends in the ventral quadrant to one of several thalamic nuclei including the VPL nucleus via the brain stem¹⁸⁾. However, Stevens et al.¹⁹⁾ reported that the axon of the STT ascends in the dorsal region of the lateral funiculus of both the ipsilateral and

contralateral cord.

Present results suggest that the deep afferents ascending through the dorsal quadrant of the spinal cord may be more important to activate the pain inhibitory mechanisms. Neurons that receive both skin and deep tissue inputs were observed in the thalamic VPL of cats. Cervical cordotomy confirmed that information from some peripheral receptors in both skin and deep structures ascend through several distinctive pathways in the spinal cord and project to the same thalamic neuron. Skin and deep inputs were seen to converge at the thalamic level in cats.

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References

- 1) Chiang, C.-Y., Chang, C.-T., Chu, H.-L., Yang, L.-F.: Peripheral afferent pathway for acupuncture analgesia. *Sci. Sinica*, 16: 210-217, 1973
- 2) The Academy of Traditional Chinese Medicine: *An Outline of Chinese Acupuncture*. Foreign Languages Press, Peking, 1975
- 3) Chang, H.-T.: Integrative action of thalamus in the process of acupuncture for analgesia. *Sci. Sinica*, 16: 25-60, 1973
- 4) Hashimoto, T. and Aikawa, S.: Manual acupuncture and its peripheral mechanisms: involvement of nociceptors in muscle. *J. Jap. Soci. Acu.* 44: 191-200, 1994
- 5) Andersson, S. A.: Pain control by sensory stimulation. In: J.J. Bonica et al. (Eds.) *Advances in Pain Research and Therapy*, Vol. 2, Raven Press, New York, pp.569-585, 1979
- 6) Saburi, M., Niki, K., Kobayashi, S. and Aikawa, S.: A magnetically coupled multimicroelectrode system. *IEEE Transactions on Biomedical Engineering*, BME-30: 341-346, 1983
- 7) Jasper, H. H. and Ajmone-Marsan, C.: *A stereotaxic atlas of the diencephalon of the cat*. National Research Council of Canada, Ottawa., 1954
- 8) Amassian, V. E., Berlin, L., Macy J. Jr. and Waller, H. J.: Simultaneous recording of the activities of several individual cortical neurons. *Trans. N.Y. Acad. Sci.*, 21: 395-405, 1959
- 9) Andersson, S. A., Landgren, S. and Wolsk, D.: The thalamic relay and cortical projection of group 1 muscle afferents from the forelimb of the cat. *J. Physiol.*, 183: 576-591, 1966
- 10) Jones, E. G.: Synaptic organization. In: *The Thalamus*, Plenum Press, New York and London, pp.153-223, 1985
- 11) Foreman, R. D., Schmidt, R. F. and Willis, W. D.: Convergence of muscle and cutaneous input onto primate spinothalamic tract neurons. *Brain Res.*, 124: 555-560, 1977
- 12) Millar, J.: Convergence of joint, cutaneous and muscle afferents onto cuneate neurons in the cat. *Brain Res.*, 175: 347-350, 1979
- 13) Kniffki, K. D. and Mizumura, K.: Responses of neurons in VPL and VPL-VL region of the cat to algescic stimulation of muscle and tendon. *J. Neurophysiol.*, 49: 649-661, 1983
- 14) Heath, C. J., Hore, J. and Phillips, C. G.: Inputs from low threshold muscle and cutaneous afferents of hand and forearm to area 3a and 3b of baboon's cerebral cortex. *J. Physiol.*, 257: 199-227, 1976
- 15) Ma, W., Peschanski, M. and Ralston, H. J. III.: The differential synaptic organization of the spinal and lemniscal projections to the ventrobasal complex of the rat thalamus. Evidence for convergence of the two systems

- upon single thalamic neurons. *Neuroscience*, 22: 925-934, 1987
- 16) Hongo, T.: Some functional aspects of the spinocervical tract. *Jap. J. EEG EMG*, 1: 52-60, 1972
- 17) Hirsberg, R. M., Al-Chaer, E. D., Lawand, N. B., Westlund, K. N. and Willis, W. D.: Is there a pathway in the posterior funiculus that signals visceral pain? *Pain*, 67: 219-305, 1996
- 18) Willis, W. D. and Coggeshall, R. E.: *Sensory Mechanisms of the Spinal Cord*. 2nd ed., Plenum, New York, 1991
- 19) Stevens, R. T., Hodge, C. J. Jr. and Apkarian, A. V.: Medial, intralaminar, and lateral terminations of lumbar spinothalamic tract neurons: A fluorescent double-label study. *Somatosens. Mot. Res.*, 6: 285-308, 1989

ネコ視床後外側腹側核ニューロンへの 皮膚および深部構造よりの脊髄内伝導路

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要 旨

針刺激による鎮痛効果発現には刺激部位の皮膚組織よりも深部組織からの入力に関与していることが指摘されている。そこで、ネコ視床後外側腹側核（VPL）への皮膚および深部組織よりの求心性入力の投射経路について、皮膚および深部構造への選択的通電刺激による誘発反応を指標に検討した。一部のVPLニューロンは皮膚および深部構造よりの入力の収束が認められた。深部入力は脊髄背側部を上行し、皮膚入力は背側部および両側の腹外側部を上行することが頸髄レベルでの部分切除術により明らかになった。両入力を受けるVPLニューロンへの脊髄内上行性伝導路は一部で異なることから皮膚および深部入力は視床レベルでも収束していることが確認された。

キーワード：視床VPL、皮膚入力、深部入力、脊髄切断、脊髄内伝導路