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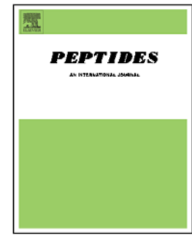

Effect of arginine vasopressin on acupuncture analgesia in the rat

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ABSTRACT

Arginine vasopressin (AVP) has been proven to be involved in the process of pain regulation. This communication was designed to investigate the effect of AVP on acupuncture analgesia in the rat model. The results showed that intraventricular injection (*icv*) of AVP could enhance acupuncture analgesia in a dose-dependent manner, whereas *icv* of anti-AVP serum decreased acupuncture analgesia. However, neither intrathecal (*ith*) nor intravenous injection (*iv*) of AVP or anti-AVP serum could influence acupuncture analgesia. Electrical acupuncture of "Zusanli" points (St. 36) decreased AVP concentration in the hypothalamic paraventricular nucleus (PVN), and increased AVP concentration in the hypothalamic supraoptic nucleus (SON), periaqueductial gray (PAG), caudate nucleus (CdN) and raphe magnus nucleus (RMN), but did not change AVP concentration in the pituitary, spinal cord and plasma. The effect of AVP on acupuncture analgesia was partly reversed by pretreatment with naloxone, an opiate receptor antagonist. These data suggested that AVP in the brain played a role in the process of acupuncture analgesia in combination with the endogenous opiate peptide system.

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1. Introduction

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 surgeries of pulmonary, brain, heart, gastrointestinal tract and thyroid since it was used in the tonsillectomy first time in 1958. The mechanism of acupuncture analgesia is a dynamic balance process between nociceptive and antinociceptive substances in the body especially in the central nervous system [33].

Arginine vasopressin (AVP), a nonapeptide posterior pituitary hormone, is mainly synthesized in the hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON). This hormone, combined with an apparent carrier protein

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(neurophysin), is transported along the hypothalamo-hypophyseal pathway to the neurohypophysis, in which it is stored for subsequent release. The remarkable functions of AVP include body fluid homeostasis, hormone regulation, cardiovascular control, learning and memory [9]. A lot of work has reported that AVP influences pain modulation in both human and nonhuman species [2–4,8]. Our previous study showed that intraventricular injection (*icv*) of AVP increased pain threshold and *icv* administration of anti-AVP serum decreased pain threshold, but neither intrathecal (*ith*) nor intravenous injection (*iv*) of AVP or anti-AVP serum could influence pain threshold [22].

PVN and SON, the main source of AVP, play roles in acupuncture analgesia [16,19,26,27]. In the process of PVN regulating acupuncture analgesia, AVP has been shown more important rather than oxytocin and endogenous opiate peptides [20,21,23]. AVP in the periaqueductal gray (PAG), caudate nucleus (CdN) and raphe magnus nucleus (RMN) relates to pain modulation [15,17,18,28,29]. Depending on the data above, we presume that AVP is involved in the process of acupuncture analgesia. Of course, it needs to be clarified in which level (e.g. the brain, the spinal cord or the systemic circulation) AVP takes part in acupuncture analgesia.

2. Materials and methods

2.1. *Animals*

Adult male Sprague–Dawley rats weighing 180–220 g were used in all experiments (Second Military Medical University, Shanghai, China and Nanfang Medical University, Guangzhou, China). Animals were housed in a colony room under controlled temperature, humidity and a 12 h light/dark cycle (light on at 6:00 a.m.), with food and water available *ad libitum*. All procedures were conducted according to the guidelines of the International Association for the Study of Pain [34].

2.2. *Materials*

AVP was obtained from Peninsula Laboratories, San Carlos, CA, USA; ^{125}Iodine was from Amersham Pharmacia, Buckinghamshire, UK; the other chemicals were from Sigma Co., St. Louis, MO, USA.

Rabbit anti-rat AVP serum was made by Department of Neurobiology, Second Military Medical University, Shanghai, China. The specificity of the antiserum was more than 99.98% cross-reactivity with AVP and less than 0.12% cross-reactivity with oxytocin, vasotocin, lysine-vasopressin, vasoactive intestinal peptide, neurotensin, leucine-enkephalin, methionin-enkephalin, β-endorphin and dynorphinA_{1-13}. The dilution of the antiserum was more than 1:40,000 for the radioimmunoassay [6].

2.3. *Surgery*

2.3.1. *For intraventricular injection (icv)*

With Pellegrino L.J. rat brain atlas as reference, we used the stereotaxic apparatus (Jiangwan I-C, Shanghai, China) to implant a stainless steel guide cannula of 0.6 mm outer diameter into the left lateral ventricule (AP 0.3 mm, LR −0.5 mm, H 3.0 mm) for the *icv* under the pentobarbital sodium (35 mg/kg, intraperitoneal injection) anesthesia. The guide cannula was fixed to the skull by dental acrylic.

2.3.2. *For intrathecal injection (ith)*

Under pentobarbital sodium anesthesia (35 mg/kg, intraperitoneal injection), the rat was implanted a chronic intrathecal catheter (PE-10, 12 cm in length, 0.6 mm outer diameter) extending into the lumbar enlargement of the spinal cord for *ith*.

All operations above were carried out under the aseptic condition and the animals needed at least 14 days for recovery after the surgery.

2.4. *Nociceptive tests*

All animals were tested under the condition of free activity in the small cages (30 cm in diameter, 25 cm in high) from 8:00 to 10:00 a.m. The potassium iontophoresis inducing tail-flick served as pain stimulus. The small wet cotton with the solution of potassium iontophoresis was set on the tail skin. The cotton was given the direct electrical current, and the anode led the potassium iontophoresis to permeate the tail skin. If the current was strong enough, the permeated potassium iontophoresis caused the animal feeling the pain stimulation. When the rat began to flick his tail to response this feeling, the intensity of current at the moment of the response was recorded as the pain threshold, which was expressed as mA (WQ-9E Pain Threshold Measurer, Shanghai, China). The intensity of direct electrical current from WQ-9E was increased lineally.

2.5. *Electrical acupuncture*

The stainless needles (0.2 mm in diameter and 3 mm in long) were placed at the bilateral points of ‘‘Zusanli’’ (St. 36). The stimulated electrical current was passed the bilateral points with alternating polarities and a dense-disperse wave (JSD-731-C electro-stimulator, pulse width $f_1 = 10$ Hz, $f_2 = 20$ Hz) for 30 min. The intensity was adjusted until the animals appeared comfortably and the local muscle contractions were seen ($I = 10–15$ mA).

2.6. *Microinjection or intravenous injection (iv)*

2.6.1. *icv*

The animal was gently handled and a stainless steel needle with 0.4 mm diameter for *icv* was directly inserted into the guide cannula, 1 mm beyond the tip. Ten μl of serum (anti-AVP serum or normal rabbit serum) or solution (AVP solution or artificial cerebrospinal fluid) was gently injected into the lateral ventricule over 10 min.

2.6.2. *ith*

Ten μl of serum or solution was gently injected into the lumbar enlargement of the spinal cord through the chronic intrathecal catheter over 10 min.

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