CROATICA CHEMICA ACTA CCACAA **68** (3) 511–520 (1995)

ISSN 0011-1643
CCA–2250

Original Scientific Paper

Physiological Characteristics of Some Monoamine Metabolites in Cat Cerebrospinal Fluid

Darko Orešković,[a] Mauricio Sanković,[a] Ana Fröbe[a] and Marijan Klarica[b]*

[a]*Department of Chemistry, »Ruđer Bošković« Institute, Bijenička 54, HR-10 000 Zagreb, Croatia*

[b]*Department of Pharmacology, Medical Faculty University of Zagreb, Šalata 11, HR-10 000 Zagreb, Croatia*

Received January 10, 1995; revised March 30, 1995; accepted May 12, 1995

The concentrations of main metabolites of serotonin and dopamine, 5-hydroxyindoleacetic acid and homovanillic acid, respectively, were measured in cisternal cerebrospinal fluid of cats by high performance liquid chromatography with an electrochemical detector. Higher concentrations of homovanillic acid and a wide interindividual oscillation for both parameters have been found. However, samples collected at four different time intervals showed stabile intraindividual concentrations of the metabolites.

The existence of a concentration gradient of both parameters inside the cat cerebrospinal fluid system was confirmed in experiments with a provoked artificial flow of cerebrospinal fluid inside physiological limits. These experiments also suggested that the hydrodynamics of cerebrospinal fluid is the factor responsible for the concentration gradient existence. It appears that the absence of cerebrospinal fluid circulation is the main hydrodynamic reason for the maintenance of 5-hydroxyindoleacetic acid and homovanillic acid gradients between various parts of the cerebrospinal fluid system.

INTRODUCTION

Monoamine dopamine (DA) and serotonin (5-HT) are important neurotransmitters of the central nervous system (CNS) which have been implicated in a variety of the CNS disorders in humans. DA is most often linked

* Author to whom correspondence should be addressed.

to schizophrenia and Parkinson's disease and 5-HT is related primarily to mood disorders.[1] Thus, information concerning the neurotransmitter metabolism in the brain is of great clinical interest, yet difficult to investigate because »in vivo« experiments on human brain are limited for ethical and technical reasons. Since the cerebrospinal fluid (CSF) is in immediate contact with all CNS structures, and the metabolism of 5-HT and DA is reflected in the CSF as a change of concentrations of 5-hydroxyindoleacetic acid (5-HIAA; main metabolite of 5-HT) and homovanillic acid (HVA; main metabolite of DA),[2] the analysis of CSF constituents should represent a promising tool for assessing the CNS function and pathology. Unfortunately, the still insufficient knowledge of the CNS and CSF physiology and pathophysiology limits the interpretation of the results of CSF analysis.

Although animal models have been widely used to measure the CSF levels of 5-HIAA and HVA in different experimental conditions in order to understand the underlying pharmacological, physiological and pathophysiological principles, there is still little information available on the sex differences, stability in time, concentration gradients and the intercorrelation of 5-HIAA and HVA in CSF. In addition, some basic principles of CSF hydrodynamics are still not completely understood. It appears that the pulsation of CSF and the exchange of substances between the CSF and the surrounding tissue in the whole CSF system widely influence the hydrodynamics of CSF.[3,4,5] Thus, for a correct interpretation of the results, it is necessary to investigate all these parameters under controlled and defined conditions of CSF hydrodynamics.

In this study, we have measured physiological concentrations of 5-HIAA and HVA in cisternal CSF, their sex dependence, stability in time, and the effect of the artificial constant flow of CSF (at rates within the physiological limits) on the levels of these metabolites and their mutual relationship in cats. This was undertaken to advance our understanding of 5-HIAA and HVA levels in CSF as a mirror of monoamine function in the brain.

EXPERIMENTAL

Experiments were performed with domestic cats of either sex weighing 2.2–4.2 kg. The animal quarter was maintained at a temperature of 23 ± 3 °C, with natural light-dark cycles, and entered between noon and two p.m. for cleaning and supplementing fresh food and water. The animals were housed in individual cages, sized 0.80 m × 0.45 m × 0.40 m, and were fed commercial cat food with one half of boiled horsemeat. Before any experimental procedure, the cats were placed in quarantine for 30 days.

All experimental treatments were performed on animals anesthetized with sodium thiopental (Nesdonal, Specia, Paris; 60 mg/kg), and the anesthesia was maintained by administration of the anesthetic via a polyethylene cannula in the femoral vein. Tracheotomy was performed if experiments continued for more than one and

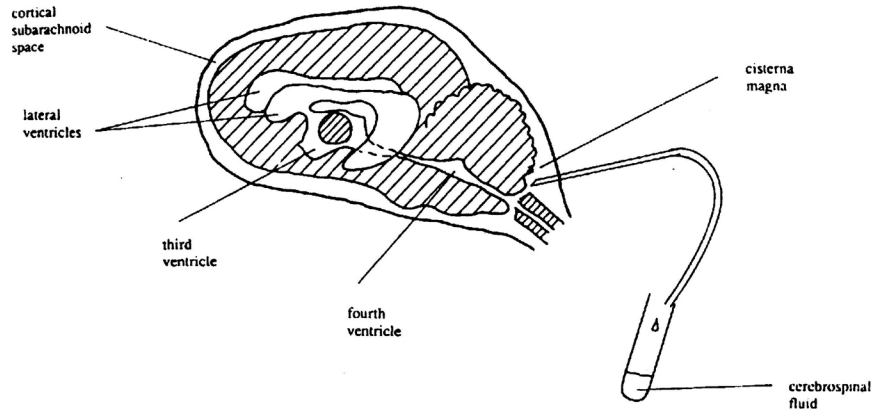

Figure 1. Scheme of experimental model showing the position of cannula in the cisterna magna

a half hours. The cats were positioned in a stereotaxic frame with their heads elevated, the external auditory meatus being 15 cm above the stereotaxic table (sphing position). The cat was kept in that position for an hour before CSF sampling. The neck hair was cut and, through intact skin, 0.5 ml of CSF was drawn by a 22-gauge needle on 1 ml plastic syringe introduced by hand from cisterna magna through atlanto-occipital foramen at 9^{30}–11^{00} a.m. for any cat.

Animals in chronic experiments were treated at the end of sampling with synthetic penicillin (Benzapen, Pliva, Zagreb; 3 ml i.p.) and 5% glucose (Glukoza 5%, Blood Transfusion Institute, Zagreb; 10 ml/kg, i.p.). During unconsciousness, the body temperature was maintained by an electric thermophore. The body weight during chronic experiments was measured on the day of the next collection of CSF sample.

Animals in acute experiments were subjected to the same starting procedure as the chronic animals. For maintenance of constant artificial flow of CSF, a 22-gauge needle was placed in the cisterna magna (see above) and was connected *via* polyethylene tubing with a glass tube. By adjusting the level of spontaneous flow out of CSF at –1 k Pa (–10 cm H_2O), we were able to reach the rate of artificial flow within physiological limits. The level of the external auditory meatus was taken as pressure zero. Blood pressure was monitored in the femoral artery using a Statham transducer and a Grass 7D polygraph. Body temperature was maintained at 310.15 K using an infrared lamp connected to an electronic thermometer placed in the rectum. No changes in monitored physiological parameters were observed in cats during the experimental procedure. At the end of experiment, the animal was sacrificed by an anesthetic overdose.

After collection, samples were centrifuged at 3000 r.p.m. for 5 min to remove particulate matter and stored at –20 °C until the time of analysis (3–7 days).

The frozen samples of CSF were thawed, mixed 5:1 with 0.1 M perchloric acid, 0.2 mM EDTA and 0.4 μM $Na_2S_2O_5$ solution, centrifuged at 10 000 r.p.m. for 15 min, and filtered (MSI Cameo HPLC nylon filters, 0.2 μm). Twenty μl aliquots were used for HPLC-ED analysis.

The high performance liquid chromatography (HPLC) system consisted of a delivery pump (LKB 2150, Sweden), a sample injector (Rheodyne 7125, U.S.A.), C18 reverse phase column (LKB Ultropac Column TSK ODS-120 T, 250 × 4.6 mm, 5 μm particle size), and Micro-Guard column (40 × 4.6 mm, Bio-Rad Laboratories, U.S.A.). An electrochemical detector (ED; HP-ED 1049A, Hewlett-Packard, U.S.A.) with a glassy carbon electrode was used at +0.55 V versus reference electrode. All chromatograms were recorded on a pen recorder (LKB 2210 Potentiometric Recorder, Sweden). Concentrations were determined from peak heights against external standards. The mobile phase contained 0.1 M Na_2HPO_4; 0.05 M citric acid, 12% methanol (*v*/*v*); 0.1 mM EDTA, 1 mM KCl and pH 4.6. The flow rate was maintained at 0.8 ml per min at a pressure of 140 × 10^2 k Pa.

The CSF concentrations of 5-HIAA and HVA were expressed as ng metabolites per ml of CSF and given as mean values (M) ± standard error of a mean (S.E.M.). The realiabilities of the results were determined by calculating the coefficients of variation (C.V.). Statistical comparisons of mean values were carried out using Student's *t*-test.

RESULTS

Table I shows the concentration of 5-HIAA and HVA in cisternal CSF in the examined group of cats and the concentration in male and female animals. The intercorrelations of monoamine metabolites show that the difference between the concentrations of 5-HIAA and HVA in CSF was statistically significant, with that of HVA being approximately 40% higher. No sex dependence on the levels of these metabolites was observed, but only a tendency towards higher values in females as compared with males. The frequency distribution of individual values of the measured parameters was wide, with the coefficient of variation in the whole population of 44.5% and 35.6% for 5-HIAA and HVA, respectively (data not shown).

TABLE I

Mean (± S.E.M.) CSF concentrations (ng/ml) of 5-HIAA and HVA in cats of both sexes (n = 18), males (n = 13) and females (n = 5)

Sex	5-HIAA	HVA
Both	80.09 ± 10.19*	117.69 ± 9.63*
Male	79.24 ± 13.12	112.60 ± 11.45
Female	82.25 ± 13.29	130.95 ± 16.16

Differences between CSF concentrations of 5-HIAA and HVA are statisticaly significant (*$p < 0.02$)

TABLE II

Mean (± S.E.M.) CSF concentrations (ng/ml) of 5-HIAA and HVA and the respective coefficients of variation (C.V.) of CSF samples taken once a week from four cats (3 female and 1 male)

Time (day) Cat	5-HIAA						HVA					
	1st	7th	14th	21th	M ± S.E.M.	C.V. %	1st	7th	14th	21th	M ± S.E.M.	C.V. %
A	84.48	87.70	68.20	95.07	83.86* ± 4.91	11.34	103.68	120.50	97.20	94.20	103.90* ± 5.09	11.30
B	65.90	70.20	69.30	67.00	68.10^{x} ± 0.86	1.99	166.90	130.50	150.20	180.20	156.93^{x} ± 9.30	13.71
C	128.64	110.15	13.30	115.20	120.07^{x} ± 4.30	9.94	202.08	198.88	230.20	189.20	205.09^{x} ± 7.63	8.62
D	51.60	60.14	50.20	59.10	55.26^{o} ± 2.20	5.09	71.04	68.20	78.15	69.17	71.64^{o} ± 1.95	6.34
M ± S.E.M.	82.65 ± 14.49	82.05 ± 9.51	79.50 ± 15.15	84.10 ± 11.20			135.90 ± 25.72	129.53 ± 23.26	138.94 ± 29.47	133.19 ± 26.18		
C.V. %	40.51	26.72	44.00	30.74			43.40	41.47	48.49	45.39		

Differences between CSF concentrations of 5-HIAA and HVA are statistically significant ($^{*}p < 0.05$; $^{o}p < 0.01$; $^{x}p < 0.001$)

The concentrations of 5-HIAA and HVA in samples of cisternal CSF, taken once a week from four cats during four weeks, are given in Table II. It can be seen that the concentration of 5-HIAA and HVA showed no statistically significant variation with time, and neither a statistically significant difference between the concentrations of 5-HIAA and HVA. The intraindividual intercorrelations between the concentrations of 5-HIAA and HVA show that the concentration of HVA is significantly higher than the concentration of 5-HIAA for any examined animal. The frequency distribution of measured concentrations was narrow, with the coefficients of variation approximately 3 to 4 times lower than the coefficients of variation for the whole group (interindividual).

Under the artificial CSF flow conditions (Table III), the concentrations of both 5-HIAA and HVA increased significantly during the first hour, but then remained constant for the duration of the experiment (up to six hours). The rate of artificial flow was stabile during the experiment, except for the stabilization period (first hour), because the CSF pressure was immediately decreased from +1 kPa to −1 kPa by adjusting the level of spontaneous flow out of CSF.

DISCUSSION

Despite a number of analyses of CSF concentration of 5-HIAA and HVA in humans, as indicators of metabolic changes of 5-HT and DA in CNS, some important factors still need critical evaluation in order to clarify the relationship between CNS, 5-HT, DA and theirs metabolites in CSF. For exam-

TABLE III

Mean (± S.E.M.) CSF concentartions (ng/ml) of 5-HIAA and HVA in cats (n = 7) during artificial flow (µl/min).

Time (hours)	5-HIAA	HVA	Flow
0	76.19 ± 10.06^x	110.14 ± 17.06^x	Ø
0–1	98.11 ± 9.53^x	106.93 ± 14.32^x	36.70 ± 2.75
1–2	207.67 ± 14.63	248.96 ± 25.06	17.80 ± 0.63
2–3	192.27 ± 15.79	230.36 ± 34.27	16.40 ± 0.83
3–4	214.19 ± 15.68	254.30 ± 42.84	16.27 ± 1.11
4–5	193.83 ± 20.61	246.56 ± 36.02	15.70 ± 0.98
5–6	184.37 ± 11.95	249.62 ± 28.38	15.55 ± 1.27

The concentrations of 5-HIAA and HVA at the begening of the experiment and after the first hour of CSF collection were significantly different from the ones obtained at later times ($^x p < 0.001$)

ple, for the same pathological states in CNS, literature data on CSF concentrations of 5-HIAA and HVA are contradictory; no exchange, an increase or a decrease of examined parameters is found.[1,6,7] The reasons for these differences are probably in the methodology, ignorance of the behaviour of the investigated substances in CSF and CNS and insufficient knowledge of the CSF physiology. Also, the dependence on sex, age, food, pharmacologic treatment, period of day or season have not, in our opinion, received adequate consideration. To avoid many of these factors, which can influence the CSF concentrations of 5-HIAA and HVA, we have standardized the procedure of cisternal puncture (anaesthesia, volume of CSF drawn, time of collection, body position), feeding (feeding time, type of food), CSF sample manipulation and made the animal selection according to age (adult cats) and sex (see Methods).

The knowledge of the CNS-CSF relationship of these substances in animals is also insufficient because not enough experimental work has been done to elucidate the problems mentioned. There is little or no data on the concentrations of 5-HIAA and HVA in CSF of cats, on their mutual relationship, sex differences and intraindividual stability of these metabolites. Our results (Table I) show that the CSF concentration of HVA is higher than that of 5-HIAA, which corresponds to the results in humans and dogs[8,9,10] but is opposite to the results in sheep.[11] The influence of sex on these metabolites is not statistically significant (Table I), as it is the case in humans and monkeys,[12,13] and only a tendency towards higher values in females is noted. If the sex difference for 5-HIAA and HVA obtained in humans by lumbar puncture of CSF is a consequence of the fact that men are usually taller than women, as suggested by Bertilsson,[14] and that interindividual variability of these parameters is high (see Results), it seems understandable why sex dependence in cats has not been observed with this number of animals.

The concentrations of 5-HIAA and HVA in samples of cisternal CSF, taken once a week from four cats (Table II), did not show any statistically significant variation with time. In addition, the data's large coefficients of variation tend to make the difference between the concentrations of 5-HIAA and HVA statistically nonsignificant in all of the days examined. When the concentrations of 5-HIAA and HVA in individual cats were compared (Table II), the higher levels of HVA measured gain statistical significance. In other words, interindividual variability and intraindividual stability have been observed for the concentrations of 5-HIAA and HVA in CSF. However, for a more quantitative evaluation of these phenomena, it is necessary to extend this study to a larger sample. These results closely correspond to the results in humans and monkeys.[9,13] The number of animals required to detect a significant change in the mean levels of each monoamine metabolite has been calculated taking into account the extent of intraindividual variation, *i.e.* to monitor changes of CSF concentrations of 5-HIAA and HVA, it is better to use autocontrol than control animals.

It is known that concentration gradients for both metabolites exist in CSF, but it is not clear which mechanism is responsible for the maintenance of these phenomena.[8,11,15] The highest concentration[9] of 5-HIAA and HVA are inside the brain ventricles and diminish towards the lowest concentration inside the lumbar sack. Since a probenecid-sensitive organic acid transport system in the CSF actively moves 5-HIAA and HVA from CSF to venous circulation, we have recently investigated in dogs if the concentration gradient of 5-HIAA is a consequence of this system.[16] It was shown that after blocking the active transport by probenecid, the concentration gradient of metabolite in CSF persisted. The same result was obtained on intercorrelation between 5-HIAA and HVA concentrations in humans,[17] suggesting that the common transport system does not account for the concentration gradient in CSF. The study of these phenomena in cats is delicate, considering the difficulty of collecting CSF samples from cat's brain ventricles. However, if the concentration gradient from ventricles to cisterna magna exists, an increase of metabolites concentration should be observed under a provoked artificial flow of CSF out of cisterna magna, as compared with the cisternal concentrations of 5-HIAA and HVA before artificial flow. Furthermore, with this model, it is possible to examine if and how a controlled constant flow of CSF influences the concentrations of 5-HIAA and HVA.

Data in Table III show a statistically significant increase of the CSF concentrations of 5-HIAA and HVA in samples taken during the artificial flow (from 1 to 6 hours) as compared to the CSF samples taken before the artificial flow (zero time). It is evident that this increase is a result of a high concentration of metabolites upstream of the artificial flow, and that the concentration gradient in cats exists at least between the cisternal CSF and the rest of the CSF system. This finding is in agreement with the previous results in humans, dogs and sheep.[8,11,15]

It is interesting to note (Table III) that the concentration of 5-HIAA and HVA, after the artificial flow was established, increased more than twice, and that no significant variation for each metabolite was observed during the experiment. It is also necessary to point out that the rate of artificial flow of CSF was within physiological limits.[3,18] The question arises, if CSF circulates at the same rate as the experimentally provoked flow, how is it possible that the concentration gradients exist inside the CSF system? In other words, if CSF circulates as it is generally believed, then the provoked flow of CSF (in physiological range) should not increase the concentrations of metabolites in cisternal CSF. These results, therefore, suggest that the absence of a CSF circulation is one of the crucial hydrodynamic reasons for this phenomenon. Our data agree closely with the recently published results that secretion, circulation and absorption of CSF do not exist as generally believed but that the main regulator of CSF volume is an interplay of osmotic and hydrostatic forces operating at the blood-brain barrier.[3,4,19,20]

Thus, the approach by experimental CSF flow enabled us to provide a potential explanation for the existence of concentration gradients in the CSF system. Our results suggest that it is necessary to abandon the classical framework of thinking in CSF physiology, in order to explain how metabolites in CSF reflect the function of CNS.

Acknowledgement. – We are grateful to Katarina Karlo and Vlado Vraneša for skillful technical assistance. This work was supported by the Ministry of Science and Technology of Croatia.

REFERENCES

1. M. Scheinin, *Med. Biol.* **63** (1985) 1.
2. A. T. B. Moir, G. W. Aschcroft, T. B. B. Crawford, D. Eccleston and H. C. Guldberg, *Brain* **93** (1970) 375.
3. D. Orešković, P. S. Whitton, V. Lupret, *Neurosci.* **41** (1991) 773.
4. M. Bulat, *Dynamics and statics of the cerebrospinal fluid: The classical and a new hypothesis*, in: C. J. J. Avezaat, A. I. R. van Eijndhoven and J. T. J. Taus (Eds.), *Intra-cranial Pressure VIII*, Springer-Verlag, Berlin, Heidelberg, New York, 1993, p. 726.
5. D. Orešković and M. Bulat, *Hydrostatic force in regulation of CSF volume*, in: C. J. J. Avezaat, A. I. R. van Eijndhoven and J. T. J. Taus (Eds.), *Intracranial Pressure VIII*, Springer-Verlag, Berlin, Heidelberg, New York, 1993, p. 731.
6. D. P. van Kammen, J. Peters and R. Pleak, *Cerebrospinal fluid monoamine metabolism in shizophrenia*: An update, in: P. Burrows, R. Norman and D. Rubinstein (Eds.), *Handbook of Studies on Shizophrenia, Part 2*, Elsevier Science Publishers, 1986, p. 219.
7. I. G. Csernansky, R. I. King, W. O. Faustman, J. A. Moses, M. E. Poscher and K. F. Faull, *Brit. J. Psychiat.* **156** (1990) 501.
8. H. C. Guldberg, G. W. Aschcroft and T. B. B. Crawford, *Life Sci.* **5** (1966) 1571.
9. I. Hildebrand, F. Burgeois, M. Buyse, S. Przedborski and S. Goldman, *Acta Neurol. Scand.* **81** (1990) 427.
10. K. Hyland, R. A. H. Surtees, I. R. Heales, A. Bowron, D. W. Howels and I. Smith, *Pediatr. Res.* **34** (1993) 10.
11. M. Ruckenbusch and J. F. Sutra, *J. Physiol.* (London) **384** (1984) 457.
12. S. N. Young, S. Gauthier, G. M. Anderson and W. C. Purdy, *J. Neurol. Neurosurg. Psychiat.* **43** (1980) 438.
13. M. J. Raleigh, G. L. Brammer, M. T. McGuire, D. B. Pollack and A. Yuwiler, *Neuropsychopharmac.* **7** (1992) 295.
14. L. Bertilsson, *Life Sci.* **41** (1987) 821.
15. I. Degrell and E. Nagy, *Biol. Psychiat.* **27** (1990) 891.
16. M. Bulat, A. Vladić, D. Orešković and V. Lupret, *Concentration gradients of 5-hydroxyindoleacetic acid in the cerebrospinal fluid system*, in: M. Bulat (Ed.), *Pharmacological Communications*, Croatian Pharmacological Society, Zagreb, 1993, 48.
17. M. Jibson, K.F. Faull and G. Csernansky, *Biol. Psychiat.* **28** (1990) 595.
18. M. Pollay, *J. Neurosurg.* **42** (1975) 665.

19. E. A. Bering, *Pathophysiology of hydrocephalus*, in: K. Shulman (Ed.), *Workshop in Hydrocephalus*, University of Pennsylvania Press, Philadelphia, 1966, p. 9.
20. M. Bulat, V. Lupret and D. Orešković, *Iugoslav. Physiol. Pharmacol. Acta* **24**, *Suppl 6* (1988) 39.

SAŽETAK

Fiziološke karakteristike nekih metabolita monoamina u cerebrospinalnom likvoru mačke

Darko Orešković, Mauricio Sanković, Ana Fröbe i Marijan Klarica

Koncentracija glavnih metabolita serotonina i dopamina, 5-hidroksiindoloctene kiseline i homovanilne kiseline, određivana je u cerebrospinalnom likvoru mačke. Opažena je intraindividualna stabilnost i interindividualna oscilacija koncentracije metabolita. Također je pokazano da je hidrodinamika likvora odgovorna za koncentracijski gradijent oba promatrana parametra u likvorskom sustavu.