

CEREBROSPINAL FLUID CONCENTRATIONS OF ATRIAL NATRIURETIC PEPTIDE IN CHILDREN

T. TULASSAY, A. KHOÓR, M. BALD⁺, J. RITVAY,
A. SZABÓ, W. RASCHER⁺

1st Department of Paediatrics Semmelweis University Medical School, Budapest, Hungary; University Children's Hospital, Essen, FRG⁺

Received 17 April 1989

Concentrations of atrial natriuretic peptide (ANP) in plasma and in cerebrospinal fluid (CSF) were measured in preterm neonates, in infants and in children with hydrocephalus. Plasma ANP in preterm neonates were elevated compared to infants and children with hydrocephalus. CSF-ANP in all groups were lower than plasma levels. ANP concentrations in the liquor exhibited higher values in children with hydrocephalus. No correlation was found between plasma and CSF-ANP levels while CSF-pressure and ANP concentration in the liquor correlated positively. Our data provide evidence for the existence of a cerebral ANP system in humans. The CSF-ANP system seems to be independent from the systemic, atrial ANP. CSF-ANP may be of great importance in the regulation of water and ion content of central nervous system and probably liquor formation.

INTRODUCTION

Atrial natriuretic peptide (ANP) is a peptide hormone exerting potent diuretic, natriuretic and vasorelaxant activity /1,2/. It was originally isolated from cardiac atria, from where it is secreted into the blood stream. The hormone regulates blood pressure, and salt-water balance through specific receptors in target organs, such as kidney, adrenal gland and great arteries. Several lines of evidence recently presented indicate, however, that the biological activity of ANP is not limited to the cardiovascular and renal systems. Specific ANP receptors, as well as immunoreactive (ir-) ANP and mRNA of ANP precursor have been shown to exist in brain

/3,4,5/. Furthermore, dense distribution of ir-ANP and ANP receptors are observed in several brain regions which are known to be involved in the control of cardiovascular homeostasis /6/. Cerebroventricular injection of ANP has been reported to induce inhibition of water intake and salt appetite /6/. These facts indicate the potential role of this peptide in the neural control of body fluid homeostasis.

The present study was designed to establish whether ANP is measurable in cerebrospinal fluid (CSF) in children. Furthermore, the relationship between ANP concentrations in plasma and CSF, as well as the relationship between CSF pressure and CSF-ANP content were also examined.

PATIENTS AND METHODS

Twelve children enrolled into three groups were examined. Four of them were preterm infants within the neonatal period (Neonatal group), whose lumbal puncture was carried out because of neonatal seizure. However, cerebral diseases could not be proven by liquor analysis, cranial ultrasonography and follow-up neurological examinations. Mean gestational age was 33 weeks (range 30-35), birth weight 1912 (1300-2300) gram and postnatal age at the time of the examinations 5.3 (3-7) days.

Four infants exhibited the symptoms of meningismus but CSF examinations excluded bacterial meningitis and routine chemical and bacteriological analysis revealed normal values (Infant group). Mean age of these children was 20 (7-46) months.

Four children with hydrocephalus were also examined (Hydrocephalus group). All of them were born as premature babies, but they were investigated at different postnatal age (24th, 28th day, and 6th, 7th month). Hydrocephalus was diagnosed by cranial ultrasonography and by clinical symptoms of the disease. None of them had cerebroventricular shunt at the time of investigation.

Blood and liquor samples were taken between 9.00 and 11.00 a.m. Liquor was drawn by lumbal puncture; only samples not contaminated with blood were examined. Blood samples for the measurements of ANP were drawn into EDTA tubes, centrifuged immediately at 4°C. All samples for hormone determination were frozen at -30 °C until assayed. A specific radioimmunoassay for ANP was used, as described elsewhere /7/. ANP levels were measured in extracted plasma and CSF by radioimmunoassay to alpha ANP with a lower limit of detectability of 4 fmol/ml.

Concentrations of sodium, potassium, chloride, as well as creatinine, and total protein were measured in the plasma and liquor by conventional laboratory methods (Technicon

Autoanalyser), osmolality in plasma and liquor was determined by freezing point method (Knauer's osmometer). CSF pressure was measured by an opening pressure system.

Data are expressed as mean and range. Statistical analysis of the data was carried out by using ANOVA test. Correlation coefficient was calculated by regression analysis. Informed parental consent was obtained in each patient.

RESULTS

Plasma concentrations of ANP were elevated in the Neonatal group being 141 (49.3-391), fmol/l, while in the Infants and Hydrocephalus groups the values were within the physiological range 27.5 (16.1-34.8) and 31.9 (18.1-38.0) fmol/ml, respectively (Table). Although the statistical analysis did not reveal significant differences between the groups, all hormone values in the Neonatal group exceeded those obtained in the Infants group. No difference was observed among the groups in the plasma osmolality. Concentrations of sodium in the serum were also normal (Neonates: 140 (138-141), Infants: 139 (137-140), Hydrocephalus: 139 (137-141) mmol/l).

Concentrations of ANP in liquor samples exhibited much lower values than in the plasma. In spite of the difference in plasma ANP between neonates and infants, no differences were found in the liquor level of the hormone between the two groups being 5.3 (4.0-6.2) in neonates and 4.0 (3.9-4.1) fmol/ml in infants, respectively. In contrast, all children with hydrocephalus had elevated liquor ANP levels ranging from 9.9 to 91.8 fmol/l which differs significantly from the values obtained in both groups ($p < 0.01$). Liquor osmolality was normal in all patients and no difference was found among the groups. No differences were noted in the liquor sodium concentrations between the groups being Neonatal 144 (141-151), Infant: 146 (142-148), Hydrocephalus: 145 (140-149) mmol/l. Mean liquor pressure was three times higher in the Hydrocephalus group than in neonates and infants ($p < 0.01$). Significant positive correlation was found between liquor pressure and liquor concentration of ANP ($y = 215 + 4.8x$; $r = 0.68$; $p < 0.01$).

TABLE

Concentrations of atrial natriuretic peptide (ANP) in plasma and cerebrospinal fluid, plasma and liquor osmolality and liquor pressure in 3 different groups of children

	Plasma		ANP (fmol/ml)	Liquor	
	ANP (fmol/ml)	osm (mosm/kg)		osm (mosm/kg)	pressure (mmH ₂ O)
Neonatal group					
1	391.0	300	5.6	300	152
2	51.0	297	6.2	300	172
3	72.9	298	4.0	297	141
4	49.3	300	5.6	303	158
Infant group					
1	27.6	297	4.0	300	181
2	16.1	299	4.0	296	146
3	34.8	301	4.0	306	191
4	31.5	298	4.0	308	210
Hydrocephalus group					
			++		++
1	38.0	300	12.8	306	450
2	18.1	299	10.0	312	556
3	35.2	297	91.8	314	620
4	36.5	299	9.9	316	398

++ p < 0.01 vs neonatal and infant groups

DISCUSSION

Our data show that ANP is present in the liquor in a measurable quantity. However, the concentrations of the hormone are below that of measured simultaneously within the plasma. The data presented here provide also some evidence that CSF-ANP represents an independent hormone system. If CSF-ANP were only a consequence of atrial ANP production and an accumulation within the CSF, then hormone concentrations in the liquor would have been different in neonates and infants. Namely, preterm infants have five-times higher plasma ANP level than infants but their CSF-ANP concentrations were identical. Therefore, there is strong suggestion that ANP is secreted directly into the liquor, independently from cardiac ANP production. Our data are not in agreement with those of Levin (1988) who measured CSF-ANP in human subjects undergoing myelograms for various neurological disorders. In this study, the CSF levels exceeded the circulating plasma ANP levels in most subjects /8/. Further investigations are needed to clarify whether neurological disorders resulted in an elevated CSF-ANP or children have different liquor ANP concentration as adults.

Our hypothesis, namely that ANP is secreted directly into CSF is supported by the data of Levin et al (1987) who demonstrated that the uptake of radiolabelled ANP into the brain is minimal and supported the idea that local synthesis of ANP predominantly accounts for the brain pool of this peptide /9/.

The exact role of CSF-ANP has not been fully elucidated. Since the function of a given neuropeptide in the brain is often complementary to its peripheral action, a central effect of CSF-ANP in the cardiovascular control has been suggested. In fact, antagonistic relationship between brain ANP and brain renin-angiotensin systems on the regulation of blood pressure, drinking, salt appetite and on ACTH and vasopressin release has been demonstrated in the rat /6/. Atrial ANP plays also an essential role in the regulation of extracellular fluid volume /2,7/. Extracellular fluid movement into and out of the brain occurs mainly at the capillary endothelium (blood brain

barrier) and at the choroid plexus epithelium (blood-CSF barrier). Steardo and Nathanson (1987) showed that the secretory cells of the choroid plexus contain ANP receptors /10/. Furthermore, an inhibitory effect of intraventricular ANP on CSF production rate was also demonstrated /10/. In a more recent study, intracerebroventricular administration of ANP prevented accumulation of water in the brain after the infusion of hypotonic solution /11/. Thus, a central influence of ANP upon the water and ion balances of the nervous tissue can also be hypothesized. Concerning these experimental data, the elevated CSF-ANP level observed in children with hydrocephalus in our study may represent a regulatory mechanism in CSF production trying to dampen liquor secretion.

The regulation of CSF-ANP has not been established yet. Because osmolality and concentrations of sodium in the plasma and also in the liquor did not differ in our patients with different CSF-ANP levels, other factors are responsible for the regulation of intracerebral hormone secretion. The positive correlation between CSF-pressure and ANP level suggests that an analogue mechanism to atrial ANP regulation may play a regulatory role within the brain, too. In contrast, Broderich et al (1988) could not demonstrate significant relationship between ANP concentration in the liquor and CSF-pressure in healthy adults /12/. Further studies are needed to establish the exact regulatory mechanisms of CSF-ANP secretion.

In conclusion, our study provided evidence for the existence of a cerebral ANP system. The CSF-ANP seems to be independent from the systemic, atrial ANP. Hormone values in the liquor are lower than in the plasma and with increasing CSF-pressure CSF-ANP levels become also higher.

REFERENCES

1. Needleman P, Greenwald JE: Atriopeptin: a cardiac hormone intimately involved in fluid, electrolyte and blood-pressure homeostasis. *N Eng J Med* 314: 828, 1986
2. Lang RE, Unger T, Ganten D: Atrial natriuretic peptide: a new factor in blood pressure control. *J Hypertension* 5: 255, 1987

3. Morii N, Nakao K, Sugawara A, Sakamoto M, Suda M, Shimokura M, Kiso Y, Kihara M, Yamori Y, Imura H: Occurrence of atrial natriuretic polypeptide in brain. *Biochem Biophys Res Commun* 127: 413, 1985
4. Ueda S, Sudoh T, Fukada K, Kangawa K, Minamino N, Matsuo H: Identification of alpha atrial natriuretic polypeptide (4-28) and (5-28) in porcine brain. *Biochem Biophys Res Commun* 149: 1055, 1987
5. Sudoh, T, Kangawa K, Minamino N, Matsuo H: A new natriuretic peptide in porcine brain. *Nature* 332: 78, 1988
6. Nakao K, Morii N, Itoh H, Yamada T, Shiono S, Sugawara A, Saito Y, Mukoyama M, Arai H, Sakamoto M, Imura H: Atrial natriuretic polypeptide in brain - implication of central cardiovascular control. *Klin Wochenschr* 65 (Suppl VIII): 103, 1987
7. Rascher W, Tulassay T, Lang RE: Atrial natriuretic peptide in plasma of volume-overloaded children with chronic renal failure. *Lancet* II: 303, 1985
8. Levin ER: Atrial natriuretic factor is detectable in human cerebrospinal fluid. *J Clin Endocrinol Metab* 66: 1080, 1988
9. Levin ER, Frank HJ, Weber MA, Ismail M, Mills S: Studies on the penetration of the blood brain barrier by atrial natriuretic factor. *Biochem Biophys Res Commun* 147, 1226, 1987
10. Steardo L, Nathanson JA: Brain barrier tissues: end organs for atriopeptins. *Science* 235: 470, 1987
11. Dóczy T, Joó F, Szerdahelyi P, Bodosi M: Regulation of brain water and electrolyte contents: the possible involvements of central atrial natriuretic factor. *Neurosurgery* 21: 454, 1987
12. Broderick JP, Schwab TR, Heublein DM, Whisnant JP, Burnett JC: Human cerebrospinal fluid concentrations of atrial natriuretic factor: is there a relationship to CSF pressure of plasma ANF? (Abstract). *Kidney Int* 33: 256, 1988

T. TULASSAY, MD

H-1083 Budapest
Bókay J. u. 53.