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The Electrophysiological Study on the Influence of the Morphine Microiontophoresis on Impulsive Activity of Neurons in Nucleus Related to Acupuncture Analgesia

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Abstract We carried out the experiments on microiontophoresis of morphine to neurons in VLPAG (ventrolateral periaqueductal gray), RMG (raphe magnus nucleus) and Gi (gigantocellular reticular nucleus). Three-barreled glass micropipettes were used for the microiontophoresis of morphine. The position of tip of electrode was verified by methylene blue (MB) labeling. As a result, the rates of the excited neurons in the VLPAG, RMG and Gi were increased by microiontophoresis of morphine. This shows that the mechanism of the latency and aftereffect of acupuncture are related to amplified impulse activity of neurons in the VLPAG, RMG and Gi by morphine-type substance.

Key words acupuncture, microiontophoresis, microelectrode

Introduction

The great leader Comrade **Kim Jong Il** said as follows.

"Developing medical science and technology is an important task facing the public health sector today."("ON THE FURTHER IMPROVEMENT OF THE HEALTH SERVICE" P. 17)

Acupuncture plays an important role in traditional Koryo medicine. The most important effect of acupuncture is analgesia [11, 12]. Many nucleus such as PAG (periaqueductal gray), RMG, and Gi are concerned in acupuncture analgesia. And the endorphin and serotonin in cerebrospinal fluid (CSF) were multiplied by acupuncture [1, 2]. If hypophysis which secretes the endorphin was destroyed, the analgesia by acupuncture disappeared [2]. This shows that the role of humoral factors, especially morphine-type substance is very important in acupuncture analgesia. When a rat was stimulated by electroacupuncture at the Zusanli point (ST36) of a rat, the effect of analgesia was persisted for a while after stop of electroacupuncture [3]. By the way, many nucleuses such as PAG, RMG, and thalamus are concerned in the acupuncture analgesia on Zusanli point[5]. The obvious analgesia of morphine had appeared, when the morphine was microinjected into magnocellular nucleus, lateral reticular nucleus of midbrain [7]. And neurons with different characteristics exist there [8, 10]. Microinjection of morphine on the thalamus inhibited effectively the reaction of neurons in posterior horn of spinal cord by formalin nociceptive stimulus. And the effect was significantly decreased by microinjection of naloxone (1, 0.5μg), antagonist of opioid receptor [9].

In this paper, we introduce the reactionary characters of neurons in VLPAG, RMG and Gi by microiontophoresis of morphine to them, in order to find the neurohumoral mechanism of analgesia by acupuncture on the Zusanli point.

1. Objects and Methods

1.1. Objects

Wistar rats (200～230g) were used in experiments.

1.2. Methods

1.2.1. Determination of tail flick latency (TFL) by radiant heat in a rat.

A rat was put in small plastic box except the tail. When the tail of a rat was stimulated by using radiant heat, tail flick latency (TFL), threshold of the painful response on account of the radiant heat was determined [10].

1.2.2. The local stimulus and destroy of some brain structures in a rat.

Anesthesia of rats was induced with a single injection of thiopental.(60mg/kg)

A rat was fixed on the stereotaxic apparatus, then electrodes were inserted into the brain of a rat according to the stereotaxic atlas of a rat brain(Paxinos & Watson). After that, the electrodes were fixed on the bones of skull by using acrylic resins [4]. The stimulus experiments were performed after 3 to 5 days after inserting the electrodes.

By using the current stimulator "ST-3", the brain nucleus were stimulated with continuous impulse (0.1～0.2mA, 0.2ms, 50Hz). In some experiments we destroyed the brain nucleuses of a rat by passing the current. (1mA, 40s)

1.2.3. Microiontophoresis

Three-barrelled glass micropipettes were used for experiment. The current intensity for microiontophoresis is 30nA, time is 60s . The sites of tip of electrode were verified by methylene blue (MB) labeling [6]. The current of 50nA passed through electrode with methylene blue for 100s. The quantity of electricity passed through electrode was 500nC.

2. Results and Discussion

2.1. The influence of electroacupuncture of the Zusanli point (ST36) on the tail flick latency (TFL)

The influence of electroacupuncture at ST36 on TFL by radiant heat in a rat was observed. We divided the rats into two groups i.e. effective and uneffective group on acupuncture, analgesia effect in effective group was 1.2 times higher than control. We did the experiment in the effective group. When a rat was stimulated by electroacupuncture (1～2Hz, 20min) at the ST36 point, TFL changed as follows(Table 1).

As shown in the table 1, when a rat was stimulated by electroacupunncture (1～2Hz, 20min) at (ST36) point of a rat, the analgesia gradually appeared in a rat.

And the analgesia effect was the highest at 30min after acupuncture, the effect of analgesia persist for a while after stop of electroacupuncture.

Table 1. Changes of TFL by acupuncture on the ST36 point in a rat

Items	Before stimulus (control)	After stimulus/min						
		5	10	20	30	40	50	60
TFL/s	2.56±0.47	2.72± 0.47	3.17*± 0.20	3.77**± 0.48	4.63**± 0.34	4.06**± 0.34	3.56**± 0.56	3.27*± 0.59
Elongation rate of TFL%	100.0	106.3	123.8	147.3	180.8	158.6	139.1	127.7

* $p<0.05$, ** $p<0.01$ (compare with control)

2.2. The influence of PAG to acupuncture analgesia on ST36 point in effective group on acupuncture analgesia of rats

The influence of PAG to acupuncture analgesia on ST36 was observed in effective group on acupuncture analgesia of rats.

First of all, the changes of TFL of a rat on radiant heat were observed in the effective group on acupuncture analgesia, when a DLPAG (dorsolateral periaquedutal gray) was locally stimulated in a rat. (table 2)

When DLPAG was locally stimulated in a rat, the analgesia gradually appeared, its character assembled with that of ST36 point acupuncture.

Table 2. Changes of TFL of a rat, when exciting DLPAG

Items	Before stimulus (control)	After stimulus/min						
		5	10	20	30	40	50	60
TFL/s	20.00±0.37	2.30± 0.23	2.46*± 0.19	2.94**± 0.18	3.14**± 0.36	2.95**± 0.33	3.09*± 0.26	2.74*± 0.10
Elongation rate of TFL/%	100.0	115.0	123.0	147.0	157.0	147.5	152.5	137.0

* $p<0.05$, **$p<0.01$(compare with control)

This shows DLPAG is associated with analgesia by acupuncture on ST36 point. As a matter of fact, when ST36 point was stimulated after destroy of DLPAG, the analgesia didn't appear (table 3).

Table 3. The changes of TFL of a rat by ST36 point acupuncture after destroying DLPAG

Items	Before stimulus (control)	After stimulus/min						
		5	10	20	30	40	50	60
TFL/s	1.96±0.09	2.03± 0.54	2.06± o.67	2.06± 0.67	2.08± 0.06	2.09± 0.14	1.69± 0.09	2.06± 0.12
Elongation rate of TFL/%	100.0	103.6	105.1	105.1	106.6	106.6	100.0	105.1

The character that appears some latency and persistence in the analgesia by ST36 point acupuncture shows that the analgesia by acupuncture is related to humoral mechanism. But the TFL was elongated by local stimulus of VLPAG (Ventrolateral periaqueductal gray) only when it was excited(table 4).

Table 4. The changes of TFL of a rat by local stimulus of VLPAG

Items	Before stimulus control	After stimulus/min						
		5	10	20	30	40	50	60
TFL/s	1.98±0.19	3.82*± 0.55	2.27± 0.37	2.06± 0.09	2.00± 0.15	2.01± 0.19	1.99± 0.22	2.07± 0.15
Elongation rate of TFL/%	100.0	192.9	114.6	104.0	100.0	101.5	100.5	104.5

* $p<0.01$ (compare with control)

As shown in the table 4, when VLPAG in a rat was stimulated , TFL was elongated in the period stimulating the VLPAG (192.9%). That is, the analgesic effect appeared in the only period when VLPAG was stimulated, but didn't appear after stop of stimulation.

2.3. Influence of RMG and Gi stimulus to TFL of a rat on radiant heat

The characters of analgesia observed by local stimulus to VLPAG also appeared in RMG and Gi.

Table 5. Influence of RMG and Gi stimulus to TFL of a rat

Nucleuses	Items	Before stimulus control	After stimulus/min						
			5	10	20	30	40	50	60
RMG	TFL/s	1.99± 0.17	3.01*± 0.53	2.21± 0.36	2.06± 0.09	2.02± 0.14	2.01± 0.19	1.98± 0.17	2.07± 0.15
	Elongation rate of TFL/%	100.0	111.1	103.5	101.5	101.5	101.0	99.5	104.5
Gi	TFL/s	2.55± 0.14	3.94*± 0.66	2.43± 0.12	2.55± 0.18	2.55± 0.14	2.49± 0.10	2.52± 0.13	2.72± 0.31
	Elongation rate of TFL/%	100.0	154.5	95.5	100.0	100.0	97.6	98.8	106.6

* $p<0.05$ (compare with control)

As shown in the table 5, when RMG and Gi were individually simulated in a rat, TFL were elongated 151.3, 154.5% respectively. But the elongation of TFL didn't appear after stimulus. That is, there weren't the analgesia after stop of stimulus. When above data of table 4, 5 were analyzed with characters of ST36 point acupuncture analgesia, it showed that VLPAG, RMG, Gi were sites acting endogenic opioid substance and were concerned in the acupuncture analgesia. So we studied the change of impulse activity of neurons in VLPG, RMG and Gi of a rat by microiontophoresis of morphine.

2.4. The influence of morphine microiontophoresis to impulse activity of neurons in VLPAG, RMG and Gi

First, the changes of impulse activity of neurons in VLPAG to morphine microiontophoresis were studied. There were many neurons discharging spontaneously impulse in VLPAG. So those were divided into excited neurons, inhibited neurons and changeless neurons according to discharging frequency, and the changes of impulse activity of neurons by microiontophoresis were analyzed.

Table 6. The reactionary rates of neurons in the VLPAG by microiontophoresis of morphine

Groups	Number of neurons	Rate of excited neurons/%	Rate of inhibited neurons/%	Rate of changeless neurons/%
Control	28 (100.0%)	23.3	33.4	43.3
Experiment	26 (100.0%)	58.2	25.5	18.3

As shown in the table 6, the rate of excited neurons was increased from 23.3% to 58.2% and the rate of changeless neurons was decreased from 43.3% to 18.3% when microiontophoresis of morphine was performed in VLPAG.

And then, the changes of impulse activity of neurons by microiontophoresis of morphine in RMG and Gi were studied.

Table 7. The reactionary rates of neurons in the RMG and Gi by the micriontophoresis

Nucleuses	Groups	Number of neurons	Rate of excited neurons/%	Rate of inhibited neurons/%	Rate of changeless neurons/%
RMg	Control	28(100.0%)	21.5	28.7	49.8
	Experiment	26(100.0%)	53.8	23.1	23.1
Gi	Control	30(100.0%)	19.8	27.4	52.8
	Experiment	28(100.0%)	49.6	25.2	25.2

As shown in the table 7, the rate of excited neurons by microiontophoresis was increased than control in RMG (53.8%), and rate of excited neurons by microiontophoresis was increased than control in Gi. (49.6%) These show that the endogenous morphine-type substances are released by acupuncture and these can activate the neurons in VLPAG, RMG and Gi. As a result, the characteristic latency and the aftereffect are related with such mechanism. These are in accordance with results of experiment for local stimulus of VLPAG, RMG and Gi respectively.

Conclusions

1) The rates of the excited neurons in VLPAG, RMG and Gi are increased when microiontophoresis of morphine is performed.

2) The mechanism of the latency and the aftereffect of acupuncture on ST36 point are related to amplified impulse activities of neurons in the VLPAG, RMG and Gi by morphine-type substance.

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