Medulla Oblongata Mechanism of Inhibitory Effect of Thermal Stimulation to Nociceptive Colorectal Distention in Rats

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ABSTRACT

Objective: To discuss mechanism of moxibustion (thermal stimulation) effect and best moxibustion stimulus parameter.

Methods: Experiments were performed on 48 male Sprague-Dawley rats. Unit discharges from individual single neuron were recorded extracellularly with glass-microelectrode in Subnucleus Reticularis Dorsalis (SRD). Visceral-intrusive stimulation is done by colorectal distension. Thermal stimulation with different temperature (40°C, 42°C, 44°C, 46°C, 48°C, 50°C, 52°C) and different stimulus area (diameter of circle : 1.0 cm, 1.5 cm, 2.0 cm, 2.5 cm, 3.0 cm, 3.5 cm, 4.0cm) was applied around RN12 during nociceptive colorectal distension.

Results: SRD neurons could be activated by visceral stimulation within noxious range. Under low temperature of stimulus, especially under 45°C of pain threshold to ordinary people, visceral nociceptive afferent facilitated thermal stimulus from the body surface. While after thermal stimulation reached a harmful degree, the thermal stimulus will inhibit visceral nociceptive afferent. Moreover, statistics show that the higher the temperature is, the smaller the size of stimulation area is needed, and they correlate with each other negatively.

Conclusion: Visceral nociception could be inhibited by somatic thermal stimulation with specific parameter at medulla level. According to our finding, best thermal stimulation temperature is around 48°C and the best size of stimulation area is around 3.14-7.07cm² (with 2.0-3.0cm diameter).

Key words: Thermal stimulation, Subnucleus Reticularis Dorsalis, Stimulus Parameter

INTRODUCTION

In ancient time, the Father of Western Medicine said: disease, can be treated with metal (needle or knife) if cannot be treated with medicine; can be treated with fire if cannot be treated with metal; And it is cannot be treated with anything if cannot be treated with fire. And in traditional Chinese medicine, there were similar expressions in classics. At present, the mechanism of acupuncture analgesia have already been studied wildly and deeply. But research about the mechanism of moxibustion lag far behind.

In our study, we tried to use the SRD neuron as our research model to observe influences of moxibustion applied with different area and temperatures on the activity of these neurons in order to expound the best parameter of moxibustion treatment; if thermal sensitized acupoint is coursed under pathological circumstances; the neurological mechanism of effects of moxibustion and thermal sensitized acupoint.

MATERIALS AND METHOD

1. Animals

Experiments were performed on Sprague-Dawley rats weighing between 220-300g provided by the experimental animal center of Academy of Military Medical Science. The animals were anesthetized with an intraperitoneal injection of urethane (1.0~1.2 g·kg⁻¹). All the animal experiments in the present study were approved authoritatively in accordance with the Animal care and use principles of China Academy of Chinese Medical Sciences.

2. Surgery Preparation

Following an intraperitoneal injection of 100μg atropine sulfate, a tracheal cannula was inserted and the animals were paralyzed by intravenous injection of gallamine triethiodide (Flaxedil) and artificially ventilated.

The animals were mounted in a stereotaxic frame with the head fixed in a ventro-flexed position by means of a metallic bar cemented to the skull, and the foramen magnum was then exposed by removing the overlying musculature, atlantooccipital membrane, and dura mater dryness. The heart rate and body temperature were monitored in real time. The body temperature of rats was maintained between 36-38°C by feedback-control heating apparatus (RWDCL-8).

Visceral-intrusive stimulation is done by colorectal distension. A condom was used to make a 4–6 cm-long air sac, and it was tied to a 4 mm-diameter rubber tube. The tube was connected to a sphygmomanometer-pressure transducer with a T-tube. During the experiment, the air sac was inserted into the rat’s colorectum with a depth of 4 cm. CRD stimulation is carried out by pressure supplied by a 20–80 mm Hg sphygmomanometer for 20 s or longer. Previous research
indicated that the pressure bigger than 40 mm Hg is visceral-intrusive stimulation. In order to prevent possible sensitization triggered by over stimulation in the colorectum, the interval between two CRD stimulations should be at least more than 10 minutes.

3. Extracellular Recording
Unitary extracellular recordings were made with glass micropipettes filled with 5% NaCl and Pontamine Sky Blue solution. The micropipettes were inserted on the left side of the medulla, 1.0-2.0 mm caudal to the obex, and 0.5-1.5 mm lateral to the midline. Meanwhile, stainless steel electrodes were inserted on the tail of rats to emit harmful electronic stimulation (4-6mA, 0.66Hz, 2ms duration) to find SRD neuron. Cell electricity was amplified by oscilloscope (VC-10 Japan) and microelectrode amplifier (Photonix 8301) then processed by Electrophysiological recording system (Power-Lab) from microelectrode.

4. Procedures of experiment
First, neuron activity was recorded for 2 minutes, then 50s of CRD stimulation was given to the rats. At the same time, 5s reaction of neuron to CRD was recorded. Thermal stimulation was added after 10s of CRD stimulation for 30s, and the reactions were observed. After thermal stimulation, another 10s neuron’s reaction to CDR was recorded. In order to control the temperature of thermal stimulation accurately, water in different temperature bottleneck in different size of wild-mouth bottle (40°C, 42°C, 44°C, 46°C, 48°C, 50°C, 52°C) was used instead of moxibustion around RN12 (Zhongwan). Also, different sizes of area were stimulated: 0.785cm² (Φ1.0cm), 1.766cm² (Φ1.5cm), 3.14cm² (Φ2.0cm), 4.906cm² (Φ2.5cm), 7.065cm² (Φ3.0cm), 9.616cm² (Φ3.5cm), 12.56cm² (Φ4.0cm). There were in total 49 combinations of heat temperature and different sizes of area were stimulated: 0.785cm² (Φ1.0cm), 1.766cm² (Φ1.5cm), 3.14cm² (Φ2.0cm), 4.906cm² (Φ2.5cm), 7.065cm² (Φ3.0cm), 9.616cm² (Φ3.5cm), 12.56cm² (Φ4.0cm).

5. Histological location
After single-cell recording, the location was recorded by sending 20µA negative direct current to the glass microelectrode. The hearts of rats were retained and fixed by infusion. Then sections of icing brain tissue were obtained and observed by HE dying. The Rat Brain in Stereotoxic Coordinates, Sixth Edition (Paxions & Watson, 2007) was taken as reference to locate the microelectrode.

6. Data collection and analysis
Software such as Power-lab data acquisition system Chart 5.0, and SPSS13.0 were used for data collection and analysis. The volume of neuronal discharge per second and the activation/inhibition rate were calculated. The mean and standard deviation before and after the thermal intervention were calculated as the descriptive statistics and represented by $\bar{x} \pm SE$. The activation/inhibition rate was represented by $\delta \pm SE\%$. One-Way ANOVA was used for the comparison between groups. $P<0.05$ was considered as statistically significant.

RESULTS
1. The common features of SRD neuron activities
There are 105 neurons on the dorsal side of medulla oblongata in 48 male adult SD rats, among which there are 89 SRD neurons and 16 spinal trigeminal nucleus. Figure 1 shows the Pontamine sky blue location of part of the SRD neurons.

Over-threshold electronic stimulation (2ms duration) on any part of the body can activate SRD neurons. This activation is featured by two peaks of stimulation, before which there is a incubation period. The incubation period of stimulating the basilar part on the tail is shorter than that of stimulating 10cm from the tip of the tail. The two incubation periods of the basial tail are 12-40ms and 200-250 ms (after 50 single square wave stimulation), and those of the tip of tail are 23-60ms and 560-650ms. Statistics show that the time difference of early incubation period between the two location’s stimulation is 9.5±0.6ms, and it can be deduced that the peripheral fiber conduction velocity is 10.5±0.6m/s, which is in line with the conduction velocity of Aδ neurotransmitter fibers. Besides, the time difference of the latter incubation period between the two location’s stimulation is 145±10.2ms, and it can be deduced that the peripheral fiber conduction velocity is 0.7±0.05m/s, which is in line with the conduction velocity of C type neurotransmitter fibers. So it can be concluded that the early peak is activated by Aδ neurotransmitter fibers and the latter peak by C type neurotransmitter fibers. This is in accordance with the results drawn by Villanueva.[6-7]

All SRD neurons could not be activated by non-nocuous stimulation (such as sound, light, and proprioceptive), but they can be activated by nocuous mechanical stimulation (such as to pinch the skin with toothed forceps) or hot water with 48°C on general areas of the body.

2. Activation of CRD on SRD neuron
Like what was mentioned before, stimulation of CRD ≥40 mmHg is harmful to organs. In the experiment, CRD stimulation on 8 SRD neurons were observed. It has been recorded that the intensity of neuron activities increased from 2.85±1.72spikes/s to 10.53±3.81spikes/s, and the increase rate is 202.1±5.89%. The following Figure shows that there are significant difference ($P<0.001$) after 80mmHg stimulation.
CRD stimulation compared to the background, indicating that nocuous CRD can activate SRD neurons. (Fig. 2)

3. Influence on SRD neurons reaction under the simulation of CRD and different kinds of thermal stimulation

During stimulation with CRD, We examined SRD neurons’ responses to thermal stimulation with 40°C. Results showed that 40°C-Φ1.0cm (n=15), 40°C-Φ1.5cm (n=20) don’t have any influence on the activity of SRD induced by CRD; at the ranges of 40°C-Φ2.0cm (n=16), 40°C-Φ2.5cm (n=14), and 40°C-Φ3.0cm (n=11), the reaction of SRD can be slightly amplified with no statistical difference (P>0.05). However, when the simulation area reach the size of Φ3.5cm (n=11) and Φ4.0cm (n=8), 11±5.12% and 10.21±3.56% increase rate can be seen in SRD neuron activity, and compared with control group, there is statistical difference (P<0.05).

However, there are no statistical difference showed between the two stimulus intensities (P>0.05). (Fig. 3)

Similar to 40°C, thermal stimulation of 42°C-Φ1.0cm (n=11), 42°C-Φ1.5cm (n=13), 42°C had no effect on the activity of SRD induced by CRD. At the ranges of 40°C-Φ2.0cm (n=11) and 40°C-Φ2.5cm (n=15) the reaction of SRD can be slightly amplified with no statistical difference (P>0.05). However, at the ranges of Φ3.0cm(n=10), Φ3.5cm (n=9) and Φ4.0cm (n=7), increase rate of 10.56±4.32%, 9.38 ±4.58% and 9.27±3.94% can be seen respectively on SRD neuron activity. Compared with control group, there is statistical difference (P<0.05), while inter-group comparisons show that there are no statistical difference (P>0.05).

At 44°C, thermal stimulation of 44°C-Φ1.0cm(n=15) and 44°C-Φ1.5cm(n=11) had no effect on the activity of SRD induced by CRD, and there is no statistical difference compared to control group. At the range of 44°C-Φ2.0cm (n=10), thermal stimulation can increase 10.25±2.14% of SRD activity induced by CRD, and compared with control group there is statistical difference (P<0.05). Moreover, thermal stimulation of 44°C-Φ2.5cm(n=11) can raise 19.12 ±3.12% of SRD neurons activity induced by CRD; at the range of 44°C-Φ3.0cm(n=8), a 18.52±3.45% increase rate was seen; at the range of 44°C-Φ3.5cm(n=10), an increase rate of 22.85±2.45% was witnessed, and at the range of 44°C-Φ4.0cm(n=9), SRD neurons activity rose 20.14±1.45%. Statistics show that all these increase had statistical significance with P<0.05. (Figure 4 A & B)

At 46°C, thermal stimulation of 46°C-Φ1.0cm(n=14), 46°C-Φ1.5cm(n=11), 46°C-Φ2.0cm(n=16) and 46°C-Φ2.5cm (n=12) had no effect on activity of SRD induced by CRD, and there is no statistical difference compared to control group. At the range of 46°C-Φ3.0cm(n=10), thermal stimulation decreased SRD neurons’ discharges by 7.85±2.54%; at the range of 46°C-Φ3.5cm(n=9), thermal stimulation decreased reaction intensity of SRD neurons by 9.55±3.25%; and at the range of 46°C-Φ4.0cm(n=8), a 8.27±1.51% reduction rate was seen. Statistics show that all these increase had statistical significance with P<0.05.

At 48°C, thermal stimulation of 48°C-Φ1.0cm(n=17) can slightly increase activity of SRD neurons induced by CRD, but there is no statistical difference compared to control group. However, when the size of stimulus area is larger than Φ1.5cm(n=19), all 6 groups of thermal stimulation could inhibit activities of SRD neurons induced by CRD (Figure 5 B). To be specific, thermal stimulation of 48°C-Φ1.5cm decreased the activity of SRD neurons by 20.34...
±5.31%; at the range of 48°C-Φ2.0cm(n=11), a 25.85±4.54% decrease rate was seen. Differences of the two groups are statistically significant (P<0.01). At the ranges of 48°C-Φ2.5cm(n=10), 48°C-Φ3.0cm(n=10), 48°C-Φ3.5cm(n=6) and 48°C-Φ4.0cm(n=7), SRD neurons’ activity decreased 41.31±3.21%, 39.25±1.28%, 42.57±4.10% and 42.85±2.41% respectively. Statistics show that all these four groups have statistical difference compared to control group (P<0.001). (Figure 5 A & B).

At 50°C, the results is similar to the resulnts with 48°C. Thermal stimulation of 50°C-Φ1.0cm(n=10) reduced the activity of SRD induced by CRD by 18.68±3.71%, and there is statistical difference compared to control group (P<0.05). However, when the size of stimulus area is larger than Φ1.5cm(n=9), 6 groups of thermal stimulus could restricted the activities of SRD neurons. At the ranges of 50°C-Φ1.5cm(n=10), 50°C-Φ2.0cm(n=9), 50°C-Φ2.5cm(n=8), 50°C-Φ3.0cm(n=9), 50°C-Φ3.5cm(n=6), and 50°C-Φ4.0cm(n=6), SRD neurons’ activity decreased 36.1±4.31%, 46.23±5.32%, 56.22±4.23%, 52.52±2.32%, 51.25±3.54% and 46.57±6.12% respectively. Statistics show that all these six groups have inhibiting effect on SRD neurons and there were statistical difference compared to blank group (P<0.001).

Viewing from the results, it can be concluded that under low temperature of stimulus, especially under 45°C of pain threshold to ordinary people, viseral nocicepetive afferent facilitated thermal stimulus from the body surface and further activated SRD neurons. While after the thermal stimulation reached a harmful degree, the thermal stimulus will inhibit viseral nocicepetive afferent. Moreover, statistics show that the higher the temperature is, the smaller the size of stimulating area is needed, and they correlate with each other negatively. From Figure 6, it can be seen that at the degree of 48°C with Φ2.0cm~3.0cm, the effective rate can reach to 50%.

**DISCUSSION**

1. SRD neurons and nociceptive information processing

According to former research, much more attention has been drawn to the SRD’s function to process periphery afferent. First, all SRD neurons could accept peripheral afferent from
noxious inhibitory controls (DNIC) [8, 9]. The integrative stimulus on either part of the body was referred as diffuse That nociceptive responses can be inhibited by harmful discused under the research of spinal dorsal horn neurons.

function of SRD neurons, and it is a neurological function of higher level stimulus. The saturation as well as negative information, as SRD activities could be further activated by activation on harmful spinal stimulus by following cannot be illustrated by the saturation of afferent lines of reaction can be drawn to calculate the different stimulus sizes and intensities of heat-moxibustion. analyze the dose-effect activation on these neurons in the route of ventral lateral nerve tract.
temperature can keep above 40°C for 350s. Okazaki, M., while 20 moxa cones can reach 150°C at highest, and the temperature can keep above 40°C for 20s; in Song Dynasty, Dou Cai wrote in “Bianque Xinzhu-Wangshi Zhifa” that to save life, moxibustion should be firstly used, then pills and medicine, and the third Fuzi. In Qing Dynasty, all doctors have emphasized on moxibustion, it is also said in “Yixue Rumen” that people should perform moxibustion in every season to strengthen qi and resist diseases and that if medicine and acupuncture are impotent, moxibustion can be used.

Except for moxa, materials like sulphur, wick, mulberry twig, peach branchlet, beeswax, and pill lozenges are all often used. Also, there are thunder fire miraculous moxa roll, Taiyi miraculous moxa roll, oil-lamp moxibustion (lighting rush and oil on the skin of patients), canister moxibustion (insert bamboo or reed tube into patient’s ear to treat ear diseases) and so on. The methods of moxibustion are various, including direct contact moxibustion, indirect contact moxibustion, needle warming moxibustion, mild moxibustion, sparrow-pecking moxibustion and so on. Indirect moxibustion also can be subdivided into several types: ginger moxibustion, garlic moxibustion, Fuzi-cake moxibustion, Douchi-cake moxibustion, Pepper-cake moxibustion and so on. Clinically, indirect moxibustion and moxa cone are mostly used. In general, the heat, size and time of moxibustion are critical factors, and the amount of moxa and size of area can be adjusted. It is better that the skin keeps red and warm, but should not be scorched (except for blistering moxibustion).

As to the size of stimulation, the diameter of moxa cone often used is 1.0cm and that of moxa stick is 1.2 cm\(^{[17]}\). They can at least warm the skin area of 3cm diameter. As a special moxibustion, long snake moxibustion is also widely applied in clinical treatment. The size of area it covers is the largest mostly used. In general, the heat, size and time of moxibustion are critical factors, and the amount of moxa and size of area can be adjusted. It is better that the skin keeps red and warm, but should not be scorched (except for blistering moxibustion).

When a 2mg moxa cone is used, along with the increase of moxa cone, the highest temperature will rise and duration will extend. For example, one moxa cone can reach 100°C at highest, and the temperature can keep above 40°C for 20s; while 20 moxa cones can reach 150°C at highest, and the temperature can keep above 40°C for 350s. Okazaki\(^{[18]}\), M have observed the effect of single and multiple moxa cones on skin. He also regards that in single moxa cone, the temperature can go up with the increase of moxa cone diameters (0.5–2cm). On skin, the temperature of single moxa cone can rise to 105°C and the temperature of 3 moxa cones can rise to 125°C. Under skin, the temperature of single moxa cone can reach 56°C, which is similar to the temperature of three moxa cones. It can be seen that the moxibustion stimulation often exceed the feeling threshold of pain (45°C)\(^{[19]}\). In clinical treatment, long snake moxibustion and blister moxibustion are commonly used, which can exceed the pain threshold of human beings. Viewing from the most clinical experiments, while these stimulation can achieve well effect, they can also generate physical damage.

Visceral nociception could be inhibited by somatic thermal stimulation with specific parameter at medulla level. According to our finding, best thermal stimulation temperature is around 48°C and the best size of stimulation area is around 3.14-7.07cm\(^2\) (with 2.0-3.0cm diameter).

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