Effect of intranasal arginine vasopressin on human headache

Jun Yang, Lu Lu, Hong-Chang Wang, He-Qin Zhan, Guang-Fan Hai, Yan-Juan Pan, Qiong-Qing Lv, Da-Xin Wang, Yu-Quan Wu, Ren-Ren Li, Lei Xue, Xin-Hua Wang, Xiao-Ming Deng, Xin-Feng Liu, Yan-Ning Qian, Zhi-Kuan Deng, Zhi-Jian Zhang, Xin-Huan Zhan, Xing-Jian Zhou, Guo-Liang Wang, Jian-Xin Zhai, Jing-Cheng Wang

a College of Pharmacy, Xinxiang Medical University, Xinxiang, Henan 453003, China
b Academician Workstation for Neuropsychiatry Pharmacological Research of Henan Province, Xinxiang Medical University, Xinxiang, Henan 453003, China
c Henan Provincial Mental Hospital, Xinxiang Medical University, Xinxiang, Henan 453002, China
d Biomedical Engineering Laboratory, Jiangsu Su Bei People’s Hospital, Yangzhou University, Yangzhou, Jiangsu 225001, China
e Department of Geriatrics, 117 Hospital of People’s Liberation Army, Hangzhou, Zhejiang 310013, China
f Department of Anesthesiology, Guangdong 999 Brain Hospital, Guangzhou, Guangdong 510510, China
g Department of Hyperbaric Oxygen, Nanfang Hospital, Nanfang Medical University, Guangzhou, Guangdong 510515, China
h Department of Anesthesiology, Dongfang Hospital, Tongji University, Shanghai 200120, China
i Department of Anesthesiology, Changhui Hospital, Second Military Medical University, Shanghai 200433, China
j Department of Neurology, Nanjing General Hospital in Nanjing Military Area Command, Nanjing, Jiangsu 210002, China
k Department of Neurology, Jiangsu Provincial People’s Hospital, Nanjing, Jiangsu 210029, China
l Department of Neurology, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China
m Department of Internal Medicine, Fuzhou General Hospital in Nanjing Military Area Command, Fuzhou, Fujian 350001, China
n Department of Surgery, 95 Hospital of People’s Liberation Army, Putian, Fujian 351100, China
o Department of Internal Medicine, 101 Hospital of People’s Liberation Army, Wuxi, Jiangsu 214000, China
p Department of Brain Surgery, Guangzhou General Hospital in Guangzhou Military Area Command, Guangzhou, Guangdong 510000, China
q Department of Surgery, The Fourth People’s Hospital, Wuxi, Jiangsu 214062, China

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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 in 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.

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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 intravenous injection (iv) of AVP increased the pain threshold [5] and anti-AVP serum (iv) decreased the pain
threshold [6], but neither intrathecal (intrathecal) 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. 125Iodine 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 antisera was 100% cross-reactivity with AVP and no cross-reactivity with oxytocin, vasotocin, lysine-vasopressin, vasoactive intestinal peptide, neuropeptides, leucine-enkephalin, methionine-enkephalin, β-endorphin and dynorphin A1–13. The dilution of the antisera 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-Na2-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 antisera against human AVP. AVP was labeled 125Iodine 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 multiasampling analysis of difference followed by the χ2 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
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 \( (37.64 \pm 7.59 \text{ pg/ml} \text{ vs.} 12.45 \pm 3.43 \text{ pg/ml}, p < 0.01) \) (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).

### 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 relieving 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 \( \chi^2 \) 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 no 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.
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 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