NOTE

The Levels of Vasopressin in Cerebrospinal Fluid of Patients with Alcoholism

T. NOTO*, N. KATO*, K. INOUE*, M. KITABAYASHI*,
AND T. NAKAJIMA**

*Department of Psychiatry, Kyoto Prefectural
University of Medicine, Kyoto, Japan

**Department of Neuropsychiatry, Osaka Daini-Keisatsu
Hospital, Ibaragi, Osaka

Abstract

Concentrations of arginine-vasopressin (AVP) in the cerebrospinal fluid (CSF) of 5 alcoholic patients and 6 healthy volunteers were determined by a radioimmunoassay.

The concentrations of AVP in the CSF of alcoholic patients were 2.93–6.17 pg/ml (the mean value, 4.80 ± 1.34 pg/ml) and those of healthy volunteers 3.80–12.59 pg/ml (the mean value, 9.87 ± 3.19 pg/ml). The AVP level in the CSF of alcoholics was significantly lower than that of normals ($P < 0.02$).

Arginine-vasopressin (AVP) synthesized in the magnocells of supraoptic and paraventricular nuclei of mammals is transported to the target sites in the central nervous system (Buijs *et al.*, 1978) and is supposed to play there some important roles, i.e. in memory mechanism (Van Wimersma Greidanus *et al.*, 1975; De Wied, 1965; Ader and De Wied, 1972; King and De Wied, 1974; De Wied, 1971), biorhythmic mechanism (Zimmerman and Robison, 1976; Stetson and Watson-Whitmyre, 1976; Rusak and Zucker, 1975; Baltzer and Weiskrantz, 1975) etc. The neurosecretory fibers from the magnocells were shown to reach the wall of the lateral ventricle and the third ventricle (Buijs *et al.*, 1978; Zimmerman, 1976). Therefore, AVP may be released directly into the cerebrospinal fluid (CSF). AVP in the CSF was thought to be involved in the control mechanism of intracranial pressure by regulating the water bal-

ance in CSF (Noto *et al.*, 1978; Noto *et al.*, (1979).

The CSF is also supposed to transport neurohormones to the target site in the brain (Van Wimersma Greidanus *et al.*, 1975). On the basis of these experimental findings, AVP was clinically applied to the treatment of patients with memory disturbances.

Oliveros *et al.* (Oliveros *et al.*, 1978) reported that the hormone was effective in alleviating some memory disturbances resulting from alcoholism and head injury and we also applied it intrathecally to the patients with dementia and obtained some favorable results (Nakajima *et al.*, 1980). Recently it was also suggested to be that AVP is very effective in treating depression (Gold *et al.*, 1978).

However, there was no direct evidence that patients with memory disturbances and depression had a lower level of AVP in the brain and even in the CSF because of difficulty in the microdetermination of the hormone. Alcoholism shows withdrawal symptoms, neu-

rological signs, memory disturbances, a depressive mood and so on. AVP is effective in memory disturbances, depressive mood etc., so research on AVP levels in the central nervous system of alcoholics may be important in the therapy of alcoholism.

This paper reports the determination of AVP in the CSF and also data on the AVP level in the CSF of patients with alcoholism.

Materials and Methods

The profiles of alcoholic patients

Patient 1: A 32 year old man was admitted with muscle weakness, loss of emotional control and memory disturbances, and diagnosed as having alcoholism. He had drunk about 500 ml of whisky daily and exhibited the above symptoms for several months before admission. He always began drinking in the morning.

Patient 2: A 65 year old man was admitted with convulsions and confusion, and diagnosed as having an acute alcohol withdrawal syndrome. He had drunk about 1 liter of "Shochu" (distilled spirits with about 40% alcohol content) a day. He had started drinking about 20 years ago.

Patient 3: A 54 year old man was admitted with tremors of the fingers and hands, hallucinations and convulsions, and diagnosed as having delirium tremens. He had drunk about 500 ml of whisky daily for the past 7 years. He had started drinking 35 years ago.

Patient 4: A 55 year old man was admitted with sensory disturbances of the legs, severe insomnia, a delirious state and had attempted suicide, and was diagnosed as having delirium tremens. He had been drinking about 900 ml of "Sake" (about 15% alcohol content) a day. He started drinking about 37 years ago.

Patient 5: A 75 year old man was admitted with insomnia, loss of appetite, disturbances in his gait and memory disturbances, and was diagnosed as an alcoholic. He had started drinking about 57 years ago and had been drinking 600–900 ml/day of "Sake" for the previous 9 months.

Collection of CSF samples

CSF samples from the 5 alcoholic patients and 6 healthy volunteers who had normal liver function were collected by lumbar puncture at 9–10 am. 50 μ l of acetic acid was added to 5 ml of the collected CSF and the acidified samples were stored at -80°C until analyzed. The procedure for CSF collection was not fully standardized, but no anesthesia was employed. All the CSF samples collected were clear and their total protein content was within normal limits. The cell counts of the CSF samples were also within the normal range.

Radioimmunoassay of AVP in the CSF

Antisera were raised in white Japanese rabbits by injection of synthetic AVP (Sigma, Grade VI, 100 IU/ml) conjugated with bovine serum albumin (Sigma, crystallized) (Vance *et al.*, 1968). AVP was iodinated by Na^{125}I using chloramine T and the iodinated AVP was purified on Sephadex G-25 (Pharmacia Fine Chemicals).

1-Deamino, 8-D-arginine-vasopressin (DD-AVP) and oxytocin (Sigma, Grade III) were used for checking cross-reaction.

AVP in the CSF was absorbed by florisol (Wako Co., 100–200 mesh) activated by heating overnight at 400°C . The CSF sample (2.5 ml) was taken into a siliconized tube and 50 mg of the activated florisol was added to it. Absorption of AVP was carried out under shaking at 4°C for 30 min (Beardwell, 1971) and the mixture was then centrifuged at 3,000 rpm for 10 min. The supernatant solution was aspirated off. The precipitate was washed twice with 1.5 ml of distilled water and then twice with 1.25 ml of 0.1 N HCl. AVP was eluted from the absorbent by shaking it in 2 ml of acetone-water-acetic acid (50:49:1, by vol.) for 30 min. The suspension was centrifuged and the eluate was pooled. This extraction was repeated twice. The combined eluates in a tube were evaporated to dryness by gently blowing a constant air stream into the tube at room temperature.

The dried residue was dissolved in 500 μ l of the assay buffer (0.01 M phosphate buffer, pH 7.2, containing 0.15 M NaCl, 0.1% NaN_3 and 0.05% bovine serum albumin). 200 μ l of the solution was used for the radioimmunoassay of AVP and duplicate determinations were performed. 50 μ l of the anti-AVP serum (dilution of 1:20,000) was added to the solution and the reaction mixture was incubated at 4°C . After incubation for 24 h the iodinated AVP (50 μ l, 5,000 cpm) was added to the mixture and incubation was further continued for the following 48 h. For separation of bound and free ^{125}I -AVP 100 μ l of 1% bovine γ -globulin and 1 ml of 25% polyethyleneglycol were added to the reaction mixture, and it was then centrifuged at 3,000 rpm for 40 min. The supernatant solution was discarded and radioactivity of the precipitate was counted with an automatic gamma counting system (Nuclear Chicago, Model 4227). For the recovery check, 0, 12.5, 25, 50 pg of AVP was added at the first step of the extraction of AVP to 2.5 ml of the CSF that had little endogenous AVP destroyed by freezing and thawing many times and the AVP measurements of the samples and the above samples for the recovery check were done at the same time.

Results

Accuracy of the assay of AVP in CSF

A standard curve of AVP and cross-reaction curves of oxytocin and DD-AVP using

Fig. 1. A standard curve of AVP (●—●) and cross-reaction curves of oxytocin (×—×) and DD-AVP (▲—▲) using the anti-AVP serum. B_0 represents the cpm of ^{125}I -AVP bound to the anti-AVP serum under no cold AVP in the assay tube.

the anti-AVP serum are shown in Fig. 1. Oxytocin and DD-AVP were not combined with the anti-AVP serum under the conditions used. By this assay 1 pg of AVP/tube was detectable. This means that over 1 pg of AVP in 1 ml of CSF is able to be determined. For the calculation of recovery of AVP in this assay CSF which had endogenous AVP almost destroyed by freezing and thawing was used. The recovery rate of AVP in this assay was $65.56 \pm 2.55\%$ ($M \pm \text{SD}$, $n=30$). There was little difference among the recovery rates of the 3 concentrations of AVP.

AVP content in CSF

Table 1 shows the AVP levels in the CSF of 6 healthy volunteers and 5 alcoholic patients. The concentrations of AVP in the CSF of 6 volunteers were 3.80–12.59 pg/ml (the

mean value, 9.87 ± 3.19 pg/ml) and those of 5 alcoholic patients 3.92–6.17 pg/ml (the mean value, 4.80 ± 1.34 pg/ml). The level of AVP in the CSF of the alcoholics is significantly lower than that of the controls ($P < 0.02$).

Discussion

The AVP level in the CSF of 6 volunteers in the present study is very close to that reported by Dogterom *et al.* (Dogterom *et al.*, 1978), but it is higher value than Jenkins, *et al.* (Jenkins, *et al.*, 1980) and Luerssen, *et al.* (Luerssen, *et al.*, 1977). The reason for the difference is not clear, but the amount of AVP in the material is known to decrease quickly under such unsuitable conditions as an unsuitable container, pH, temperature and so on. Cross-reactivities of oxytocin and DD-AVP in the assay using the anti-AVP serum were very low, indicating the values obtained in this assay to be more reliable.

The lower level of AVP in the CSF of patients with alcoholism was first demonstrated in this paper. Whether alcohol acts directly on the brain to lower the AVP level in the CSF or indirectly is a matter for further research. However, it is likely that alcohol may suppress the synthesis of AVP in the supraoptic and paraventricular nuclei or/and the release of AVP into the CSF because the intake of alcohol lowers the AVP level in serum (Noto *et al.*, 1980). Since AVP is thought to play an important role in the

Table 1. AVP levels in the CSF of the alcoholic patients and health volunteers.

Controls	AVP (pg/ml)	Patients	AVP (pg/ml)
1	12.59	1	3.43
2	3.80	2	5.61
3	12.59	3	6.17
4	11.56	4	2.93
5	7.61	5	5.84
6	10.96		
Mean $\pm$ S.D.	9.87 ± 3.19 ,	Mean $\pm$ S.D.	$4.80 \pm 1.34^*$

* $P < 0.02$ alcoholics versus controls

function of the brain, the suppression of AVP level in the CSF may cause or be the result of some neuropsychiatric symptoms of alcoholism. Whichever is true, increasing the level of AVP in the CSF may be helpful in treating alcoholism and the intrathecal injection of AVP may be used in the therapy of alcoholism.

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