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Through V_2, not V_1 receptor relating to endogenous opiate peptides, arginine vasopressin in periaqueductal gray regulates antinociception in the rat

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Abstract

Our previous study has proven that central arginine vasopressin (AVP) plays an important role in antinociception, and pain stimulation raises AVP concentration in the periaqueductal gray (PAG). The nociceptive effect of AVP in PAG was investigated in the rat. The results showed that microinjection of AVP into PAG increased pain threshold, whereas microinjection of V_2 receptor antagonist-d$(CH_2)_5$[D-Ile^2, Ile^4, Ala^9-NH_2]AVP into PAG decreased pain threshold in a dose-dependent manner, but local administration of V_1 receptor antagonist-d$(CH_2)_5$Tyr(Me)AVP did not change pain threshold; Pain stimulation elevated AVP, Leucine–enkephalin (L–Ek), Methionine–enkephalin (M–Ek) and β-endorphin (β-Ep), not dynorphinA_{1-13} (DynA_{1-13}) concentrations in PAG perfuse liquid; PAG pre-treatment with naloxone, an opiate receptor antagonist or V_2 receptor antagonist completely reversed AVP-induced increase in pain threshold, however, PAG pre-treatment with V_1 receptor antagonist did not influence this effect of AVP administration. The data suggest that AVP in the PAG, through V_2 rather than V_1 receptor, regulates antinociception, which progress relates to enkephalin and endorphin.

Keywords: Arginine vasopressin; Periaqueductal gray; Vasopressin V_2 receptor; Enkephalin; Endorphin; Antinociception

1. Introduction

Arginine vasopressin (AVP), a nonapeptide posterior pituitary hormone, is synthesized in the paraventricular and supraoptic nuclei of hypothalamus [1]. 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 [2]. The remarkable functions of AVP include body fluid homeostasis, hormone probation, cardiovascular control, learning and memory [3]. Many studies have proved that AVP influences antinociception in both human and nonhuman species [4–8]. Our previous study showed that intraventricular injection of AVP increased pain threshold, and intraventricular injection of anti-AVP serum decreased pain threshold, whereas intrathecal or intravenous administration of AVP or anti-AVP serum did not influence pain threshold [9]. The central AVP plays an important role in antinociception.

Endogenous opiate peptide system includes three series-enkephalin, endorphin and dynorphin [10]. Since Hughes et al. purified and identified leucine–enkephalin (L–Ek) and methionine–enkephalin (M–Ek), one series endogenous opiate peptides in 1975 [11], the other two series-endorphin and dynorphin had been confirmed [12,13]. Endogenous opiate peptides including enkephalin, endorphin and dynorphin are traditionally thought to be bioactive substances regulating analgesia in central nervous system [14].

Histological study discovered that there are many AVP-containing fibers in periaqueductal gray (PAG) [15,16], in which a high density of endogenous opiate peptide neurons locates [14]. Endogenous opiate peptides including L–Ek, M–Ek, β-

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endorphin (β-Ep) and dynorphinA_{1-13} ($DynA_{1-13}$) in PAG have been reported to involve in antinociceptive effects [14]. Many experiments have proved that there are many affinities between AVP and endogenous opiate peptides in central nerve system [17–20]. AVP may bind to its major receptors — V_1 and V_2 receptor subtypes [21–23]. Our previous work has shown that pain stimulation can change both AVP and β-Ep concentration in PAG [19,24]. Depending on these data, we presume that AVP in the PAG may regulate antinociception, which process relates with endogenous opiate peptides.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing 180–220 g used in all experiments were obtained from the Experiment Center of Nanfang Medical University, Guangzhou, China. Animals were housed in a colony room under controlled temperature, humidity and a 12 hour 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 [25].

2.2. Materials

AVP, L–Ek, M–Ek, β–Ep, $DynA_{1-13}$, $d(CH_2)_5$[D-Ile_2, Ile_4, Ala-NH_2^9]AVP and $d(CH_2)_5$Tyr(Me)AVP were obtained from Peninsula Laboratories, San Carlos, CA, USA; ^{125}Iodine was from Amersham Pharmacia, Buckinghamshire, UK; Naloxone and other chemical reagents were from Sigma Co., St. Louis, MO, USA.

Rabbit anti-rat AVP, L–Ek, M–Ek, β-Ep or $DynA_{1-13}$ serum was made by the Department of Neurobiology, Second Military Medical University, Shanghai, China. The detailed descriptions were published before [26–29]. The specificity of each kind of antiserum was more than 99% cross-reactivity with its corresponding antigen and less than 1% cross-reactivity with other similar peptides. The effective dilution of the antiserum was 1:20,000–80,000 for radioimmunoassay.

2.3. Surgery

With the 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 (0.5 mm outer diameter) into the PAG (AP 4.2 mm, LR 0.4 mm, *H*6.2 mm) for push–pull perfusion or microinjection under the pentobarbital sodium (35 mg/kg, intraperitoneal injection) anesthetization [30]. The guide cannula was fixed to the skull by dental acrylic. All operations were carried out in the sterile condition and the animals needed at least 14 days to recover after surgery.

2.4. Nociceptive tests

The potassium iontophoresis inducing tail-flick served as pain stimulus, and 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) [31].

2.5. Pain stimulation

Through the positive electrodes (as above) producing the direct electrical current was generated from Pain Threshold Measurer to induce the potassium iontophoresis in the animal tails and result in acute pain. The intensity was fixed to 1.2–1.4 times of pain threshold (0.6–0.7 mA) for 1 min.

2.6. Microinjection

On the experimental day, a stainless steel needle with 0.3 mm diameter for PAG injection was gently inserted into the guide cannula, 1 mm beyond the tip. The 1-μl solution with artificial cerebra-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 PAG over 10 min [31].

2.7. Push–pull perfusion

A stainless steel needle, 0.3 mm outer diameter, was directly inserted into the guide cannula, 1 mm beyond the tip, for pushing ACSF into the PAG, and the guide cannula for pulling the ACSF out. The pushing ACSF was warmed at 37 °C to pass through the nucleus of 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 [30,32,33]. The pain stimulation was treated as above (Section 2.5) in this experiment.

2.8. Radioimmunoassay (RIA)

The AVP, L–Ek, M–Ek, β-Ep and $DynA_{1-13}$ concentrations were determined with specific rabbit antiserum against AVP, OXT, L–Ek, M–Ek, β-Ep and $DynA_{1-13}$. The peptides were labeled ^{125}Iodine using the chloramines-T method, and iodinated peptides were purified by Sephadex G-50 [34]. The assay sensitivities for the AVP, M–Ek, L–Ek, β-Ep or $DynA_{1-13}$ were 1.0, 3.0, 4.1, 1.2 and 6.3 pg/tube and intra- and inter-assay coefficients of variation were less than 5.1% and 8.0%, respectively [26–29].

2.9. Histologic verification

At the end of the experiments, a black dye was injection into the PAG. The rats were then sacrificed under the high dose of pentobarbital sodium (80 mg/kg, intraperitoneal injection), and the histological location of microinjection or push–pull perfusion was ascertained. The data were excluded from analysis if the needle was not at PAG [31,35].

2.10. Statistical analysis

In order to eliminate the influence of the individuation, the data for AVP, L–Ek, M–Ek, β-Ep and $DynA_{1-13}$ concentrations