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Hypoxanthine, Xanthine, Urate and Creatinine Concentration Gradients in Cerebrospinal Fluid

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ABSTRACT

The purine metabolites hypoxanthine, xanthine and urate as well as creatinine were measured in cerebrospinal fluid (CSF) from two groups of patients and a reference sample group. In one of the patient groups lumbar CSF was collected in 2 ml portions until a total volume of 14 ml was withdrawn. Every second portion was analysed for its content of the metabolites in focus. In the other patient group both cisternal CSF and a fixed volume (20 ml) of lumbar CSF were obtained and analysed.

An increase in concentration of hypoxanthine, xanthine and creatinine and a decrease in urate concentration was found in the successive CSF specimens. The mean individual increase in hypoxanthine concentration between the first and the last 2 ml portion was as high as 39.6 %, while it was lower for xanthine, 21.5 %, and creatinine, 6.7 %. The decrease in urate concentration was 17.2 %. The results from the other patient group were in good agreement with these findings. The concentrations in the cisternal CSF was 162 % of that in lumbar CSF for hypoxanthine, 155 % for xanthine, 123 % for creatinine and 80 % for urate. Mechanisms behind inter- and intraindividual differences in gradients are discussed.

INTRODUCTION

Analysis of the purine metabolites in CSF, especially hypoxanthine and xanthine, has attracted some interest during the last few years for several reasons: I) The absence of xanthine oxidase (EC 1.1.3.22) in mammalian brain tissue (1) suggests that xanthine and not urate is the end product of brain purine metabolism. II) The high content of hypoxanthine phosphoribosyl transferase (EC 2.4.2.8) in normal brain and the grave mental symptoms observed when it is deficient (the Lesh-Nyhan syndrome), suggest that hypoxanthine salvage may be especially important in this organ. III) At a reduced oxygen supply the utilization of high energy compounds such as ATP exceeds their formation leading to an accumulation of hypoxanthine and xan-

thine. This accumulation has been used as an indicator of hypoxia (13,6). IV) Experimental research has shown that purine metabolites i.e. ATP and adenosine have modulatory effects on neurotransmission (12).

Strong correlations between the concentrations of hypoxanthine, xanthine, creatinine and the monoamine metabolites 5-hydroxyindole acetic acid (5-HIAA) and homovanillic acid (HVA) in CSF have recently been reported (10). Ventriculo-lumbar gradients have been found for monoamine metabolites by fractionated CSF sampling (2,14). Indirect evidence for similar gradients of the purine metabolites i.e. a negative correlation between CSF hypoxanthine and height (15) and different mean concentrations of the purine metabolites in two reference populations where different volumes of CSF were withdrawn (8). These observations promted us to perform the present study which provides direct evidence of ventriculo-lumbal gradients for hypoxanthine, xanthine, urate and creatinine.

METHODS

The study comprised nine patients with diagnosis of depression (group I), eight patients with dementia (group II) and a reference sample group of 16 apparently healthy individuals.

The CSF was withdrawn by lumbar puncture at the L4-L5 interstice with the patient in the lateral recumbent position. The patients had been fasting overnight and were not allowed to leave their beds for 8 hours before the investigation, which took place at 8-9 a.m. In Group I the CSF was collected in 7 2-ml portions from each patient, stored frozen at -20°C, and analysed within 3 weeks. In Group II both lumbar and cisternal CSF was obtained. The cisternal CSF (4 ml) was withdrawn 1-2 weeks before the lumbar CSF, which was collected in 1 20-ml portion. Both cisternal and lumbar CSF were mixed, divided into smaller portions and kept frozen in liquid nitrogen until analysed. In the reference group 1 20-ml portion of lumbar CSF was obtained and treated as described for Group II.

The purine metabolites and creatinine were analysed using a high performance liquid chromatographic (HPLC) method (8).

Statistical methods used were Student's t-test for means and paired observations, and linear-regression analysis; p values represent two-tailed statistical inference.

RESULTS

The concentrations of hypoxanthine, xanthine and creatinine increased in the successive 2-ml portions of CSF obtained from Group I (Figure 1). The increase in concentration was most pronounced for hypoxanthine and least

pronounced for creatinine. Urate concentration, on the other hand, decreased and this decrease was of about the same order of magnitude as the increase in xanthine.

The results from the individual patients in Figure 1 are marked with roman numerals. Patients having a steep concentration gradient of one metabolite also have steep gradients of the other metabolites. This is especially obvious when patient VII and patient IX are compared. It is also obvious from Figure 1 that no steady state level has been reached for any of the four metabolites in this limited number of CSF portions.

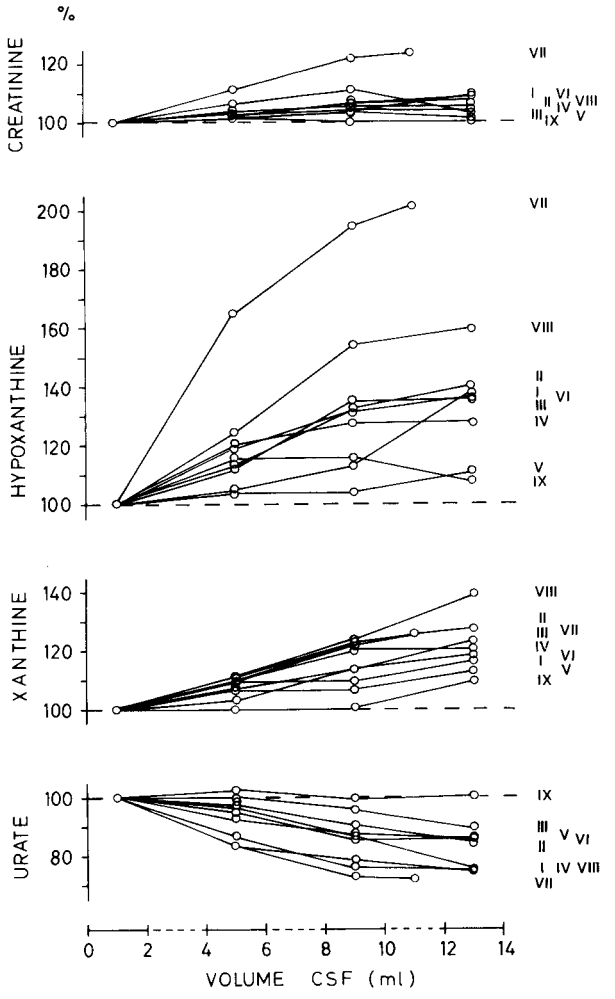

Fig.1. Percental differences of hypoxanthine, xanthine, urate and creatinine concentration, between the first and every second successive 2-ml portion of cerebrospinal fluid from Group I. The individual patients are marked with roman numerals, n = 9.

The expected mean concentration of hypoxanthine, if the CSF had been collected and mixed in one volume of 14 ml, was calculated and found to be 90.1 % (range 82.0 - 102.6 %) of the concentration in the last 2-ml portion. Corresponding figures for creatinine, xanthine and urate were 97.8% (95.3 - 102.4), 92.3% (85.1 - 102) and 109.6% (99.8 - 118.7), respectively.

The difference in mean concentration between the first and the other fractions was significant, $p<0.0125$ for urate, $p<0.0025$ for creatinine and hypoxanthine, and $p<0.0005$ for xanthine.

Mean concentrations $\pm$ SD in fractions 1 and 7 of the 4 analytes are given together with the percental differences of the means in Table 1.

Table 1. Mean concentrations of purine metabolites and creatinine in the first and the last 2 ml fraction of CSF and the percental difference between the means. A total volume of 14 ml has been withdrawn (patient group I, n = 9).

	Concentration ($\bar{x} \pm$ SD, umol/l)		% diff.
	Fraction 1	Fraction 7	
Hypoxanthine	2.23 + 1.09	3.06 + 1.42	+37.2
Xanthine	1.69 $\pm$ 0.44	2.04 $\pm$ 0.49	+20.7
Urate	18.7 $\pm$ 6.2	15.3 $\pm$ 4.7	-18.2
Creatinine	55.5 $\pm$ 9.6	59.2 $\pm$ 10.4	+6.7

The mean values of the percental individual changes are similar to the percental differences of the means: hypoxanthine + 39.6%, xanthine + 21.5%, creatinine + 6.7% and for urate -17.2%.

In Table 2, the mean values $\pm$ SD of the concentrations found in cisternal and lumbar CSF from group II are shown as well as the corresponding lumbar values from the reference sample group. These results are in good agreement with the results obtained in Group I with higher concentrations of hypoxanthine, xanthine and creatinine in cisternal than in lumbar CSF. For urate no significant difference between cisternal and lumbar CSF was observed although the cisternal concentration tended to be lower.

Table 2. Concentrations ($\bar{x}$ + SD, umol/l) of the purine metabolites and creatinine in cisternal (Cp) and lumbar (Lp) CSF from Group II (n = 8) and in lumbar CSF from the reference sample group (Group III, n = 16)

	Group II				Group III
	Cp	p	Lp	p	Lp
Hypoxanthine	4.7 $\pm$ 0.5	<0.001	2.9 $\pm$ 0.6	ns	3.1 $\pm$ 0.58
Xanthine	3.4 $\pm$ 0.6	<0.001	2.2 $\pm$ 0.4	<0.05	1.9 $\pm$ 0.42
Urate	14.5 $\pm$ 7.3	ns	18.2 $\pm$ 5.5	ns	14.7 $\pm$ 7.1
Creatinine	78.9 $\pm$ 9.0	<0.02	64.3 $\pm$ 10.9	<0.005	53.8 $\pm$ 8.9

Significant correlations were found between the concentrations in cisternal and lumbar CSF, for hypoxanthine $r = 0.38$, $p<0.001$, for xanthine $r = 0.71$, $p<0.001$ and for creatinine $r = 0.51$, $p<0.001$. The correlation for urate, $r = -052$ was not significant.

No major differences were found between concentrations in lumbar CSF from Group II and the reference group, except for creatinine.

Table 3 shows the correlations between the four metabolites in focus in cisternal and lumbar CSF in Group II as well as in lumbar CSF from the reference sample group. The correlations between xanthine and creatinine is quite consistent as is the finding of a negative correlation between hypoxanthine and urate.

Table 3. Correlation coefficients (Pearson r) between all the purine metabolites and creatinine in cisternal and lumbar CSF in Group II (n = 8) and in lumbar CSF in the reference Sample group (Group III, n = 16)

	Urate	Hypoxanthine	Xanthine
Cisternal CSF, Group II			
Hypoxanthine	-0.416		
Xanthine	+0.736	+0.093	
Creatinine	+0.648	+0.296	+0.635
Lumbar CSF, Group II			
Hypoxanthine	-0.150		
Xanthine	+0.032	+0.459	
Creatinine	+0.200	+0.695	+0.753
Lumbar CSF, Group III			
hypoxanthine	-0.704		
Xanthine	-0.186	+0.370	
Creatinine	+0.076	+0.206	+0.715

DISCUSSION

The intraindividual differences in steepness of the CSF gradients for the four metabolites studied can probably be explained by differences in metabolism and transport processes. Active transport of hypoxanthine from blood to CSF has been shown (4). This is in line with the observation of higher hypoxanthine concentration in CSF than in blood plasma of man (7). Hypoxanthine, an intermediate metabolite in the purine turnover can be reutilized for nucleotide synthesis in most cells. Alternatively, it can be oxidized to xanthine by xanthine oxidase. The absence of xanthine oxidase in brain tissue (1), however, excludes the latter alternative. Ventriculo-lumbar gradients of hypoxanthine could be explained by i) reutilization in cells surrounding the CSF space and ii) by diffusion, along the concentration gradient, back to the blood as the CSF flows along the spinal canal and iii) by CSF turnover-rate. A low CSF flow-rate would result in long equilibrium times and could be expected to result in low concentrations in the first fractions and possibly steep gradients and vice versa.

Xanthine can not be reutilized to any significant extent in man and is expected to be eliminated from the CSF as the end product of brain purine metabolism. In addition to elimination via CSF bulk flow other transport mechanisms must exist in order to explain the gradients observed, but whether these are pure passive diffusion or if active transport processes are involved is not clear at present.

Urate is formed on the blood side of the "blood brain barrier" i.e. mainly in the liver and the small intestine. The concentration of urate in blood plasma is about 20 times higher than in the CSF and a positive linear correlation exists between these two as long as the barrier permeability is normal (5). Urate is considered to penetrate into the CSF via passive diffusion. The concentration reached is dependent on plasma concentration, the "barrier" permeability and probably also the CSF turnover rate.

Creatinine is formed on both sides of the "blood-brain barrier". Normally the CSF concentration of creatinine is close to the plasma concentration. With increasing age and normal renal function the CSF concentration rises but the serum concentration does not. In uremic patients there was a pronounced gradient with higher concentration in serum. The CSF concentration was, however, also elevated (9). It seems that creatinine is not freely diffusible across the blood-brain barrier although the CSF concentration might be influenced by the serum concentration in certain circumstances. In fact, an active transport mechanism of creatinine from the CSF has been proposed (3).

The finding of steep gradients for all the metabolites in some individuals and minor gradients in others, suggest that the individual gradients are influenced by some common factors. Two such factors could simply be the CSF flow-rate and the volume of the cerebrospinal canal. This hypothesis is supported by the fact that the gradients of xanthine and urate are of the same order of magnitude despite opposite transport directions across the CSF-blood barrier. According to this hypothesis measurement of the gradients of urate and xanthine may have the potential of providing information about these two factors.

Further studies are now addressed to the question whether the low CSF concentrations of 5-hydroxyindole acetic acid, xanthine and creatinine found in depressed patients with a suicidal ideation (15,16) can be explained by aberrant CSF flow (or volume of the cerebrospinal canal) rather than by decreased neuronal activity. In this context it is interesting to note that aberrant CSF flow-rate has been found in another mental disease namely schizofrenia (11).

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