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Research report

Effect of hypothalamic supraoptic nucleus on acupuncture analgesia in the rat

Jun Yang [a,*], Yu Yang [a,1], Jian-Min Chen [a], Wen-Yan Liu [b], Bao-Chen Lin [c]

[a] *Institute for Pharmaceuticals and Medical Science, Guangdong Bangmin Pharmaceutical Co. Ltd., Jianhai Distract, Jianmen, Guangdong 529080, China*
[b] *Department of Physiology, Jining Medical College, Jining, Shangdong 272113, China*
[c] *Department of Neurobiology, Second Military Medical University, Shanghai 200433, China*

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Abstract

Hypothalamic supraoptic nucleus (SON) has been demonstrated to involve in pain modulation. Acupuncture analgesia is a very useful clinical skill for pain relief, which has over 2500-year history in China. The present study investigated the effect of SON on acupuncture analgesia in the rat. Electrical stimulation of the SON or microinjection of a small dose L-glutamate sodium into the SON enhanced acupuncture analgesia in a dose-dependent manner, while cauterization of the SON weakened acupuncture analgesia. Pituitary removal did not influence the effect of L-glutamate sodium that enhanced acupuncture analgesia in the SON. The data suggested that the neurons and not the nerve fibers in the SON played an important role in acupuncture analgesia, which effect might be through the central nervous system rather than the hypothalamo-neurohypophyseal system.

Keywords: Hypothalamic supraoptic nucleus; Acupuncture analgesia; Nucleus stimulation; Nucleus cauterization; Central nervous system; Rat

1. Introduction

The hypothalamic supraoptic nucleus (SON) is a very important neural structure that synthesizes and secretes arginine vasopressin (AVP) and oxytocin (OXT) [8,12]. Intraventricular injection of AVP or OXT increases the pain threshold, whereas local administration of anti-AVP serum or anti-OXT serum decreases the pain threshold [22,32–34]. Pain stimulation changes AVP and OXT concentrations in the SON [21,32,33]. The SON, then, might influence pain modulation through its bioactive substances such as AVP and OXT.

Acupuncture analgesia, which has over 2500-year history in China, is a method of pain relief by using acupuncture. As a very useful clinical skill, acupuncture analgesia has been used widely in many kinds of surgery including pulmonary, brain, heart, gastrointestinal tract, and thyroid since it was applied in the tonsillectomy first time in 1958 [43]. In 1979, Lin et al. has reported that SON lesion influences the effect of acupuncture analgesia [9]. The present study used SON stimulation and cauterization to investigate the effect of SON on acupuncture analgesia and eliminates the pituitary role to research the mechanism of SON-regulated acupuncture analgesia, i.e. whether the effect of SON on acupuncture analgesia was via the central nervous system and/or the hypothalamic-neurohypophyseal system in the rat.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing 180–220 g were used in all of the experiments (Nanfang Medical University, Guangzhou, China). Animals were housed in a colony room under controlled temperature, humidity and in a 12 h light:12 h dark cycle (light on at 6:00 am), with food and water available *ad libitum*. All procedures were conducted according to the guidelines of the International Association for the Study of Pain [44].

2.2. Surgery

Under pentobarbital sodium (35 mg/kg, intraperitoneal injection) anesthesia and with Pellegrino L.J. rat brain atlas as a reference [13], we used a stereotaxic apparatus (Jiangwan I-C, Shanghai, China) to insert bipolar

* Corresponding author at: 10-6655 Fielding Avenue, Montreal, Que. H4V 1N4, Canada. Tel.: +1 514 481 0631.
E-mail address: yj6676@yahoo.com (J. Yang).
[1] Volunteer, a student from École secondaire Saint-Luc, Montreal, Que. H3X 2H2, Canada.

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electrodes into the SON on both sides (AP −1.0 mm, LR ± 1.8 mm, H 7.0 mm) for electrical cauterization of the nucleus, or to place a bipolar electrode into the right SON for electrical stimulation of the nucleus, or to implant a stainless steel guide cannula of 0.5 mm outer diameter into the right SON for microinjection of the nucleus. The electrode or the guide cannula was fixed to the skull by dental acrylic. All operations were carried out under aseptic conditions.

For the SON stimulation or microinjection experiments, the animals needed at least 14 days for recover after surgery.

2.3. SON cauterization

For the electrical cauterization of the SON, an electrical current (direct current) of 1.0 mA was injected into the SON over 10 s during surgery. The rats needed at least 7 days to recover after SON cauterization.

2.4. Pituitary removal

One week after implantation of the guide cannula, the pituitary gland of the rat was aspirated through the left ear channel using a special needle of 1.2 mm outer diameter under diethyl ether anesthesia. In the control group, the animal underwent a sham surgery, i.e. the operation was carried out but the pituitary gland was not removed. The rats needed at least 7 days to recover after pituitary removal.

2.5. SON stimulation

Electrical stimulation of the SON was done with a Shanghai JSD-731-C electro-stimulator. Applying the bipolar electrode, the right SON was stimulated with a square pulse of electrical current (direct current, 30 Hz, 0.3 ms) of 3.12, 12.5, 50 or 200 μA (the experimental group) for 2 min. In a control group, no electrical current was passed [40]. Each animal was given the stimulation only once.

2.6. SON microinjection

For the SON microinjection, a stainless steel needle with 0.3 mm diameter was directly inserted into the guide cannula, 1 mm beyond the tip. 1 μl of a solution of artificial cerebrospinal fluid (ACSF, containing 0.1 M NaCl, 1.0 mM KH_2PO_4, 4.0 mM KCl, 2.0 mM $MgSO_4$, 2.0 mM $CaCl_2$, 2.1 mM $NaHCO_3$ and 8.0 mM Glucose), which contained 0.02, 0.08, 0.32 or 1.28 μM L-glutamate sodium, was gently microinjected into the SON over 10 min. In a control group, no L-glutamate sodium was dissolved in the ACSF. Each animal was microinjected only one time.

2.7. Nociceptive tests

All animals were tested under the condition of free activity in the small cages (30 cm in diameter, 25 cm in height) from 8:00 to 10:00 am. Potassium iontophoresis inducing tail-flick served as the nociceptive stimulus. A small wet cotton pad saturated with a solution of potassium was set on the skin of the tail. The cotton was exposed to electrical current (direct current), and the anode led the potassium to permeate the skin of the tail. If the current was strong enough, the permeating potassium resulted in the animal feeling the nociceptive stimulation. The intensity of the electrical current at the moment of the response was recorded as the nociceptive threshold, which was expressed as mA (WQ-9E Pain threshold Measurer, Shanghai, China) [39]. The nociceptive stimulus was terminated at once when the rat showed a response to it.

2.8. Electrical acupuncture

The bilateral points of "Zusanli" (St. 36) were used for electrical acupuncture [29]. The bilateral points were passed the stimulated electrical current with alternating polarities and a dense-disperse wave (JSD-731-C electro-stimulator, $f_1 = 10$ Hz, $f_2 = 20$ Hz) for 30 min. The intensity was adjusted until the animal showed comfortably that the local muscle appeared a little contraction ($v = 2$–3 V) [29].

2.9. Experimental protocol

The standard experimental protocol for electrical stimulation and microinjection of the SON was as follows: after measuring the basic pain threshold, the SON was electrical stimulated (maintained over 2 min) or the solution with L-glutamate sodium was microinjected (over 10 min); then, the pain threshold was measured 5 min after treatments. The time between the two measurements of pain threshold was 10 min.

2.10. Histological verification

At the end of the experiments, the rats were sacrificed under a high dose of pentobarbital sodium (80 mg/kg, intraperitoneal injection), and the histological locations of SON microinjection, electrical stimulation or cauterization were ascertained (Fig. 1). The data were rejected if the histological locations were not accurate.

2.11. Statistical analysis

Data were expressed as mean ± standard error of the mean (S.E.M.) and were analyzed between groups by two-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

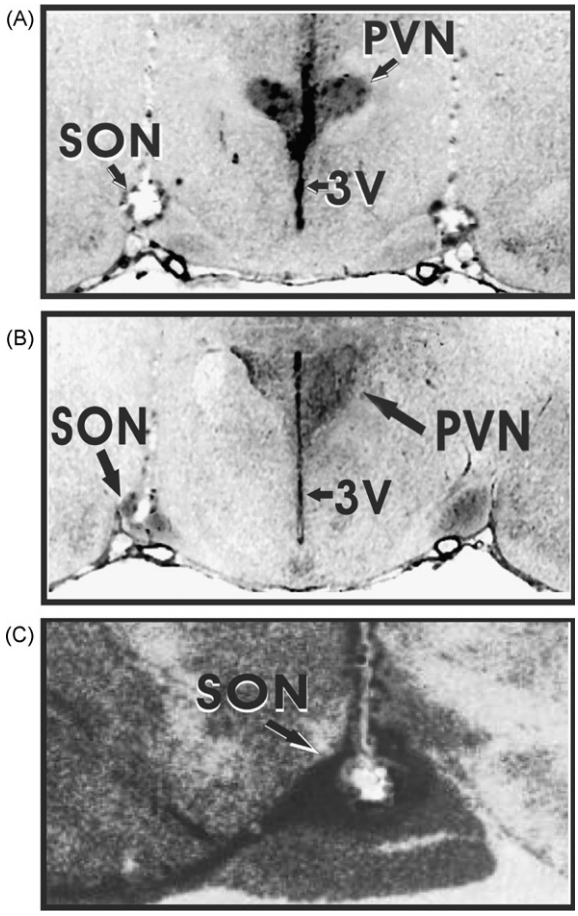

Fig. 1. Histological verification of the hypothalamic supraoptic nucleus (SON). The microphotograph shows the brain location of SON in AP −1.0 mm. (A) SON cauterization; (B) SON electrical stimulation; (C) SON microinjection.