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Effect of arginine vasopressin in the nucleus raphe magnus on antinociception in the rat

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ABSTRACT

Previous work has shown that arginine vasopressin (AVP) regulates antinociception through brain nuclei rather than the spinal cord and peripheral organs. The present study investigated the nociceptive effect of AVP in the nucleus raphe magnus (NRM) of the rat. Microinjection of AVP into the NRM increased pain threshold in a dose-dependent manner, while local administration of AVP-receptor antagonist-d$(CH_2)_5$Tyr(Et)DAVP decreased the pain threshold. Pain stimulation elevated AVP concentration in the NRM perfuse liquid. NRM pretreatment with AVP-receptor antagonist completely reversed AVP's effect on pain threshold in the NRM. The data suggest that AVP in the NRM is involved in antinociception.

1. Introduction

Arginine vasopressin (AVP), a nonapeptide posterior pituitary hormone, is synthesized in the paraventricular and supraoptic nuclei of the hypothalamus [12]. This hormone, combined with an apparent carrier protein (neurophysin), is transported along the hypothalamo-hypophyseal pathway to the neurohypophysis, where it is stored for subsequent release [41]. The remarkable functions of AVP include body fluid homeostasis, hormone regulation, cardiovascular control, learning and memory [13]. AVP has been identified as an important factor governing analgesia in both human and nonhuman species [3,5–7,18–20,23]. Intraventricular injection of AVP increases pain threshold, and administration of anti-AVP serum decreases it [40].

The hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON), which direct integrated and adaptive responses to pain [21,36,39], are the main neural structures of AVP synthesis and secretion [8]. AVP plays an important role in the analgesia induced via the PVN and SON [37,38]. The PVN and SON project AVP-containing nerve fibers to the nucleus raphe magnus (NRM), which affects pain modulation [2,14,30,32]. There are a number of other peptides, including endogenous opiate peptides, in the NRM, and classic neurophysiology of the NRM (ON and OFF cells) modulates pain inhibition and pain facilitation [9,31]. Acupuncture,

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which induces analgesia, causes an increase in AVP concentration in the NRM [35]. Thus we presume that the AVP in the NRM is involved in antinociception.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing 180–220 g were used in all of the experiments (Experiment Center of Second Military Medical University, Shanghai, China). Animals were housed in a colony room under controlled temperature, humidity and in a 12 h light/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 [42].

2.2. Materials

AVP and d$(CH_2)_5$Tyr(Et)DAVP were obtained from Peninsula Laboratories, San Carlos, CA, USA; ^{125}Iodine was from Amersham Pharmacia, Buckinghamshire, UK; other chemical reagents were from Sigma Co., St. Louis, MO, USA.

Rabbit anti-rat AVP serum was prepared by the Department of Neurobiology, Second Military Medical University, Shanghai, China [22]. The specificity of the antiserum was more than 99% cross-reactivity with its corresponding antigen and less than 1% cross-reactivity with other similar peptides such as oxytocin, vasotocin and lysine vasopressin.

2.3. Surgery

With the Pellegrino L.J. rat brain atlas as reference, we used a stereotaxic apparatus (Jiangwan I-C, Shanghai, China) to implant a stainless steel guide cannula (0.5 mm outer diameter) into the NRM (AP 8.4 mm, LR 0.5 mm, H 8.2 mm) for push–pull perfusion or microinjection under sodium pentobarbital (35 mg/kg, intraperitoneal injection) anesthetization [34]. The guide cannula was fixed to the skull by dental acrylic. All operations were carried out under sterile conditions and the animals needed at least 14 days to recover after surgery.

2.4. Neciceptive tests

Potassium iontophoresis inducing tail-flick served as the pain stimulus, and the intensity of the current at the moment of response was recorded as the pain threshold, which was expressed as mA (WQ-9E Pain Threshold Measurer, Shanghai, China) [18].

2.5. Pain stimulation

Through the positive electrode, Pain Threshold Measurer (as above) producing the direct electrical current induced the potassium iontophoresis to the tails as the pain stimulation, which was acute pain. The intensity was fixed to 1.2–1.4 × the pain threshold (0.6–0.7 mA), and the duration time was 1 min.

2.6. Microinjection

All microinjection experiments were carried out as double-blind studies. For NRM injection, a 0.3 mm diameter stainless steel needle was gently inserted into the guide cannula, 1 mm beyond the tip. The 1 μl solution with artificial cerebral spinal 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) was gently injected into the NRM over 10 min [18].

2.7. Push–pull perfusion

A stainless steel needle, 0.3 mm in outer diameter, was directly inserted into the guide cannula, 1 mm beyond the tip, for pushing ACSF into the NRM, and the guide cannula for pulling the ACSF out. The pushing ACSF was warmed at 37 °C to pass through the nuclei of the conscious rats (0.05 ml/min), and the extracted fluid was collected at 0 °C every 10 min. The samples were stored at −80 °C for assay [25,26,34]. The pain stimulation was treated as above (Section 2.5) in this experiment.

2.8. Radioimmunoassay (RIA)

The AVP concentration was determined with specific rabbit anti-rat AVP serum. The effective dilution of the antiserum was 1:60,000. The peptide was labeled with ^{125}Iodine using the chloramines-T method, and the iodinated peptide was purified by Sephadex G-50. The assay sensitivity for the AVP was 1.0 pg/tube. Intra- and inter-assay coefficients of variation were less than 3.6% and 6.0%, respectively [22].

2.9. Histological verification

At the end of the experiments, a black dye was injected into the NRM. The rats were then sacrificed under a high dose of sodium pentobarbital (80 mg/kg, intraperitoneal injection), and the histological location of microinjection or push–pull perfusion was ascertained (Fig. 1). Data were rejected if histological locations were not accurate or the black dye diffused out of the NRM [36].

2.10. Statistical analysis

In order to eliminate the influence of individuation, the data for the AVP concentration in the NRM perfuse liquid were converted to relative changed value. The value was calculated as follows:

$$\text{changed value}\,(\%) = \frac{\text{present value} - \text{value before treatment}}{\text{value before treatment}} \times 100$$

All data were expressed as mean ± standard error of the mean (SEM) and were analyzed between groups by analysis of variance (ANOVA), with two-way ANOVA followed by the Bonferroni test and one-way ANOVA followed by the Dunnett test and the Newmann–Keuls test. $P < 0.05$ was considered statistically significant.