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The antinociceptive role of central arginine vasopressin is involved in the endogenous opiate peptide, serotonin and acetylcholine systems

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ABSTRACT

Our previous work has demonstrated that arginine vasopressin (AVP) plays a role in pain modulation. The present study investigated which kinds of neuropeptides and neurotransmitters in central nervous system might be involved in AVP antinociceptive role in the rat. The results showed that (1) intraventricular injection (*icv*) of V_1 receptor antagonist [d$(CH_2)_5$-Tyr(Me)AVP] and V_2 receptor antagonist [d$(CH_2)_5$-[D-Ile^2, Ile^4, Ala^9-NH_2]AVP] blocked the antinociceptive effect induced by AVP (*icv*), (2) the opiate recaptor antagonist (naloxone) reversed the antinociceptive effect induced by AVP (*icv*), and (3) both the serotonin receptor antagonist (cypoheptadine) and M receptor antagonist (atropine) could attenuate the antinociceptive effect induced by AVP (*icv*); but (4) oxytocin, dopamine, N-methyl-D-aspartate (NMDA), γ-aminobutyric acid (GABA), N, α or β receptor antagonist did not influence the antinociceptive effect induced by AVP (*icv*). The data suggested that AVP antinociceptive role was involved in the endogenous opiate peptide, serotonin and acetylcholine systems in central nervous system.

Keywords: Arginine Vasopressin; Antinociception; Endorgenous Opiate Peptide; Serotonin; Acetycholine

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 showed that AVP influences antinociception in both human and nonhuman species [1,4-7]. Intraventricular injection (*icv*) of AVP increases the pain threshold, while anti-AVP serum (*icv*) decreases the pain threshold, but intrathecal injection (*ith*) or intravenous injection (*iv*) of either AVP or anti-AVP serum does not influence the pain threshold [8,9]. Pain stimulation could change AVP concentration in some brain nuclei, but did not change AVP concentration in the spinal cord and serum [8,9]. The antinociceptive effect of AVP is limited to the brain nuclei, not the spinal cord and peripheral organs.

Many studies have proven that most of neuropeptides (such as endogenous opiate peptides) and neurotransmitters (such as serotonin, acetylcholine, norepinephine and epinephrine) are involved in pain modulation [10]. For example, oxytocin (*icv*) could increase the pain threshold and enhance acupuncture analgesia, while anti-oxytocin serum (*icv*) decreases the pain threshold and weakens acupuncture analgesia [11-13]. However, it is not clear the interaction between AVP and other neuropeptides or neurotransmitters in pain modulation. The present study investigated which neuropeptides and neurotransmitters in central nerve system might be involved in AVP antinociceptive effect in the rat.

2. MATERIALS AND METHODS

2.1. Animals

Adult male Sprague-Dawley rats weighing 180-220 g, which were obtained from Animal Center of Yangzhou University, Yangzhou, Jiangsu, China, were housed with food and water available *ad libitum* in a colony room under controlled temperature, humidity and a 12 hours

light/dark cycle (light at 6:00 AM and dark at 6:00 PM). All the procedures were approved by Animal Care Committee of Yangzhou University and conducted according to the guidelines of the International Association for the Study of Pain [14].

2.2. Materials

AVP, $d(CH_2)_5Tyr(Me)AVP$, $d(CH_2)_5[D\text{-}Ile^2, Ile^4, Ala^9\text{-}NH_2]AVP$ and $[1\text{-}D(CH_2)_5,Tyr(ME)_2,Thr^4,Tyr\text{-}NH2(9)]$ ornithine vasotocin were obtained from Peninsula Lab, San Carlos, CA, USA. Naloxone, cypoheptadine, atropine, 6-OH gallamine, fluperidol, phentolamine, propranolol, MK801, bicuculline, 5-amino valeric acid (5AVA), 3-aminoproyl phossphonic acid (3APPA). and the other chemicals were bought from Sigma Co., St. Louis, MO, USA.

2.3. Surgery

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 0.5 mm outer diameter into the right lateral ventricle (AP 0.3 mm, LR 0.5 mm, H 3.0 mm) for *icv* under the pentobarbital sodium (35 mg/kg, intraperitoneal injection) anaesthesia. The guide cannula was fixed to the skull by dental acrylic. All operations were carried out in the aseptic condition and the animals were allowed to recover for at least 14 days after the surgery.

2.4. Intraventricular Injection (*Icv*)

On the day of experiment, a stainless steel needle with 0.3 mm diameter for *icv* was directly inserted into the guide cannula, with 1mm beyond the tip of the latter. The 10 ml of antiserum or solution was injected into the lateral ventricle gently over 10 min.

2.5. 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. Depending on the 30-year experience of studying pain in our laboratory, we used the potassium iontophoresis inducing tail-flick served as pain stimulus. The small wet cotton with the potassium iontophoresis was set on the skin of the tail. The cotton was exposed to direct electrical current, and the anode led the potassium iontophoresis to permeate the skin of the tail. If the current was strong enough, the permeated potassium iontophoresis resulted in the animal feeling the pain stimulation. 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 duration between consecutive stimuli was 10 min, and the pain stimulus was terminated at once when the rat showed response to this stimulus.

2.6. Histological Verification

At the end of the experiments, the rat was sacrificed under the high dose of pentobarbital sodium (80 mg/kg, intraperitoneal injection), and the histological location of *icv* was ascertained. The data were excluded from analysis if the positions were not accurate.

2.7. Statistical Analysis

All values were expressed as mean ± standard error of the mean (SEM) and were analyzed between groups by analysis of variance (ANOVA) and χ^2 test. $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Effect of the Neuropeptide Receptor Antagonist on Pain Threshold Increase Induced by AVP (*icv*)

Table 1 showed that 100 ng AVP (*icv*) could increase the pain threshold from 0.52 ± 0.03 mA to 0.77 ± 0.04 mA ($P < 0.001$).

Although *icv* of 2 μg $d(CH_2)_5Tyr(Me)AVP$ (V_1 receptor antagonist), 2 μg $d(CH_2)_5[D\text{-}Ile^2, Ile^4, Ala^9\text{-}NH_2]AVP$ (V_2 receptor antagonist), 2 μg $[1\text{-}D(CH_2)_5,Tyr(ME)_2,Thr^4,Tyr\text{-}NH2(9)]$ ornithine vasotocin (oxytocin receptor antagonist) or 2 μg naloxone (opiate receptor antagonist) decreased the pain threshold (all $p < 0.01 \sim 0.001$), ventricular pretreatment with V_1 receptor antagonist, V_2 receptor antagonis, opiate receptor antagonist could reverse the antinociceptive effect induced by 100 ng AVP administration (*icv*), and ventricular pretreatment with oxytocin receptor antagonist did not influence the antinociceptive effect induced by 100 ng AVP administration (*icv*) (**Table 1**).

3.2. Effect of the Neurotransmitter Receptor Antagonist on Pain Threshold Increase Induced by AVP (*icv*)

Table 2 showed that *icv* of 2 μg 5-HT receptor antagonist (cypoheptadine), 2 μg M receptor antagonist (atropine), 2 μg N receptor antagonist (6-OH gallamine), 2 μg α receptor antagonist (phentolamine) or 2 μg β receptor antagonist (propranolol) decreased the pain threshold (all $p < 0.01 \sim 0.001$), but *icv* of 2 μg dopamine receptor antagonist (fluperidol), 2 μg N-methyl-D-aspartate (NMDA) receptor antagonist (MK801), 2 μg γ-aminobutyric acid $(GABA)_a$ receptor antagonist (bicuculline), 2 μg $GABA_b$ receptor antagonist (5-amino valeric acid) or 2 μg $GABA_c$ receptor antagonist (3-aminoproyl phossphonic acid) did not influence the pain threshold.

Pretreatment with either 5-HT receptor antagonist or M receptor antagonist (*icv*) could attenuate the antinociceptive

Table 1. Effect of neuropeptide receptor antagonist (*icv*) on the pain threshold increase induced by the central AVP.

Treatment	n	Before injection	After 1st injection	After 2nd injection
ACSF + ACSF	10	0.50 ± 0.03	0.51 ± 0.02	0.52 ± 0.03
ACSF + AVP	10	0.51 ± 0.03	0.52 ± 0.04	$0.77 \pm 0.04^{111\ 222}$ ***
V_1receptor antagonist + ACSF	10	0.49 ± 0.03	0.41 ± 0.04^{1}	0.46 ± 0.04
V_1receptor antagonist +AVP	10	0.51 ± 0.04	0.40 ± 0.03^{11} ○○○	$0.49 \pm 0.04^{111\ 2}$ ○○○
V_2receptor antagonist + ACSF	10	0.51 ± 0.03	0.41 ± 0.02^{111} **	0.43 ± 0.03^{11} *
V_2receptor antagonist +AVP	10	0.50 ± 0.02	0.40 ± 0.02^{111} ○○○	0.45 ± 0.02^{1} ○○○
OXT receptor antagonist + ACSF	9	0.49 ± 0.03	0.39 ± 0.03^{11} ***	0.41 ± 0.04^{1} **
OXT receptor antagonist + AVP	9	0.51 ± 0.03	0.40 ± 0.03^{111} ○○○	$0.74 \pm 0.04^{11\ 222\ aaa}$
Opiate receptor antagonist + ACSF	10	0.54 ± 0.04	0.37 ± 0.01^{111} ***	0.35 ± 0.03^{111} ***
Opiate receptor antagonist + AVP	10	0.50 ± 0.02	0.34 ± 0.03^{111} ○○○	$0.62 \pm 0.03^{11\ 222\ aaa}$ ○○○

ACSF, 10 μl artificial cerebrospinal fluid; AVP, 100 ng arginine vasopressin; V_1 receptor antagonist, 2 μg $d(CH_2)_5$Tyr(Me)AVP; V_2 receptor antagonist, 2 μg $d(CH_2)_5$[D-Ile^2, Ile^4, Ala^9-NH_2]AVP; OXT (oxytocin) receptor antagonist, 2 μg [1-D$(CH_2)_5$,Tyr(ME)$_2$,Thr^4,Tyr-NH2(9)] ornithine vasotocin; Opiate receptor antagonist, 2 μg naloxone. All values are expressed as mean ± standard error of the mean (SEM). The unit was mA. N indicates the animal number of the group. Before injection denotes the animal before the treatment; First injection denotes the animal given first intraventricular injection (*icv*) of ACSF or receptor antagonist; Second injection denotes the animal given second *icv* of ACSF or AVP in 10 min after first injection. $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ are for the comparison of the pain threshold from marked group and ACSF + ACSF group; ° $P < 0.05$, °° $P < 0.01$ and °°° $P < 0.001$ are for the comparison of the pain threshold from marked group and ACSF + AVP group; ^{1} $P < 0.05$, ^{11} $P < 0.01$ and ^{111} $P < 0.001$ are for the comparison of the pain threshold from marked value and the value before injection; ^{2} $P < 0.05$, ^{22} $P < 0.01$ and ^{222} $P < 0.001$ are for the comparison of the pain threshold from marked value after 1st injection and the value after 2nd injection; ^{aaa} $P < 0.001$ is for the comparison of the pain threshold from receptor antagonist + AVP group and receptor antagonist + ACSF group (corresponding control group).

Table 2. Effect of classical neurotransmitter receptor antagonists (*icv*) on the pain threshold increase induced by the central AVP.

Treatment	n	Before injection	After 1st injection	After 2nd injection
ACSF + ACSF	10	0.50 ± 0.03	0.51 ± 0.02	0.52 ± 0.03
ACSF + AVP	10	0.51 ± 0.03	0.52 ± 0.04	$0.77 \pm 0.04^{111\ 222}$ ***
5-HT receptor antagonist + ACSF	10	0.52 ± 0.03	0.27 ± 0.02^{111} ***	0.23 ± 0.01^{111}***
5-HT receptor antagonist + AVP	10	0.47 ± 0.03	0.30 ± 0.03^{111} ○○○	0.31 ± 0.05^{111} ○○○
M receptor antagonist + ACSF	9	0.50 ± 0.03	0.30 ± 0.02^{111} ***	0.29 ± 0.02^{111}***
M receptor antagonist + AVP	9	0.51 ± 0.03	0.33 ± 0.02^{111} ○○○	$0.60 \pm 0.04^{111\ 222\ aaa}$ ○○○
N receptor antagonist + ACSF	9	0.48 ± 0.03	0.47 ± 0.03	0.49 ± 0.03
N receptor antagonist + AVP	9	0.49 ± 0.03	0.50 ± 0.04	$0.84 \pm 0.06^{111\ 222\ aaa}$
DA receptor antagonist + ACSF	9	0.52 ± 0.03	0.52 ± 0.04	0.51 ± 0.03
DA receptor antagonist + AVP	9	0.52 ± 0.03	0.51 ± 0.03	$0.82 \pm 0.05^{111\ 222\ aaa}$
α receptor antagonist + ACSF	9	0.48 ± 0.03	0.38 ± 0.03^{1} ***	0.33 ± 0.04^{11} ***
α receptor antagonist + AVP	9	0.49 ± 0.03	0.37 ± 0.03^{1} ○○○	$0.81 \pm 0.06^{111\ 222\ aaa}$
β receptor antagonist + ACSF	9	0.47 ± 0.04	0.38 ± 0.03^{1} ***	0.36 ± 0.04^{1} ***
β receptor antagonist + AVP	9	0.48 ± 0.03	0.39 ± 0.03^{11} ○○○	$0.78 \pm 0.05^{111\ 222\ aaa}$
NMDA receptor antagonist + ACSF	8	0.51 ± 0.03	0.49 ± 0.04	0.50 ± 0.03
NMDA receptor antagonist + AVP	8	0.50 ± 0.03	0.48 ± 0.03	$0.76 \pm 0.05^{111\ 222\ aaa}$
$GABA_a$ receptor antagonist + ACSF	9	0.52 ± 0.03	0.49 ± 0.03	0.48 ± 0.04
$GABA_a$ receptor antagonist + AVP	9	0.50 ± 0.04	0.52 ± 0.03	$0.82 \pm 0.05^{111\ 222\ aaa}$
$GABA_b$ receptor antagonist + ACSF	9	0.48 ± 0.03	0.50 ± 0.04	0.47 ± 0.04
$GABA_b$ receptor antagonist + AVP	9	0.50 ± 0.04	0.49 ± 0.03	$0.79 \pm 0.05^{111\ 222\ aaa}$
$GABA_c$ receptor antagonist + ACSF	9	0.51 ± 0.03	0.50 ± 0.03	0.47 ± 0.04
$GABA_c$ receptor antagonist + AVP	9	0.50 ± 0.04	0.52 ± 0.03	$0.83 \pm 0.05^{111\ 222\ aaa}$

ACSF, 10 μl artificial cerebrospinal fluid; AVP, 100 ng arginine vasopressin; 5-HT (serotonin) receptor antagonist, 2 μg cypoheptadine; M receptor antagonist, 2 μg atropine; N receptor antagonist, 2 μg 6-OH gallamine; DA (dopamine) receptor antagonist, 2 μg fluperidol; α receptor antagonist: 2 μg phentolamine; β receptor antagonist: 2 μg propranolol; NMDA (N-methyl-D-aspartate) receptor antagonist: 2 μg MK801; GABAa (γ-aminobutyric acid) receptor antagonist: 2 μg bicuculline; GABAb receptor antagonist: 2 μg 5-amino valeric acid (5AVA); GABAc receptor antagonist, 2 μg 3-aminoproyl phossphonic acid (3APPA). All values are expressed as mean ± standard error of the mean (SEM). The unit was mA. N indicates the animal number of the group. Before injection denotes the animal before the treatment; First injection denotes the animal given first intraventricular injection (*icv*) of ACSF or receptor antagonist; Second injection denotes the animal given second *icv* of ACSF or AVP in 10 min after first injection. $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ are for the comparison of the pain threshold from marked group and ACSF + ACSF group; ° $P < 0.05$, °° $P < 0.01$ and °°° $P < 0.001$ are for the comparison of the pain threshold from marked group and ACSF + AVP group; ^{1} $P < 0.05$, ^{11} $P < 0.01$ and ^{111} $P < 0.001$ are for the comparison of the pain threshold from marked value and the value before injection; ^{2} $P < 0.05$, ^{22} $P < 0.01$ and ^{222} $P < 0.001$ are for the comparison of the pain threshold from marked value after 1st injection and the value after 2nd injection; ^{aaa} $P < 0.001$ is for the comparison of the pain threshold from receptor antagonist + AVP group and receptor antagonist + ACSF group (corresponding control group).

effect induced by 100 ng AVP administration (*icv*) (all $P < 0.001$), but the other studied neurotransmitter recaptor antagonists did not influence the antinociceptive effect induced by the administration of 100 ng AVP (*icv*) (**Table 2**).

4. DISCUSSION

AVP is synthesized within cells located in the brain and in certain peripheral organs of the body. In the brain, AVP is synthesized in cell groups within the hypothalamus; several of these cell groups release hormones into the systemic circulation or into the portal circulation of the anterior pituitary gland and others release neurotransmitters at synaptic targets within the brain. AVP is also synthesized in certain extrahypothalamic brain sites, such as limbic system structures in the forebrain. In peripheral tissues, there is evidence that AVP is synthesized in the anterior pituitary, adrenal, and thymus glands and in male and female reproductive structures (ovaries, uterus, and testes) [3]. However, most of AVP is synthesized in hypothalamic paraventricular nucleus (PVN) and hypothalamic supraoptic nucleus (SON) [2,15]. It has been proven that PVN and SON play an important role in analgesia [16-20], and AVP, which may be from PVN and SON, is involved in pain modulation [21,22].

Our present study showed that (1) not only V_1 receptor antagonist [$d(CH_2)_5$Tyr(Me)AVP] and V_2 receptor antagonist [$d(CH_2)_5$[D-Ile^2, Ile^4, Ala^9-NH_2]AVP] blocked the antinociceptive effect induced by AVP (*icv*), but also the opiate receptor antagonist (naloxone), 5-HT receptor antagonist (cypoheptadine) and M receptor antagonist (atropine) could reserve the antinociceptive effect induced by AVP (*icv*); (2) oxytocin, dopamine, NMDA, GABA, N, α and β receptor antagonist did not influence the antinociceptive effect induced by AVP (*icv*). The data suggested that AVP antinociceptive effect was related with the endogenous opiate peptide, serotonin and acerycholine systems.

Histological study has shown that there are many AVP containing fibers in the periaqueductal gray (PAG), which come from PVN neurons [23,24]. AVP enhances the synthesis and secretion of endogenous opiate peptides in the PAG [25,26].

The nucleus raphe magnus (NRM) is a serotonergic nucleus located in the rostral ventromedial medulla of the brainstem. Axons of the NRM project to the spinal cord [27], terminating primarily in the dorsal horn [28]. Brainstem nuclei that project to the dorsal horn of the spinal cord can function to inhibit afferent nociceptive transmission [29-31]. Activation of these descending antinociceptive pathways may be triggered by physiological stimuli [32] as well as by pharmacological agents [33]. Antinociception involving the NRM has been studied after either electrical stimulation or direct administration of pharmacological agents [34-36]. The NRM is a key neural structure for pain modulation, in which serotonin (5-HT) is a major site for pain regulation [10]. AVP and 5-HT interaction in the brain controls many animal behaviors [37,38].

There are many bioactive substances in the caudate nucleus (CdN) including dopamine (DA) and acetylcholine (Ach), which show interaction with AVP [35,39-41]. DA and Ach in CdN are important bioactive substances in pain modulation and the CdN is showing an important neural structure in pain modulation [38].

Our pervious study has shown that AVP in the PAG, NRM and CdN could regulate the pain process [18,42,43], and pain stimulation changes the AVP concentration in the PAG, NRM and CdN [15,40]. So we could imagine that AVP regulating the pain process might be involved in the endogenous opiate system in the PAG, serotonin system in the NRM and acetylcholine system in the CdN. However, it needs to be confirmed.

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