대한침구학회지 제22권 제2호(2005년 4월)
The Journal of Korean Acupuncture & Moxibustion Society

원저

Differential changes of nicotinamide adenine dinucleotide phosphate-diaphorase, neuropeptide Y and vasoactive intestinal peptide in the cerebral cortex of the rat after repeated electroacupuncture

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Abstract

This study was undertaken to investigate the effects of electroacupuncture(EA) on Choksamni(ST36), a well-known acupuncture site, on nicotinamide adenine dinucleotide phosphate-diaphorase(NADPH-d), neuropeptide Y(NPY) and vasoactive intestinal peptide(VIP) in the cerebral cortex of spontaneously hypertensive rats(SHR). EA on Choksamni was applied using 2Hz electrical biphasic pulses of 10min, 3 times a week for a total of 10 sessions. Thereafter we evaluated changes in NADPH-d-positive neurons histochemically and changes in NPY and VIP-positive neurons immunohistochemically.

The optical density of NADPH-d-positive neurons in the Choksamni group was significantly lower in all areas of the cerebral cortex than in the control group. However, the optical density of NPY-positive neurons in the Choksamni group was similar to that of the controls in most areas of the cerebral cortex, with the exception of the primary motor and visual cortices. The optical density of VIP-positive neurons in the Choksamni group was significantly decreased as compared to the control group in most areas of the cerebral cortex, with the exception of the cingulate cortex.

The present results demonstrated that EA on Choksamni changes the activity of the NO system, and that stimulation at the same level, causes selective changes within the peptidergic system in the cerebral cortex of SHR.

Key words : Electroacupuncture, Nicotinamide adenine dinucleotide phosphate-diaphorase, Neuropeptide Y, Vasoactive intestinal peptide, Cerebral cortex

· **Acceptance** : 2005. 1. 11. · **Adjustment** : 2005. 3. 4. · **Adoption** : 2005. 3. 17.

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Ⅰ. Introduction

Acupuncture is one of the most significant treatment methods in eastern medicine, and is based on the use of special kinds of needles, which are used to stimulate certain acupoints. Moreover, acupuncture has been shown to be effective in the treatment of various diseases. The traditional explanation of it's mechanism centers upon meridian affecting certain organs. Scientifically it has been proven, by positron emission tomography[1] and functional magnetic resonance imaging[2] that acupuncture affects the central nervous system(CNS). Molecular biological and chemoarchitectural methods have also been widely introduced to identify the effects of acupuncture on the CNS[3-5].

Nitric oxide(NO) is an important cellular mediator in the CNS[6]. Release of NO has been implicated in a variety of neuronal processes, including enhanced neurotransmitter release, synaptic plasticity, and the regulation of gene expression, neuroprotection and neurotoxicity[7]. NO is synthesized in cells by nitric oxide synthase (NOS), and the nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) is a selective histochemical marker for NOS in the brain[8-10].

Although several reports have describing in detail the distribution and ontogeny of NADPH-d[7,9], neuropeptide Y (NPY)[11] and vasoactive intestinal peptide (VIP)[12], little information is available on changes of the species in neurons of the cerebral cortex on EA stimulation.

Previously, we reported that EA at the Choksamni acupuncture point changes the activity of the NO system in the brainstem of SHR, and the site of EA administration is of importance in terms of this suppressive effect[4]. However, no report is available on the effects of EA on the NO and peptidergic systems in the cerebral cortex. Therefore, we designed the present study to investigate the effects of EA on NADPH-d, NPY and VIP in the cerebral cortex of the spontaneously hypertensive rat(SHR).

Ⅱ. Materials and methods

Twenty-six male SHRs, weighing 280-310g, were used in this study. They were acclimatized for 2 weeks in a cage with water and food ad libitum, under an ambient temperature of 21℃ before the experiment was started.

The animals were randomly divided into two groups: the Choksamni group and the control group. The control group was placed under standard conditions without any stimulation, and the Choksamni group was given EA for 10 min, three times a week, from the third to the fifth experimental week. The point chosen for stimulation was Choksamni(ST36 located 5mm lateral and distal to the anterior tubercle of tibia). Needles(0.15mm) were inserted to a depths of 5mm, and electrical stimulation was provided by a pulse stimulator(Ito Co, Japan), which produced a bipolar square wave of 2Hz frequency. The current intensity was adjusted so that the localized muscle contractions could be seen[4].

Following the EA, the rats were anesthetized with pentobarbital sodium (60mg/kg, i.p.) and perfused with 4% freshly prepared paraformaldehyde in 0.1M phosphate buffer(PB), pH 7.4. The brains were then removed and frozen sections of 40m thickness were taken in the coronal plane. The sections

were stained to detect NADPH-d according to the histochemical method of Vincent and Kimura[10]. Free-floating sections were incubated at 37℃ for 60min in 0.1M PB, pH 7.4, containing 0.3% Triton X-100, 0.1mg/ml nitroblue tetrazolium and 1.0mg/ml -NADPH.

Following numerous rinses in PBS, the sections were processed for the immunohistochemical detection of NPY and VIP. Free-floating sections were incubated for 48h in PBS (4℃) containing anti-NPY and anti-VIP antiserum(1:2000 dilution), 0.3% Triton X-100, 0.05% bovine serum albumin and 1.5% normal goat serum. The sections were then incubated with biotinylated anti-rabbit IgG secondary antibody(1:200, Vectastain-Elite kit) for 1h and with avidin-biotin-peroxidase complex(1:100, Vectastain-Elite kit) for 1h at room temperature. They were then reacted with 0.02% 3,3'-diaminobenzidine tetrahydrochloride and 0.01% H2O2 for 3min. The staining intensity of the neurons was determined by measuring the optical density of NADPH-d-, NPY- and VIP-positive neuronsin 10 sections through the rostrocaudal extent for a minimum of 50 neurons. The optical density of these stained neurons were determined using a microdensitometry based method supported by an image analyzer(Multiscan, Fullerton, USA).

The data collected were expressed as means SEM of the average optical density for each cerebral cortex area. The Student's t-test was used to compare the optical density of the stained cells in the cerebral cortex of rats. The level of statistically significant difference was defined as $P < 0.05$.

III. Result

The optical density of the NADPH-d-positive neurons in the Choksamni group was significantly lower in all areas of the cerebral cortex than in the control group. However, the optical density of NPY-positive neurons in the Choksamni group was not significantly changed versus the control group in most areas of the cerebral cortex, with the exception of the primary motor cortex and the visual cortex. The optical density of

Table 1. Optical Densities of NADPH-d-Positive Neurons, Neuropeptide Y-Positive Neurons and Vasoactive Intestinal Peptide-Positive Neurons in the Cerebral Cortex of SHR after Repeated Electroacupuncture at Choksamni(ST36).

	NADPH-d		Neuropeptide Y		Vasoactive intestinal peptide	
	Control	Choksamni	Control	Choksamni	Control	Choksamni
M1	172.6±2.7	107.0±6.8*	128.5±2.2	121.0±1.4*	130.9±2.6	122.0±2.2*
S1	170.0±2.6	101.0±2.5*	124.8±1.7	125.4±2.5	133.6±1.9	117.3±2.8*
Vi	170.7±2.4	106.2±2.6*	130.9±1.6	118.0±1.7*	137.0±2.4	114.2±0.9*
Au	173.9±4.0	125.5±2.9*	118.8±1.5	112.0±1.5	135.0±2.1	119.6±1.4*
Cg	164.9±3.2	91.2±3.7*	126.1±2.0	125.9±1.2	135.1±2.7	127.0±2.2
PRh	189.1±2.2	113.0±2.1*	122.5±1.8	124.1±2.6	134.7±2.4	122.5±1.5*
Ins	177.4±3.6	125.5±3.1*	122.8±2.7	117.0±2.9	135.4±2.4	116.6±1.3*

Data are means ± SEM of the average optical density. n=50 per each brain area. * $P < 0.05$ compared with the control group (Student's t-test). M1, primary motor cortex; S1, primary somatosensory cortex; Vi, visual cortex; Au, auditory cortex; Cg, cingulate cortex; PRh, perirhinal cortex; Ins, insular cortex. Control, Group without electroacupuncture; Choksamni, Group given electroacupuncture at Choksamni(ST36).

VIP-positive neurons of the Choksamni group was significantly decreased versus the control group, in most areas of the cerebral cortex, with the exception of the cingulate cortex(Table 1).

Ⅳ. Discussion

Many neurophysiological articles have explored the pain-relieving effects of acupuncture[13]. The underlying mechanisms are often discussed in relation to eastern medicine, and relatively few have dealt with the effects acupuncture in the CNS, other than its overt pain-relieving effect. Previous studies have shown that acupuncture exhibits a variety of activating functions in the CNS, for example, it triggers the release of neuropeptides, regulates neuronal gene expression, and enhances neurogenesis[3,5,14].

Articles on EA-related changes on the NO system in CNS have reported that EA inhibits NO expression in the hippocampus of seizure-induced rats[15] and that it has protective effects against the neural damages induced by brain ischemia[16]. In our present study, the optical density of NADPH-d-positive neurons in the Choksamni group were significantly decreased in all areas of the cerebral cortex versus the control. This result is in line with the findings of our previous study, in which electroacupuncture stimulation at Choksamni showed significant decreases of the activity of NO in areas of the brainstem[4].

In previous studies, repeated treatments with EA have produced increased concentrations of NPY in the hippocampus and the occipital cortex of Sprague-Dawley rats[17], and EA was found to increase the concentration of NPY, but not to affect the concentration of VIP in the hippocampus of SHR[18]. However, these studies only dealt with changes within the occipital cortex of Sprague-Dawley rats, and did not explore changes in the peptidergic system of SHR on EA stimulation in areas of the cerebral cortex.

In this study of changes in the cortex, EA at Choksamni was not found to significantly change the optical density of NPY-positive neurons, but showed significant changes in the optical density of VIP-positive neurons in most areas of the cerebral cortex of the SHR. Our results are not in line with those of Bucinskaite et al[17-18]. These discrepancies may arise from the different acupoints(ST36; was close to the knee joint, BL11; close to the shoulder and BL54; close to the hip joint), and regions chosen,(i.e., we explored the cerebral cortex,and the others the hippocampus). The different animal models chosen(i.e., Sprague-Dawley rat vs. SHR) may also have influenced the results from the occipital region. These different changes also suggests the possibility that EA on specific acupoints may be lead to regional changes of the neuropeptides of the CNS.

Our results show that repeated EA at Choksamni decreased the activity of NADPH-d and on stimulation of equal quality on same acupoint, had selective changes within the peptidergic system in the cerebral cortex of SHR.

Ⅴ. Acknowledgement

This work was supported by a Research Grant from the Ministry of Health and Welfare

of Korea (HMP-99-0-11-0002-B).

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