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Effect of intranasal arginine vasopressin on human headache

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ABSTRACT

Arginine vasopressin (AVP), a nonapeptide hormone of posterior pituitary, reaches the central nervous system from systemic blood circulation with a difficulty because of the blood–brain barrier (BBB). The interest has been expressed in the use of the nasal route for delivery of AVP to the brain directly, exploiting the olfactory pathway. Our previous study has demonstrated that AVP in the brain rather than the spinal cord and blood circulation plays an important role in rat pain modulation. For understanding the role of AVP on pain modulation in human, the communication tried to investigate the effect of intranasal AVP on human headache. The results showed that (1) AVP concentration in both plasma and cerebrospinal fluid (CSF) increased significantly in headache patients, who related with the headache level; (2) there was a positive relationship between plasma and CSF AVP concentration in headache patients; and (3) intranasal AVP could relieve the human headache in a dose-dependent manner. The data suggested that intranasal AVP, which was delivered to the brain through olfactory region, could treat human headache and AVP might be a potential drug of pain relief by intranasal administration.

1. Introduction

Arginine vasopressin (AVP), a nonapeptide posterior hormone of the pituitary, is mainly synthesized and secreted in the hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON). 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]. The remarkable functions of AVP include body fluid homeostasis, hormone regulation, cardiovascular control, learning and memory [7].

AVP has been proven as an important factor governing analgesia in both human and nonhuman species [3,4,11,12]. In 1968, Aziz et al. observed that AVP could prevent lumbar puncture-induced headache [2]. Kendler et al. reported that pain interacted plasma AVP concentrations in surgical emergency of men [10]. Some studies discovered that intraventricular injection (*icv*) of AVP increased the pain threshold [5] and anti-AVP serum (*icv*) decreased the pain

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threshold [6], but neither intrathecal (*ith*) nor intravenous injection (*iv*) of AVP or anti-AVP serum influenced the pain threshold [22,25]. The data indicated that AVP in the brain rather than the spinal cord and blood circulation participated in antinociception.

AVP reaches the central nervous system from systemic blood circulation with a difficulty because of the blood–brain barrier (BBB) [1]. For developing AVP-related drugs in the field of pain relief, it is very important to find one way that delivers AVP from systemically administration to central nervous system rapidly. The interest has been expressed in the use of the nasal route for delivery of peptides to the brain directly, exploiting the olfactory pathway [8,9,14,15]. However, few studies reported that intranasal AVP influenced on pain modulation in human. The communication tried to investigate the effect of intranasal AVP on the human headache for understanding the role of AVP on pain modulation in human.

2. Materials and methods

2.1. 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-human arginine vasopressin (AVP) serum was made by Department of Neurobiology, Second Military Medical University, Shanghai, China. The specificity of the antiserum was 100% cross-reactivity with AVP and no cross-reactivity with oxytocin, vasotocin, lysine-vasopressin, vasoactive intestinal peptide, neurotensin, leucine-enkephalin, methionine-enkephalin, β-endorphin and dynorphin A_{1-13}. The dilution of the antiserum was more than 1:40,000 for radioimmunoassay.

2.2. Participants

2.2.1. Headache patients

One hundred and twelve outpatients including 49 male and 63 female, 20–62 years old, average 44.5 ± 8.2 years old, who suffered with headaches, were asked to participate in the study between May 2010 and November 2011. The patients were only diagnosed as tension-type headache and migraine, which were classified as primary headaches 1–4 level depending on the International Headache Society's International Classification of Headache Disorders (ICHD). The patients, which headache history was 4–12 months (average 5.4 ± 2.1 months), did not accepted any treatments before the experiment.

2.2.2. Health volunteers

One hundred and three health volunteers including 42 male and 61 female, 19–64 years old, average 45.6 ± 8.1 years old were asked to participate in the study between May 2010 and November 2011. They have not been suffering from any headaches.

2.2.3. Inclusion criteria

Inclusion criteria were as follows: (a) agreement to sign the informed consent form; (b) eligibility was checked before the experiments (exclusion criteria: pregnancy, tumor, cardiovascular, gastrointestinal, respiratory, brain, endocrine, psychiatric or other diseases, smoking, intake of drugs); (c) participants were asked not to drink any alcohol, caffeine containing beverages and analgesic medication during the experiment; (d) participants were asked not to eat anything before collecting the blood and cerebrospinal fluid during the day of sample collection; (e) all experimental sessions were carried out between 08:00 a.m. and 09:00 a.m.; and (f) over 18 years old.

All experiments were approved by the relative hospital Ethics Committees and carried out according to the Declaration of Helsinki.

2.3. Procedure

The experiments were only carried out during the patients filling ill of headache. Participants were instructed to abstain from smoking, caffeine and analgesic medication. Subsequently, participants completed a set of questionnaires and were checked with the physical examination. The experimental sessions were conducted in a double-blind and placebo controlled within-subject cross-over design. AVP or the placebo was administered intranasal. Following a standardized protocol, the participants self-administered three puffs of AVP per nostril (with 100 ng, 200 ng or 400 ng AVP) or placebo (containing all ingredients except for the peptide) under the supervision of the study coordinator. The total time of an experimental session was 3 h. All participants received monetary compensation after completion of the study.

The health volunteers were done as the patients except the headache.

2.4. Sample collection

2.4.1. Blood sample

Blood was taken by vein-puncture between 08:00 a.m. and 09:00 a.m. The blood was collected using the EDTA·Na_2-treated vacutainer and immediately placed on ice.

2.4.2. Cerebrospinal fluid (CSF) sample

After the blood collection, CSF was taken by lumbar puncture between 08:00 a.m. and 09:00 a.m. The CSF was collected using the silicone oil-treated tube and immediately placed on ice.

2.4.3. Sample treatment

After the centrifugation at 10,000 × *g* for 20 min at 4 °C, the supernatants were withdrawn and stored at −80 °C until AVP determination.

2.5. AVP assay

AVP concentration was determined by radioimmunoassay with specific rabbit antiserum against human AVP. AVP was labeled ^{125}iodine using the chloramines-T method and iodinated peptide was purified by Sephadex G-50. The assay sensitivity of AVP was 1.0 pg/tube and the normal range for plasma AVP was 1–64 pg/ml. The intra- and inter-assay coefficients of variation were less than 5.1% and 8.0%, respectively.

2.6. Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and performed with the SPSS 17.0 statistical package, with two-way analysis of variance (ANOVA) followed by the Bonferroni test and multisampling analysis of difference followed by the χ^2 test. Significance was accepted at $p < 0.05$.

3. Results

3.1. Change of plasma AVP concentration in headache patients

Comparing with the health volunteers, plasma AVP concentration was increased significantly in headache patients (25.63 ± 5.75 pg/ml vs. 7.94 ± 1.82 pg/ml, $p < 0.01$) (Fig. 1). It showed a positive relationship between headache level and

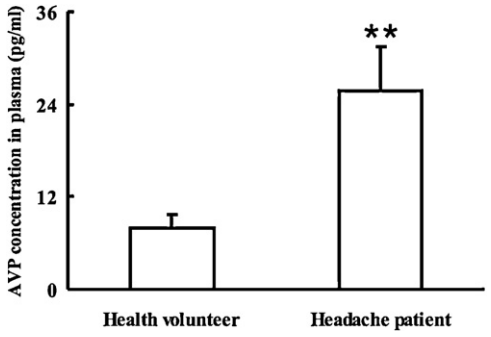

Fig. 1. Change of plasma arginine vasopressin (AVP) concentration in the headache patients. The results are shown as mean ± SEM. $^{**}p < 0.01$ is used for the comparison of plasma AVP concentration from health volunteer group ($N = 103$) and headache patient group ($N = 112$).

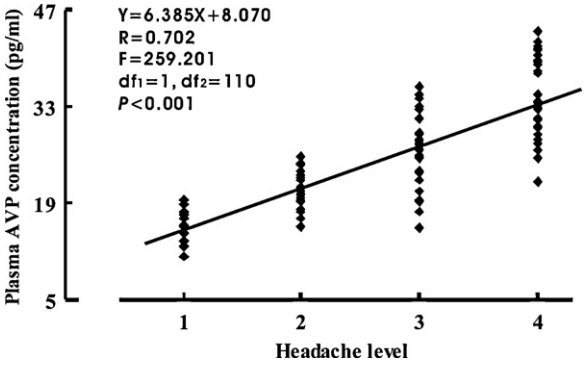

Fig. 2. The relationship between the headache level and plasma arginine vasopressin (AVP) concentration in headache patients.

plasma AVP concentration in headache patients ($Y = 6.385X + 8.070$, $R = 0.702$, $p < 0.001$) (Fig. 2).

3.2. Change of CSF AVP concentration in headache patients

Comparing with the health volunteers, CSF AVP concentration was increased significantly in headache patients (37.64 ± 7.59 pg/ml vs. 12.45 ± 3.43 pg/ml, $p < 0.01$) (Fig. 3). It showed a positive relationship between headache level and CSF AVP concentration in headache patients ($Y = 7.347X + 19.043$, $R = 0.842$, $p < 0.001$) (Fig. 4).

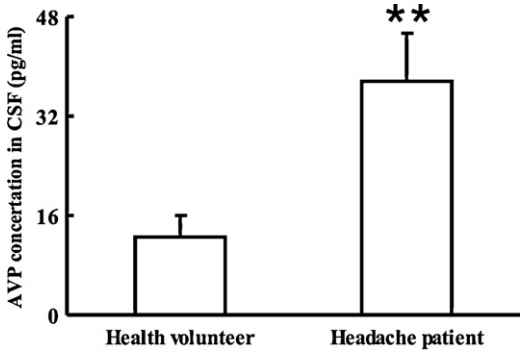

Fig. 3. Change of cerebrospinal fluid (CSF) arginine vasopressin (AVP) concentration in the headache patients. The results are shown as mean ± SEM. $^{**}p < 0.01$ is used for the comparison of plasma AVP concentration from health volunteer group ($N = 15$) and headache patient group ($N = 17$).

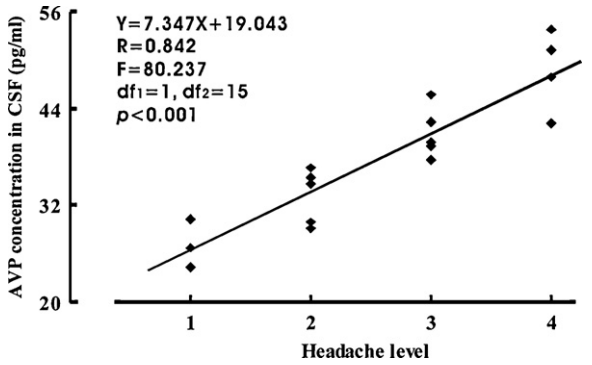

Fig. 4. The relationship between the headache level and arginine vasopressin (AVP) concentration in cerebrospinal fluid (CSF) in headache patients.

3.3. Relationship between plasma and CSF AVP concentration in headache patients

In headache patients, there was a positive relationship between plasma and CSF AVP concentration ($Y = 1.706X - 5.824$, $R = 0.934$, $p < 0.001$) (Fig. 5).

3.4. Effect of intranasal AVP on human headache

Intranasal AVP could relieve the human headache in a dose-dependent manner. It took 60–180 min (average 124.5 ± 41.7 min) for the intranasal AVP reliving the headache during 240 min follow-up. The effect of intranasal AVP 400 ng in 28 cases of headache patients was complete remission 21 cases (75.0%), partial remission 6 cases (21.4%) and invalid remission 1 case (3.6%); the effect of intranasal AVP 200 ng in 28 cases of headache patients was complete remission 13 cases (46.4%), partial remission 12 cases (42.9%) and invalid remission 3 cases (10.7%); the effect of intranasal AVP 100 ng of headache patients in 28 cases was complete remission 8 cases (28.6%), partial remission 9 cases (32.1%) and invalid remission 11 cases (39.3%); and the effect of intranasal placebo in 28 cases of headache patients was complete remission 2 cases (7.1%), partial remission 7 cases (25.0%) and invalid remission 19 cases (67.9%); in which χ^2 tests for the comparison between two groups showed all $p < 0.01$ (Table 1). The remission meant that the patient suffered less than one headache attacks during 120 min after AVP treatment.

The patients with tension-type headache and migraine had no allodynia after the AVP treatment, there were not correlations between the AVP alterations and the sex, the terms of female and the age. It showed that there were no side-effects of the AVP treatment in this study.

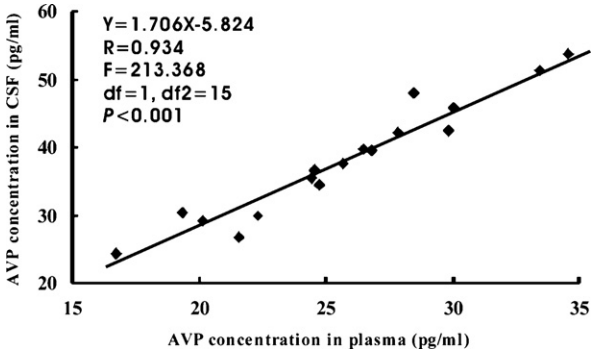

Fig. 5. The relationship between plasma arginine vasopressin (AVP) concentration and cerebrospinal fluid (CSF) AVP concentration in the headache patients.

Table 1
Effect of intranasal arginine vasopressin (AVP) on human headache.

Group	Effect (case)				χ^2 tests		
	Total	Complete remission	Partial remission	Invalid remission	Placebo	Intranasal AVP 100 ng	Intranasal AVP 200 ng
Intranasal AVP 400 ng	28	21	6	1	$p < 0.01$	$p < 0.01$	$p < 0.01$
Intranasal AVP 200 ng	28	13	12	3	$p < 0.01$	$p < 0.01$	
Intranasal AVP 100 ng	28	8	9	11	$p < 0.01$		
Placebo	28	2	7	19			

4. Discussion

AVP in the brain rather than the spinal cord and blood circulation plays an important role in rat pain modulation [22,25]. However, few experiments have proven that AVP regulates the pain process in human, because a major barrier to entry of AVP into the brain is low bioavailability and presence of the BBB. Intranasal delivery of AVP provides a potentially promising alternative to other routes administration, since a direct pathway exists between the olfactory neuroepithelium and the brain [15]. Pietrowsky et al. reported that effect of AVP was facilitated after intranasal as compared to intravenous administration in human brain [14]. Although intranasal AVP did not bypass directly from the nose to the CSF [13], the present study showed that intranasal AVP could relieve the human headache in a dose-dependent manner. The data suggested that intranasal AVP, as same as the role in the animal model, effects on pain process in human brain.

AVP, which was synthesized and secreted in PVN, plays an important role in the pain modulation in rats [20,21,24]. Our previous studies observed that pain stimulation decreased AVP concentration in the PVN, meanwhile, pain stimulation increased AVP concentration in the periaqueductal gray (PAG), nucleus raphe magnus (NRM), caduate nucleus (CdN) and SON, but AVP concentration does not change in the hypothalamic arcuatus nucleus (ARN), locus coeruleus (LC), hippocampus, pituitary, thoracic spinal cord, lumber spinal cord and serum [22]. Pain stimulation also increased AVP concentration in the PAG, NRM or CdN perfusion liquid, which was relating with pain modulation in rats [18,20]. The pain stimulation could enhance the PVN synthesis and secretion of AVP [20], which was transferred to other nervous structures, such as PAG [23], NRM [18,29] and CdN [17] to regulate the pain process in rats. The present results showed that AVP concentration in both the plasma and the CSF increased significantly in headache patients, who related with headache level; and there was a positive relationship between the plasma and the CSF AVP concentration. The data suggested that blood AVP might be from the brain during the headache.

Our previous studies also discovered that AVP enhanced the PAG synthesis and secretion of the endogenous opiate peptides including leu-enkephalin, met-enkephalin, β-endorphin and dynorphin A_{1-13} [19,26,27], induced the NRM release of serotonin (5-HT) [28] and CdN released of acetylcholine (Ach) [16] to participate in pain modulation in rats. It may be the neurobiological mechanism of central AVP regulating pain process. Bypassing the BBB to the brain through olfactory region, intranasal AVP may influence the endogenous opiate peptide, 5-HT and Ach systems to regulate pain process in human brain. However, it was very difficult to prove which brain structures and bioactive substances intranasal AVP related with in human headache relief, and to measure the half-life of the AVP in the present study. It needs to be studied in near future.

In conclusion, our present study made it clear that (1) AVP concentration in both plasma and CSF increased significantly in headache patients, who related with the headache level; (2) there was a positive relationship between plasma and CSF AVP concentration in headache patients; and (3) intranasal AVP could relieve the human headache in a dose-dependent manner. The data suggested that brain AVP, which was delivered through olfactory region, could treat human headache and AVP might be a potential drug of pain relief by intranasal administration.

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References

[1] Antunes JL, Zimmerman EA. The hypothalamic magnocellular system of the rhesus monkey: an immunocytochemical study. J Comp Neurol 1978;181:539–65.
[2] Aziz H, Pearce J, Miller E. Vasopressin in prevention of lumbar puncture headache. Br Med J 1968;4:677–8.
[3] Berkowitz BA, Sherman S. Characterization of vasopressin analgesia. J Pharmacol Exp Ther 1982;220:329–34.
[4] Berson BS, Berntson GG, Zipf W, Torello MW, Kirk WT. Vasopressin-induced antinociception: an investigation into its physiological and hormonal basis. Endocrinology 1983;113:337–43.
[5] Bernson GG, Berson BS. Antinociceptive effects of intraventricular or system administration of vasopressin in the rat. Life Sci 1980;26:455–9.
[6] Bodnar RJ, Nilaver G, Wallace MM, Badillo-Martinez D, Zimmerman EA. Pain threshold changes in rats following central injection of beta-endorphin, met-enkephalin, vasopressin or oxytocin antisera. Int J Neurosci 1984;24:149–60.
[7] McEwen BB. The role of vasopressin and oxytocin in memory processing, vol. 50. Amsterdam: Elsevier Science; 2004.
[8] Dhuria SV, Hanson LR, Frey 2nd WH. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. J Pharm Sci 2010;99:1654–73.
[9] Illum L. Is nose-to-brain transport of drugs in man a reality. J Pharm Pharmacol 2004;56:3–17.
[10] Kendler KS, Weitzman RE, Fisher DA. The effect of pain on plasma arginine vasopressin concentrations in man. Clin Endocrinol (Oxf) 1978;8:89–94.
[11] Kordower JH, Bodnar RJ. Vasopressin analgesia: specificity of action and non-opioid effects. Peptides 1984;5:747–56.
[12] Kordower JH, Sikorszky V, Bodnar RJ. Central antinociceptive effects of lysine-vasopressin and analogue. Peptides 1982;3:613–7.
[13] Merkus P, Guchelaar HJ, Bosch DA, Merkus FW. Direct access of drugs to the human brain after intranasal drug administration? Neurology 2003;60(10):1669–71.
[14] Pietrowsky R, Strüben C, Mölle M, Fehm HL, Born J. Brain potential changes after intranasal vs. intravenous administration of vasopressin: evidence for a direct nose–brain pathway for peptide effects in humans. Biol Psychiatry 1996;39:332–40.
[15] Veronesi MC, Kubek DJ, Kubek MJ. Intranasal delivery of neuropeptides. Methods Mol Biol 2011;789:303–12.
[16] Wang DX, Yang J, Song CY, Liu WY, Zhang J, Wang G, et al. Arginine vasopressin induces rat caudate nucleus releasing acetylcholine to participate in pain modulation. Peptides 2010;31:701–5.

[17] Yang J, Chen JM, Liu WY, Song CY, Lin BC. Arginine vasopressin in the caudate nucleus plays an antinociceptive role in the rat. Life Sci 2006;79:2086–90.
[18] Yang J, Chen JM, Liu WY, Song CY, Lin BC. Effect of arginine vasopressin in the nucleus raphemagnus on antinociception in the rat. Peptides 2006;27:2224–9.
[19] Yang J, Chen JM, Liu WY, Song CY, Lin BC. Through V2, not V1 receptor relating to endogenous opiate peptides, arginine vasopressin in periaqueductal grey regulates antinociception in the rat. Regul Pept 2006;137:156–61.
[20] Yang J, Chen JM, Song CY, Liu WY, Wang G, Wang CH, et al. Through the central V2, not V1 receptors influencing the endogenous opiate peptide system, arginine vasopressin, not oxytocin in the hypothalamic paraventricular nucleus involves in the antinociception in the rat. Brain Res 2006;1069:127–38.
[21] Yang J, Chen JM, Yang Y, Liu WY, Song CY, Lin BC. Investigating the role of hypothalamic paraventricular nucleus in nociception of the rat. Int J Neurosci 2008;118:473–85.
[22] Yang J, Song CY, Liu WY, Lin BC. Only through the brain nuclei, arginine vasopressin regulates antinociception in the rat. Peptides 2006;27:3341–6.
[23] Yang J, Yang Y, Chen CM, Xu HT, Liu WY, Lin BC. Arginine vasopressin in periaqueductal gray, which relates to antinociception, comes from hypothalamic paraventricular nucleus in the rat. Neurosci Lett 2007;412:154–8.
[24] Yang J, Yang Y, Chen JM, Xu HT, Liu WY, Wang CH, et al. Arginine vasopressin is an important regulator in antinociceptive modulation of hypothalamic paraventricular nucleus in the rat. Neuropeptides 2007;41:165–76.
[25] Yang J, Yang Y, Wang CH, Wang G, Xu HT, Liu WY, et al. Effect of arginine vasopressin on acupuncture analgesia in the rat. Peptides 2009;30:241–7.
[26] Yang J, Yang Y, Xu HT, Chen JM, Liu WY, Lin BC. Arginine vasopressin enhances periaqueductal grey synthesis and secretion of enkephalin and endorphin in the rat. Brain Res Bull 2006;71:193–9.
[27] Yang J, Yang Y, Xu HT, Chen JM, Liu WY, Lin BC. Arginine vasopressin induces periaquedictal gray release of enkephalin and endorphin relating to pain modulation in the rat. Regul Pept 2007;142:29–36.
[28] Yang J, Yuan HF, Chu JG, Yang Y, Xu HT, Wang G, et al. Arginine vasopressin antinociception in the rat nucleus raphe magnus is involved in the endogenous opiate peptide and serotonin system. Peptides 2009;30:1355–61.
[29] Yang J, Yuan HF, Liu WY, Song CY, Xu HT, Wang G, et al. Arginine vasopressin in hypothalamic paraventricular nucleus is transferred to the nucleus raphe magnus to participate in pain modulation. Peptides 2009;30:1679–82.