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Arginine vasopressin induces periaqueductal gray release of enkephalin and endorphin relating to pain modulation in the rat

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Abstract

Previous study has proven that microinjection of arginine vasopressin (AVP) into periaqueductal gray (PAG) raises the pain threshold, in which the antinociceptive effect of AVP can be reversed by PAG pretreatment with V_2 rather than V_1 or opiate receptor antagonist. The present work investigated the AVP effect on endogenous opiate peptides, oxytocin (OXT) and classical neurotransmitters in the rat PAG. The results showed that AVP elevated the concentrations of leucine–enkephalin (L–Ek), methionine–enkephalin (M–Ek) and β-endorphin (β-Ep), but did not change the concentrations of dynorphin$A_{1–13}$ (Dyn$A_{1–13}$), OXT, classical neurotransmitters including achetylcholine (Ach), choline (Ch), serotonin (5-HT), γ-aminobutyric acid (GABA), glutamate (Glu), dopamine (DA), norepinephrine (NE) and epinephrine (E), and their metabolic products in PAG perfusion liquid. Pain stimulation increased the concentrations of AVP, L–EK, M–Ek, β-Ep, 5-HT and 5-HIAA (5-HT metabolic product), but did not influence the concentrations of Dyn$A_{1–13}$, OXT, the other classical neurotransmitters and their metabolic products. PAG pretreatment with naloxone — an opiate receptor antagonist completely attenuated the pain threshold increase induced by PAG administration of AVP, but local pretreatment of OXT or classical neurotransmitter receptor antagonist did not influence the pain threshold increase induced by PAG administration of AVP. The data suggested that AVP in PAG could induce the local release of enkephalin and endorphin rather than dynophin, OXT and classical neurotransmitters to participate in pain modulation.

Keywords: Arginine vasopressin; Enkephalin; β-Endorphin; Classical neurotransmitter; Periaqueductal gray; Pain modulation

1. Introduction

Arginine vasopressin (AVP), a nonapeptide posterior pituitary hormone, is mainly synthesized in the paraventricular and supraoptic nuclei of hypothalamus. 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 [1]. AVP has been identified as an important factor governing analgesia in both human and nonhuman species [2,3]. Central AVP plays a role in antinociception [4]. AVP in the related brain regions including periaqueductal gray (PAG), caudate nucleus and raphe magnus nucleus influences pain modulation [5–7].

Endogenous opiate peptide system includes three series — enkephalin, endorphin and dynorphin, which are traditional bioactive substances of pain modulation [8,9]. Histological work has discovered that PAG, one regulating center of pain, contains not only a high density of endogenous opiate peptide neurons [10] but also many AVP-containing fibers [11–14]. Our previous study has found that microinjection of AVP into PAG raises the pain threshold, while local administration of V_2 rather than V_1 receptor antagonist reduces the pain threshold [5]. This analgesic effect of

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AVP in PAG can be reversed by local pretreatment with V_2 rather than V_1 or opiate receptor antagonist [5]. Pain stimulation induces PAG release of both AVP and endogenous opiate peptides such as leucine–enkephalin (L–Ek), methionine–enkephalin (M–Ek) and β-endorphin (β-Ep) [15]. Pain stimulation changes both AVP and β-Ep concentrations in PAG tissue [5]. These data suggest that AVP may interact with other bioactive substances, especially endogenous opiate peptides in the PAG taking part in antinociception. However, because PAG contains a lot of classical neurotransmitters, which relate with pain modulation [8,9], it is not clear which bioactive substances in PAG are involved in the analgesic process of AVP. The present study tries to investigate the effect of AVP on endogenous opiate peptides including L–Ek, M–Ek, β-Ep and dynorphin$A_{1–13}$ ($DynA_{1–13}$) and classical neurotransmitters including achetylcholine (Ach), choline (Ch), serotonin (5-HT), γ-aminobutyric acid (GABA), glutamate (Glu), dopamine (DA), norepinephrine (NE) and epinephrine (E) in the PAG during pain modulation, so as to understand the mechanism of AVP regulating analgesia deeply.

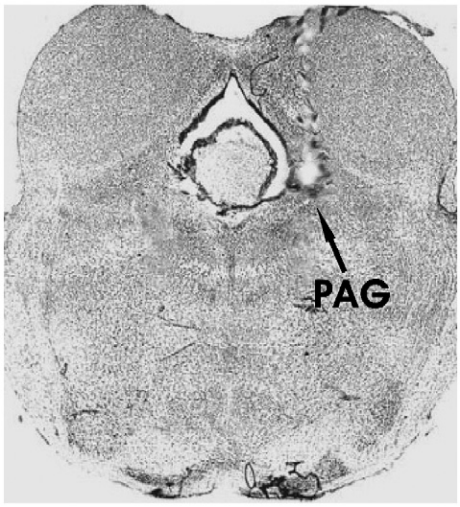

Fig. 1. Histological verification of periaqueductal gray (PAG) push–pull perfusion or microinjection.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing 180–220 g were used in all experiments (Animal experiment Center, Second Military Medical University, Shanghai, China; Animal Experiment Center, Nanfang Medical University, Guangzhou, China; Charles River Laboratories, Montreal, Quebec, Canada). Animals were housed in a colony room under controlled temperature, humidity and a 12 hour light/dark cycle (lights 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 [16].

2.2. Materials

AVP, oxytocin (OXT), L–Ek, M–Ek, β-Ep, $DynA_{1–13}$ and [1-D$(CH_2)_5$,Tyr(ME)$_2$,Thr^4,Tyr-NH2(9)] ornithine vasotocin were obtained from Peninsula Lab, San Carlos, CA, USA. ^{125}Iodine was from Amersham Pharmacia, Buckinghamshire, UK. Rabbit antiserum for rat AVP, OXT, L–Ek, M–Ek, β-Ep or $DynA_{1–13}$ was made by the Department of Neurobiology, Second Military Medical University, Shanghai, China. The antiserum specificities were summarized in Table 1 [17–21].

NE, E, DA, 3,4-dihydroxyphenylacetic acid (DOPA, DA metabolic product), homovanillic acid (HVA, DA metabolic product), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA, 5-HT metabolic product), Ach, Ch (Ach metabolic product), GABA, Glu, phentolamine (α receptor antagonist), propranolol (β receptor antagonist), atropine (M receptor antagonist), 6-OH gallamine (N receptor antagonist), fluperidol (DA receptor antagonist), cypoheptadine (5-HT receptor antagonist), bicuculline (GABA receptor antagonist), MK801 [*N*-methyl-D-aspartate (NMDA) receptor, one subunit of Glu receptor antagonist] and the other chemicals were bought from Sigma Co., St. Louis, MO, USA.

2.3. Surgery

With the Pellegrino L.J. rat brain atlas as reference [22], we used the stereotaxic apparatus (Jiangwan I-C, Shanghai, China) to implant a stainless steel guide cannula of 0.5 mm outer diameter into the right ventrolateral part of PAG (AP 0.3 mm, LR 0.5 mm, H 3.0 mm) for microinjection or push–pull

Table 1
Specificities of the antiserum

Antiserum	Cross-reactivity		Dilution for RIA	Reference
	>99.9%	<0.1%		
Anti-AVP serum	AVP	OXT, vasotocin, LVP, VIP, NT, L–Ek, M–Ek, β-Ep, $DynA_{1–13}$	1:80,000	[17]
Anti-OXT serum	OXT	AVP, vasotocin, LVP, VIP, NT, L–Ek, M–Ek, β-Ep, $DynA_{1–13}$	1:80,000	[18]
Anti-L–Ek serum	L–Ek	AVP, OXT, vasotocin, LVP, VIP, NT, M–Ek, β-Ep, $DynA_{1–13}$	1:40,000	[19]
Anti-M–Ek serum	M–Ek	AVP, OXT, vasotocin, LVP, VIP, NT, L–Ek, β-Ep, $DynA_{1–13}$	1:40,000	[19]
Anti-β-Ep serum	β-Ep	AVP, OXT, vasotocin, LVP, VIP, NT, L–Ek, M–Ek, $DynA_{1–13}$	1:20,000	[20]
Anti-$DynA_{1–13}$ serum	$DynA_{1–13}$	AVP, OXT, vasotocin, LVP, VIP, NT, L–Ek, M–Ek, β-Ep, DynB	1:30,000	[21]

AVP, arginine vasopressin; OXT, oxytocin, L–Ek, leucine–enkephalin; M–Ek, methionine–enkephalin; β-Ep, β-endorphin; $DynA_{1–13}$, dynorphin$A_{1–13}$; LVP, lysine–vasopressin; VIP, vasoactive intestinal peptide; NT, neurotensin; DynB, dynorphinB; RIA, radioimmunoassay.