0031-3998/93/3401-0010$03.00/0
PEDIATRIC RESEARCH

Vol. 34, No. 1, 1993
Printed in U.S.A.

Cerebrospinal Fluid Concentrations of Pterins and Metabolites of Serotonin and Dopamine in a Pediatric Reference Population

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ABSTRACT. Accurate diagnosis and management of inborn errors of monoamine neurotransmitter and tetrahydrobiopterin metabolism depend on reliable reference ranges of key metabolites. Cerebrospinal fluid (CSF) was collected in a standardized way from 73 children and young adults with neurologic disease, with strict exclusions. In each specimen, concentrations of homovanillic acid (HVA), 5-hydroxyindoleacetic acid (HIAA), total neopterin, 7,8-dihydrobiopterin, and tetrahydrobiopterin (BH4) were measured using HPLC. There was a continuous decrement in CSF HVA, HIAA, and BH4 during the first few years of life; this was independent of height (or length). Age-related reference ranges for each metabolite are given. Extensive correlations between HVA, HIAA, 7,8-dihydrobiopterin, and BH4 were further analyzed by multiple regression. Age and CSF BH4 were significant explanatory variables for CSF HIAA, but CSF HVA had only HIAA as a significant explanatory variable. (*Pediatr Res* 34: 10–14, 1993)

Abbreviations

CSF, cerebrospinal fluid
HVA, homovanillic acid
HIAA, 5-hydroxyindoleacetic acid
NEO, total neopterin
BH2, 7,8-dihydrobiopterin
BH4, tetrahydrobiopterin

BH4 is the cofactor required for the tryptophan, tyrosine, and phenylalanine monooxygenases, the first two being the rate-limiting steps in the biosynthesis of serotonin and dopamine (monoamines) (1). Inborn errors of biopterin metabolism may severely reduce either the biosynthesis or salvage of BH4, causing hyperphenylalaninemia, a profound deficiency of brain monoamines, and a marked reduction in CSF concentrations of the monoamine metabolites, HVA and HIAA (2). Amine deficiency leads to a characteristic neurologic syndrome (profound hypokinesis, distal chorea, myoclonic epilepsy, hypersalivation, and temperature disturbance) that appears early in life and may be responsive to amine replacement therapy using L-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan. However, inborn errors of biopterin metabolism are a highly heterogenous group of conditions and not all are associated with characteristic findings. Indeed, some affected subjects are symptomless. In addition, there are a variety of conditions that may mimic the clinical and/or biochemical picture but that are not due to a primary defect in biopterin metabolism.

In clinical practice, differential diagnosis, decisions on management, and monitoring of therapy for the patients with the inborn errors of BH4 metabolism are dependent on the correct choice and interpretation of biochemical investigations and require that reliable reference ranges are available for key metabolites. The present article documents such ranges for the following metabolites in CSF: HVA, HIAA, BH4, BH2 (a metabolite of BH4), and NEO (neopterin plus 7,8-dihydroneopterin, derivatives of the BH4 precursor dihydroneopterin triphosphate). Although pediatric reference ranges have been established for CSF amine metabolites (3–7) and for total biopterins and neopterins (8–12), these have never been generated using the same CSF samples; therefore, close study of the relationship between these variables has not been possible. These relationships are carefully examined in the present article.

MATERIALS AND METHODS

CSF samples were obtained from 73 hospitalized patients, aged 2 mo to 19 y, with various neurologic diseases in whom no disturbance of the monoamine neurotransmitter or pterin metabolic pathways was suspected and in whom examination of the CSF was indicated for other reasons. Specimens containing visible amounts of blood were excluded. Case records were reviewed at discharge to ensure no evidence of disturbance of these pathways was found after investigation. Patients with movement disorders, inborn errors of amino acid metabolism, basal ganglia calcification, liver disease, infection, immune deficiency, or hemophagocytic syndromes were excluded. The diagnoses at discharge are given in Table 1. Using the statistical methods detailed below, no differences in metabolite concentrations were found between the diagnostic groups.

Lumbar CSF was obtained with the patient in the left lateral position under sedation or light general anesthesia after a 4-h fast. The CSF was frozen at the bedside on dry ice or in liquid

Table 1. *Diagnoses at discharge of patients in reference population*

Diagnosis	*n*
Epilepsy	32
Congenital lactic acidosis	12
Leukodystrophy	5
Microcephaly	4
Cerebral palsy	4
Asphyxia	3
Other	13

Received October 29, 1992; accepted February 10, 1993.
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R.A.H.S. and A.B. were supported by the Wellcome trust, K.H. and S.J.R.H. by Action Research, and D.W.H. by the Medical Research Council.
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nitrogen and stored at −70°C until analysis. The first 0.5 mL was collected into a plain tube and used for the analysis of HVA and HIAA; the second sample (1 mL) was collected into a tube containing 1 mg of dithioerythritol and 1 mg of diethylenetriamine pentaacetic acid and used for the pterin analyses. HVA, HIAA, BH4, BH2, and NEO were measured by HPLC as previously described (13, 14).

Relationships between variables were examined using Pearson product-motion correlation, multiple regression, and analysis of variance; multiple comparisons were examined using analysis of variance and the Tukey test. Where BH2 concentrations were below the detection limit (<0.4 nM) these were excluded from the analysis of continuous data but included in the analysis of group data, where their value was assumed to be that at the detection limit. Statistical analyses were performed using Statgraphics (STSC, Inc., Rockville, MD) software.

RESULTS

Effect of age. CSF concentrations of all the metabolites examined showed a wide variance and appeared to be related to age (Fig. 1). Peak values were found during the first 3 mo of life and then concentrations generally fell to reach a plateau at around 5 y of age. On further examination of the data, however, only HVA ($r = -0.513$, $p < 0.0001$), HIAA ($r = -0.526$, $p < 0.0001$), and BH4 ($r = -0.429$, $p = 0.0002$) were significantly correlated with age. This was confirmed using analysis of variance with metabolite concentrations grouped according to age (Table 2). Using regression with the log of the metabolite concentrations as a continuous dependent variable and age as a continuous explanatory variable, best estimates were obtained for the mean and 95% confidence limit for each metabolite (Fig. 1). Again there was no significant relation between NEO and BH2 values and age.

Reference ranges for the metabolites at different ages are given in Table 2.

Relationships between metabolites. A highly significant correlation was found between the CSF concentration of HVA and HIAA ($r = 0.745$, $p < 0.0001$) (Fig. 2). Significant correlations were also found between CSF concentrations of BH4 and those of HVA ($r = 0.470$, $p < 0.0001$) and HIAA ($r = 0.647$, $p < 0.0001$) (Fig. 3). Similarly, BH2 correlated with HVA ($r = 0.391$, $p = 0.0006$) and HIAA ($r = 0.420$, $p = 0.0002$), and CSF BH4 was also correlated with BH2 ($r = 0.311$, $p = 0.007$) (not shown). There was no correlation of NEO with any of the other metabolites.

Multivariate analysis. Because of the interdependence of the metabolite concentrations, we used multiple regression to determine which variables significantly determine metabolite concentration in the CSF. In addition to age and the metabolite concentrations, we also examined the effect of height (or length) in case the effect of age is caused by rostrocaudal gradients of the metabolites along the spinal canal; identical volumes of CSF were obtained from all ages and sizes, and in the smaller patients the CSF would therefore have a more rostral source. The effects of sex and season were also examined. The data was transformed

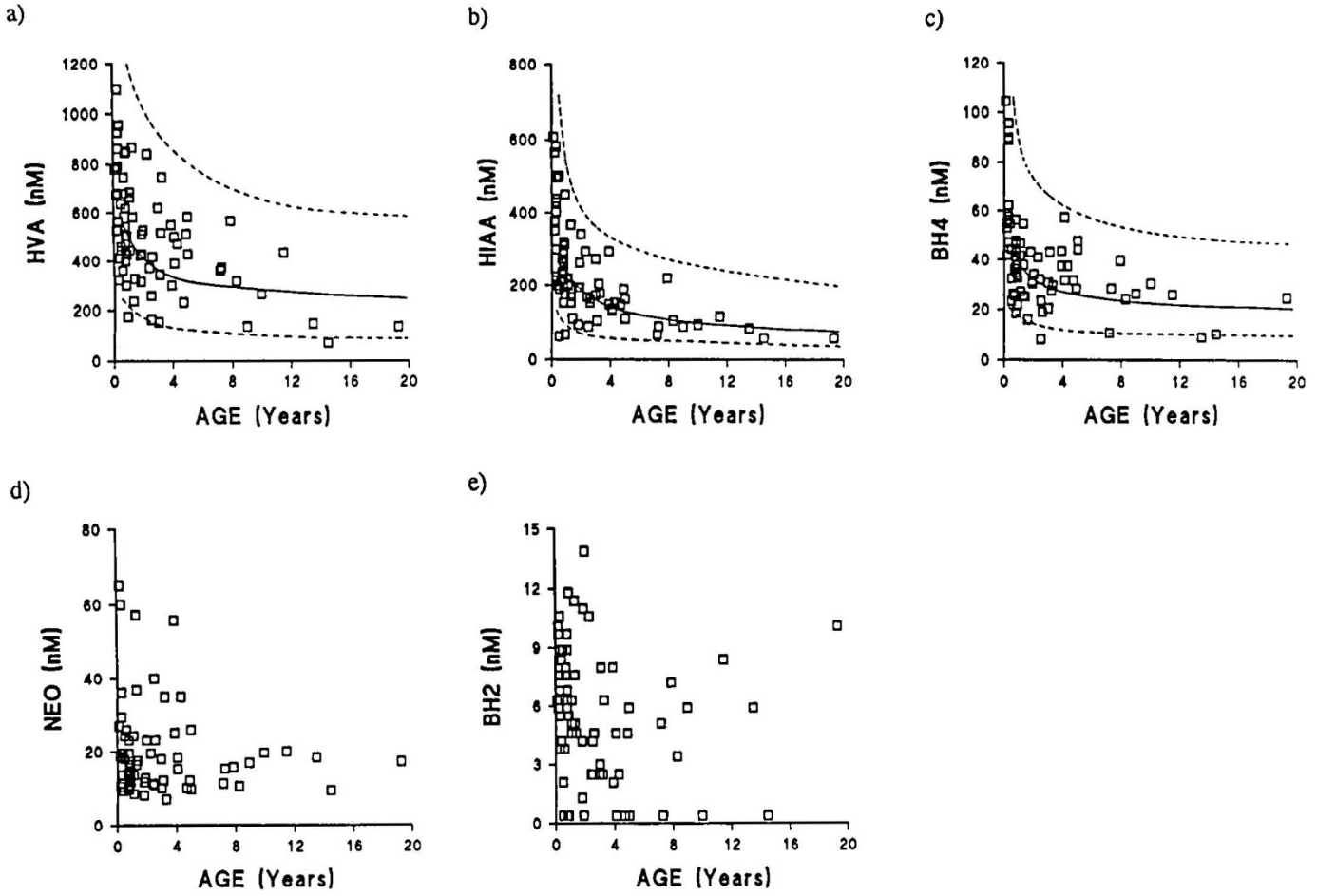

Fig. 1. The relationship between the CSF concentration of the metabolites and age. The regression curve was fitted by the method of least squares after logarithmic transformation; upper and lower curves give the predictive 95% confidence limits for the logarithmic regression. The effect of age upon CSF metabolite concentration (analysis of variance on the logarithmic regression of metabolite concentration upon age) was significant for: *a*, HVA ($F_{1,71} = 34.07$, $p < 0.0001$); *b*, HIAA ($F_{1,71} = 72.86$, $p < 0.0001$); and *c*, BH4 ($F_{1,71} = 39.33$, $p < 0.0001$). There was no significant effect of age upon CSF metabolite concentration for BH2 (*d*) ($F_{1,71} = 0.360$, $p = 0.78$) or NEO (*e*) ($F_{1,71} = 1.07$, $p = 0.30$).

Table 2. *Reference ranges for lumbar CSF concentration of HVA, HIAA, BH4, BH2, and NEO**

Metabolite	Age (y)	Mean	SD	Minimum	Maximum	*n*
HVA	0–0.33	714[a]	205	324	1098	12
	0.34–0.66	587[a]	203	362	955	8
	0.67–1.0	508[a]	196	176	851	12
	1.1–5.0	465[b]	181	154	867	31
	5.1–20	281[b]	158	71	565	10
HIAA	0–0.33	417[a]	132	215	608	12
	0.34–0.66	271[b]	154	63	503	8
	0.67–1.0	250[b]	98	68	451	12
	1.1–5.0	185[b]	71	89	367	31
	5.1–20	98[c]	47	58	220	10
BH4	0–0.33	67[a]	21	27	105	12
	0.34–0.66	37[b]	13	23	55	8
	0.67–1.0	38[b]	11	19	56	12
	1.1–5.0	33[b]	11	8	57	31
	5.1–20	23[c]	10	9	40	10
BH2	All	5.6	3.4	<0.4	13.9	73
NEO	All	19	12	7	65	73

* All values are in nmol. Analysis of variance showed a significant effect of age upon CSF HVA ($F_{3,69} = 8.35$, $p < 0.0001$), HIAA ($F_{3,69} = 13.21$, $p < 0.0001$), and BH4 concentration ($F_{3,69} = 13.81$, $p < 0.0001$), but not upon BH2 ($F_{3,69} = 1.53$, $p = 0.202$) or NEO ($F_{3,69} = 1.48$, $p = 0.217$). Multiple range testing using Tukey's test shows [a] is significantly ($p < 0.05$) different from [b], which is significantly different from [c].

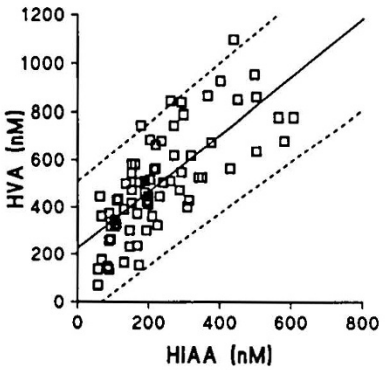

Fig. 2. The relationship between CSF concentration of HVA and HIAA. The regression line was fitted by the method of least squares (analysis of variance on the linear regression: $F_{1,71} = 88$, $p < 0.0001$); the upper and lower curves are the predictive 95% confidence limits. Isolated abnormalities of HVA or HIAA may be detected when the values fall outside the confidence limits.

to give optimal normalization for HIAA, neopterin, age and height (logarithmic transformation), and HVA, BH4, and BH2 (square root transformation) before the analysis.

After allowing for length, there was still a significant association with age, HVA, and BH4 ($p < 0.05$) for CSF HIAA (Table 3); the three variables explained over 70% of its variance. CSF HVA had only HIAA as a significant explanatory variable, accounting for almost 70% of the variance. There were significant associations of age and HIAA with CSF BH4, explaining almost 45% of the variance. CSF BH2 had only BH4 as a significant explanatory variable, but this accounted for only 10% of the variance. No significant explanatory variables were found for CSF NEO. There was no significant effect of length or height, sex or season upon any metabolite concentration.

DISCUSSION

The reference ranges presented here for CSF amine metabolites in children agree closely with those published previously (3–7). The decrement in metabolite concentrations with age over the first few years of life has been observed in all published studies that have examined the effects of age. We have found that the effect of age was independent of the length of the child and therefore unlikely to reflect decreasing concentrations along the spinal canal with increasing size.

We were careful in our sample collection to ensure that the same volume of CSF was collected and that the same portion was used for each analysis. This process removed any variation that could have occurred due to the rostrocaudal gradient that is known to exist for HVA and HIAA (15). In adults, a seasonal variation in HVA and HIAA has been reported (16); in contrast, no variation was found in a pediatric population (17). The CSF samples in our study were collected at random times over several years, and we were able to confirm the lack of seasonal variation after correcting for the effect of age. Unfortunately, detailed timing of CSF collection was not documented; therefore, we were unable to establish whether diurnal variation of metabolite concentrations occurs.

The data demonstrate that age-related reference values for amine metabolites are essential in clinical practice. The 95% confidence limits based on the logarithm of metabolite values provide the best approach to assessing the likelihood that an individual patient's results are abnormal, but we also give the complete reference range.

Previous studies have also provided pediatric reference values for total biopterins and NEO (8–12), but not for the individual biopterin species BH4 and BH2. Although the CSF NEO in the present study is in good agreement with previous work, our values for total biopterins (based on BH4 plus BH2) are considerably higher. Almost certainly this is due to the special care taken in the present study to avoid oxidative loss of reduced biopterins during sample collection, storage, and analysis. Our previous work has demonstrated that BH4 in CSF is highly unstable and readily breaks down to nonbiopterin compounds unless specimens are collected into appropriate antioxidants and stored at −70°C. These studies demonstrated that samples collected in this manner were stable for at least 6 mo at −70°C and up to 2 h at room temperature (18). For all the metabolites measured here, but particularly for BH4 and BH2, it is necessary in clinical practice to pay close attention to standardization of the site, volume, and method of collection of CSF to ensure proper interpretation of the results.

The close relation between CSF dopamine and serotonin metabolites and BH4 concentrations suggests that the metabolism of these compounds *in vivo* is normally closely linked. These results are in general agreement with those reported previously in adults (19, 20) and with *in vitro* studies in which alteration of BH4 concentration has been shown to influence monoamine metabolism (21–23). In the multiple regression model, we found that much of the variability in CSF HIAA was explained by age and BH4, yet a similar proportion of the variability in CSF HVA was explained by HIAA only. Previous studies on the interdependence of these metabolites in CSF have suggested that serotonin turnover may have a regulatory action on dopamine turnover (24), a hypothesis our data support.

In vivo, BH4 is converted to quinonoid-BH2 during the hydroxylation of tyrosine and tryptophan. The quinonoid-BH2 is then mostly recycled back to BH4 by dihydropteridine reductase; however, some of the quinonoid-BH2 escapes this regeneration process and spontaneously rearranges to form 7,8-BH2. It is therefore not surprising that some of the variability of BH2 was explained by CSF BH4. The lack of a relationship between NEO and BH4, or NEO and dopamine or serotonin metabolites, is consistent with the view that neopterins in CSF and other body fluids have little to do with amine synthesis (except in the specific instance of inborn errors of BH4 synthesis) but arise from macrophages and other immunocompetent cells (25). By far, the most common cause of raised NEO in CSF is stimulation of the macrophage system by virus infection and other immune stimuli (26). Because elevated CSF neopterin is not accompanied by an increase in urine values (27), measurement of CSF NEO is likely

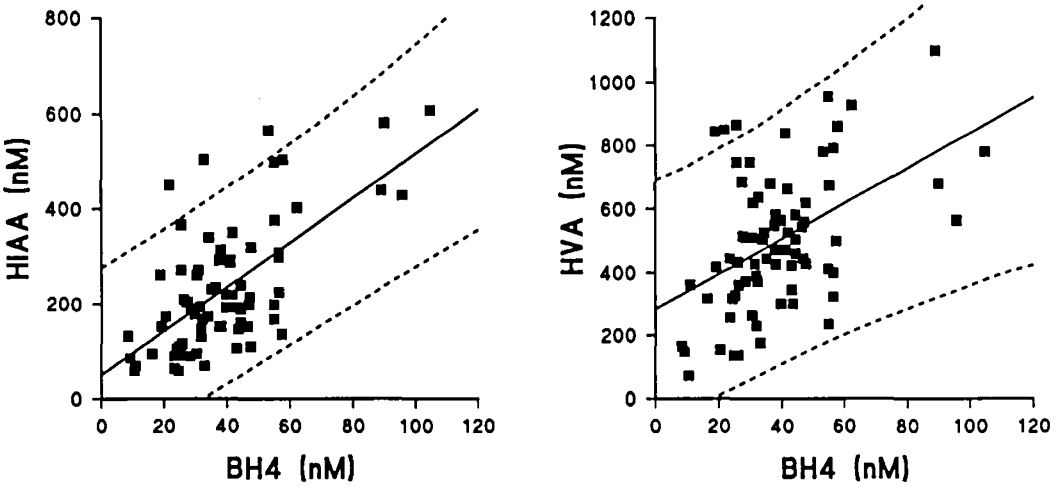

Fig. 3. The relationship between CSF concentrations of BH4 and those of HVA (*a*) and HIAA (*b*). The regression lines were fitted by the method of least squares (analysis of variance on the linear regressions: HVA $F_{1,71} = 20$, $p = 0.0003$; HIAA $F_{1,71} = 51$, $p < 0.0001$); the upper and lower curves are the predictive 95% confidence limits.

Table 3. *Partial correlation table for variables determining CSF HIAA, HVA, and BH4 found to be significant by multiple regression**

	log(HIAA)†	log(age)	$\sqrt{(HVA)}$
log(age)	−0.402		
	$p < 0.001$		
$\sqrt{(HVA)}$	0.609	−0.031	
	$p < 0.001$	NS‡	
$\sqrt{(BH4)}$	0.240	−0.319	0.032
	$p < 0.05$	$p < 0.01$	NS‡

* For each cell, above is the partial correlation coefficient, below its significance.

† Optimal normalization of each variable was determined by transforming the variable and using the Kolmogarov-Smirnov test, where the transformation with the lowest and nonsignificant D-statistic was chosen.

‡ $p > 0.05$.

to be clinically useful as an initial test to establish the presence of immune system activation, localized specifically within the CNS.

The reference values presented here have been used not only to confirm and evaluate the need for amine replacement therapy in patients with inborn errors of BH4 metabolism but also for the differential diagnosis of other disorders in which defective amine metabolism occurs without changes in BH4 (28–31). Wider application of similar CSF analysis in disorders in which there may be disturbance of dopamine or serotonin metabolism (32, 33) or in cases in which there is the possibility of CNS immune system activation (27) is likely to be of value in the future.

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