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Dorsal Column is not Involved in the Mechanism of the Hypotensive Effect by Simulating Acupuncture on Rat Hindlimb

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Abstract—**The present study investigated the role of the dorsal column (DC) in the mechanism of the hypotensive effect induced by simulating acupuncture on rat hindlimb. The femoral arterial pressure and electrocardiogram (ECG) of rats were recorded when the hypothalamic paraventricular nucleus (PVN) was electrically stimulated with or without DC lesion. Stimulation of the deep peroneal nerve (DPN) decreased the pressor response elicited by electrical stimulation of the PVN. Thirty minutes after micro-dissection of the right DC, the inhibitory effect of stimulating the right or left DPN on the pressor response induced by stimulation of the contralateral PVN was not altered. Of 6 rats tested, the inhibitory effect of stimulating the right or left DPN could still be observed five days after the right DC was destroyed. The pain responses of both hindlimbs of the rats with the right DC destroyed showed no obvious difference when compared with the sham control rats. These data suggest that the DC is not involved in the inhibitory effect of stimulating the DPN on the pressor response induced by the PVN activation.**

Keywords—**Acupuncture, deep peroneal nerve, dorsal column, hypothalamic paraventricular nucleus, pressor response**

I. Introduction

Acupuncture is a well-known Chinese traditional therapy for hypertension. Acupuncture and some of the modern rehabilitation therapeutics are based on the physiological modulatory function of the somatic afferent projection on the automatic nerve system. The somatic afferent projection or acupuncture could improve cardiac ischemia, arrhythmia and microcirculation.

The previous studies in our laboratory have shown that the deep peroneal nerve (DPN) afferent projection inhibited the pressor and ischemic responses caused by activating the periaqueductal gray (PAG) [1], dorsomedial hypothalamic nuclei (DMH) [2,3] and the rostral ventrolateral medulla (RVLM) [4,5]. It also counter-balanced the hypotensive response induced by activation of the caudal ventrolateral medulla (CVLM) [6].

The hypothalamic paraventricular nucleus (PVN) plays an important role in neuroendocrine and modulation of automatic nerve activity. It is an important modulatory area in the CNS for cardiovascular activity. However, there is no report concerning modulatory effect of the DPN on the cardiovascular effect induced by the PVN activation.

There are two somatic sensory pathways: one is the spinothalamic tract which transmits information about pain and temperature of trunk and limb; the other is the dorsal column (DC, including the gracile and cuneate fasicles) which transmits a different kind of somatic sensation, i.e., informa sition sense, such as two point discrimination, vibration and conscious proprioception [7]. Our previous study showed the somatic afferent of the DPN inhibited the ventricular arrhythmia and pressor response caused by stimulation of some nuclei in the hypothalamus through the pathway of the nucleus accumbens-ARH-ventral PAG- the raphe nuclei in the medulla (especially the nucleus raphe obscuris), and then inhibited the cardiovascular center in medulla and spinal cord [1-6,8]. However, it has not been clarified that which somatic sensory pathway is involved in the inhibitory effect of the DPN. Therefore, in the present study we determined whether destroy of DC affect signal transmission from somatic afferent by stimulating DPN in anesthetized rats.

II. Methodology

1) *Animal preparation:* Following induction of anesthesia with 1% sodium pentobarbital (50 mg/kg, i.p.), the male Sprague-Dawley rats (270~320 g) were mounted in a stereotaxic device. The femoral blood pressure, electrocardiogram (ECG) and heart rate (HR) were recorded by the MacLab system (MedEase Co., Nanjing, China). The rectal temperature was maintained between 37.5 and 38.5 ^{o}C.

2) *Electrical stimulation:* The DPN was isolated behind the knee joint for stimulation. A bipolar microelectrode (stainless steel, 24 KΩ in normal saline) was inserted stereotaxically into the PVN according to the rat brain atlas [9]. The PVN was electrically stimulated by the current pulses of 0.1~0.3 mA, 80 Hz, 0.5 ms for 10 s. The DPN was stimulated by the current pulses of 0.3~0.4 mA, 4 Hz, 0.5 ms for 5 min accompanied by stimulation of the PVN at the last 10 s.

3) *Micro-dissection of the dorsal column*: Unilateral DC was removed by a forceps after exposure of the thoracic spinal cord.

4) *Measurement of pain threshold*: We used a thermal pad in a jar with the water temperature of 56 ^{o}C to test the pain responses of the rat. The pain threshold is the time when the rats jump after they feel painful of heat. The average was calculated for three values measured for every 5 min.

5) *Anatomical confirmation*: After experiment, the direct current (100 μA, 20 s) was used to mark the stimulation site of the PVN. The rats were perfused transcardially with 0.9% saline followed by a mixture of 1%

potassium ferrocyanide and formalin saline. The brains were removed and post-fixed in formalin overnight and moved to 0.1 M phosphate buffer containing 20% sucrose. The hypothalami were cut transversally using a freezing microtome at a thickness of 50 μm. The stimulation site of the PVN and the area of the lesion of the DC were identified under microscope after the sections were stained by neutral red. The data of the inaccurate stimulating and lesion site were not included in the present results.

6) *Statistics*: The data were expressed as mean±SEM. The pressor response was calculated by the difference of the mean arterial pressure (ΔMAP) before and after stimulating the PVN.

III. Results

A. The modulatory effect of the DPN afferent projection on the cardiovascular response caused by activating the PVN

The baseline of MAP and HR of the rats were 13.46±0.33 kPa and 403±5.98 beat/min respectively. Of 8 rats tested, stimulation of the PVN caused the pressor responses, and the MAP recovered to baseline level 5-10 seconds after stimulation. After two stable pressor responses, the DPN was stimulated by the electrical current pulses for 5 min accompanied by stimulation of the PVN at the last 10 s. The pressor response caused by the PVN decreased (Table 1). The inhibitory effect of the DPN disappeared 30-45 min later.

B. The inhibitory effect of the DPN 30 min or 5 days after lesion of the right DC

Of 8 rats whose right DC was removed, the MAP increased when the left PVN was stimulated electrically and then recovered 5-10 seconds after cessation of the stimulation. After two stable pressor responses, the right DPN was stimulated by the current pulses for 5 min with stimulation of the left PVN at the last 10 s. The pressor response caused by the PVN decreased (Table 1). The inhibitory effect of the DPN lasted for 30-45 min. Stimulation of the left DPN also inhibited the pressor response induced by activation of the right PVN. Of 2 rats tested, the inhibitory effect of stimulating the right DPN before and after lesion was also not changed.

The 6 rats survived for five days after the right DC damage and were anesthetized again. The inhibitory effect of stimulating the right or left DPN could still be observed at fifth day after the right DC was destroyed (Table 1).

C. Muscle tone, flexion withdrawal reflex, pain threshold of both hindlimbs of the rats with the right DC dissected

The rats moved slowly and the muscle tension of the ipsilateral hindlimb decreased after one side of the DC was dissected (Fig. 1). The ankle joint could not crook and the resistance decreased or disappeared. When gently touched by the needle on the skin, the ipsilateral hindlimb did not withdraw compared to the contralaterl one. For all the 6 rats tested, the pain responses were sensitive to the heat plate. The pain responses of both hindlimbs of the rats with the right DC destroyed showed no significant difference when compared with the sham control rats (P>0.05).

TABLE I
THE INHIBITORY EFFECT OF STIMULATING THE RIGHT OR LEFT DPN ON THE PRESSOR RESPONSE INDUCED BY STIMULATING THE CONTRALATERAL PVN AFTER LESION OF THE RIGHT DC

Groups		ΔMAP (kPa)		
		Stimulation of PVN	Co-stimulation of DPN + PVN	Inhibitory percentage
Control (n=8)	DPN(R)	3.05±0.29	1.73±0.28**	43.29%
30 min after DC dissection (n=8)	DPN(R)	2.48±0.20	1.41±0.11**	38.64%
	DPN(L)	3.04±1.06	2.06±1.02*	39.97%
5 days after DC dissection (n=6)	DPN(R)	2.32±0.28	1.56±0.26**	33.87%
	DPN(L)	1.74±0.27	1.00±0.23*	36.86%

*P<0.05, **P<0.01 vs "stimulation of PVN".

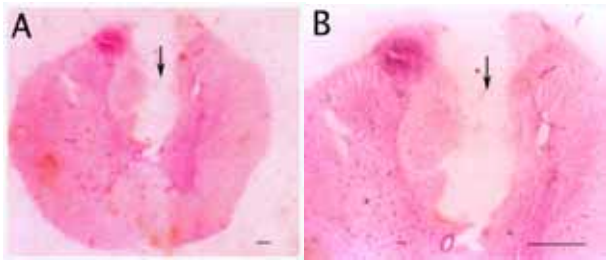

Fig. 1. The representative photomicrograph (coronal section) showed a lesion at the area of DC at thoracic level of the rat spinal cord. Magnitude: A, 15×; B, 60×. The arrow shows the destroyed region. The scale bar is 4mm.

IV. Discussion

Nowadays, scientists pay more and more attention to the central cardiovascular modulatory effect of the PVN since it was reported that the PVN has the direct fiber connection to the neurons in the intermediolateral cell column at the thoracic spinal cord [10,11]. The PVN modulates the cardiovascular activity directly through the PVN-spinal cord pathway or indirectly through brainstem A5 area to the sympathetic preganglionic neurons of the spinal cord. In this study, stimulating the PVN quickly increased the blood pressure. These results showed the PVN modulates the cardiovascular activity via automatic nerve system. The inhibitory effect of the DPN on the pressor response of the

PVN remained in rabbits with the vagus nerve cut, suggesting the sympathetic system was involved in this inhibitory effect [12].

In the spinothalamic tract, the cells whose axons form the tract are located in the spinal cord, and the last axons synapse in the thalamus (many axons synapse with other structures along the way) [13]. The axons of the dorsal column form a pair of columns located in the dorsomedial region of the spinal cord. The segment to which the DPN projects are L2-5, so we dissectioned the DC at middle-thoracic level of spinal cord to assure the afferent DC fibers have been blocked. After the inhibitory effect of the DPN on the pressor response was found to be unchanged, we tested the somatic sensory of the rats 5 days after the DC micro-dissection to make sure what we cut was the DC fibers, but not the spinothalamic tract. Of 6 rats tested, both hindlimbs had pain responses (the pain thresholds were similar) after unilateral DC lesion. This also showed the pain and temperature pathway was intact, i.e., the spinothalamic tract was not damaged. In addition, the rats showed decreased muscle tone of the hindlimb, much lower resistance of ankle joint, no flexion withdrawal reflex of the ipsilateral hindlimb. All of these suggested the DC was dissected. The lesion sites were also confirmed by neutral red stain of the sections after the experiment.

V. Conclusion

Electrical stimulation of the PVN increased arterial pressure, changed heart rate in anesthetized rats. This pressor response was inhibited by somatic afferent projection (stimulation of the DPN). The dorsal column system in the spinal cord was not involved in this inhibitory pathway.

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