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Short Communication

Concentrations of Homovanillic Acid and 5-Hydroxyindoleacetic Acid in Cerebrospinal Fluid from Human Infants in the Perinatal Period

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Abstract: To assess maturation of central serotonin and catecholamine pathways at birth, we measured lumbar CSF homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), stable acid metabolites of dopamine and serotonin, using HPLC with electrochemical detection. CSFs from 57 neonates (38 premature and 19 at term) and 13 infants 1–6 months old were studied. HVA levels increased with maturity ($p < 0.05$; ANOVA), whereas 5-HIAA levels were similar in all these subjects. HVA/5-HIAA ratios increased markedly from 1 ± 0.12 in the most premature neonates to 1.98 ± 0.17 in the older infants ($p < 0.01$; *t* test). There were no sex differences for these values. **Key Words:** Neonatal—Development—Serotonin—Dopamine—CSF. **Silverstein F. S. et al.** Concentrations of homovanillic acid and 5-hydroxyindoleacetic acid in cerebrospinal fluid from human infants in the perinatal period. *J. Neurochem.* **43**, 1769–1772 (1984).

Central catecholamine and serotonin neuronal pathways originating in the brain stem participate in regulation of vital physiological functions of human infants, including respiration, sleep, feeding, temperature, and neuroendocrine control (Gluckman et al., 1981; Yogman and Zeisel, 1983). Disruptions in these activities are common symptoms of central nervous system pathology in the neonatal period. The developmental state of neuronal metabolism in these pathways determines when they begin to have an impact on behavior (Coyle, 1977). Measurement of CSF concentrations of the stable acid metabolites of serotonin and dopamine is presently one of the few practical ways to study these pathways in humans.

Levels of monoamine metabolites in cerebrospinal fluid and brain reflect biogenic amine neurotransmitter turnover rates in the central nervous system of animals and humans (Papeshi et al., 1971; Garelis and Sourkes, 1973). Age-related changes in dopamine and serotonin metabolite levels have been reported in older children (Shaywitz et al., 1980; Seifert et al., 1980), but there are few reports of early developmental patterns in humans (Andersson and Roos, 1969; Bhat et al., 1983). To assess maturation and functional activity of catecholamine and serotonin pathways at birth, we measured levels of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), stable acid metabolites of dopamine and serotonin, in lumbar CSF from 57 neonates in the first 5 days of life. We assessed the influence of gestational age and gender on these values, and compared the levels with those in infants 1–6 months old. We also studied sequential lumbar CSFs and ventricular CSF in an infant who developed obstruction of cerebrospinal fluid flow (hydrocephalus) to learn more about bulk flow of the metabolites within the spinal fluid.

SUBJECTS AND METHODS

We assayed lumbar CSF specimens from two groups of patients, 57 neonates and 13 infants, and cerebroventricular CSF specimens from two premature infants. The neonates, 35 boys and 22 girls, were patients in the Holden Perinatal Unit of the University of Michigan Medical Center. Their gestational ages, as determined by clinical assessment, ranged from 28 to 42 weeks; there were 38 premature infants (36 weeks or less) and 19 term infants. Common medical problems in these patients in-

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Abbreviations used: ANOVA, Analysis of variance; 5-HIAA, 5-Hydroxyindoleacetic acid; HVA, Homovanillic acid.

cluded prematurity, asphyxia, respiratory distress, and infection. However, examination of the data did not reveal any relationship of these conditions to the monoamine concentrations and they were analyzed together. All subjects had a lumbar puncture in the first 5 days of life; the most common medical reason for the procedure was suspicion of infection. Typically, the total volume of CSF removed from an infant was 3–4 cc. The last aliquot (½–1 cc) was used for monoamine metabolite determinations. Fluid was also available from 13 older infants, seven boys and six girls, who received lumbar puncture for similar indications. Cerebrospinal fluids were analyzed in routine fashion and an aliquot was frozen at −20°C for neurotransmitter metabolite assay.

Cerebroventricular fluid was also obtained from two infants. In the infant who developed hydrocephalus, several lumbar spinal fluid samples were available for comparison.

HVA and 5-HIAA levels were measured using HPLC with electrochemical detection (Nielson and Johnston, 1982). To prepare samples for analysis, they were thawed, an aliquot was added to an equal amount of 0.4 *M* perchloric acid, and the sample was spun in a microfuge (Beckman) for 1 min. Twenty microliters of the supernatant was injected directly into the system, which included a pre-column (Brownlee), a C-18, 5-μm, reverse-phase column (Altex), and an electrochemical detector with glassy carbon electrode set at 0.8 V versus an Ag/AgCl electrode (Bioanalytical System). The mobile phase was 0.1 *M* phosphate-citrate buffer, pH 3, with 0.0001 *M* EDTA and 0.012% sodium octyl sulfonate. Sodium octyl sulfonate was used because we also measured CSF dopamine levels (results not reported). HVA and 5-HIAA peaks were identified by calculation of retention times and the peaks of CSF metabolites coeluted with those of added standards. Concentrations were calculated by comparison with peak heights of external standards (Sigma). Variation in measurement of the same sample over a 6-month period was about 10%.

The effects of gestational age and sex on HVA and 5-HIAA levels were tested by two-way analysis of variance (ANOVA). To assess the interrelationship of HVA and 5-HIAA levels, HVA/HIAA ratios for each subject and mean values for four age groups were calculated, and groups were compared by Student's *t* test.

RESULTS

The infants were grouped into four age groups for analysis: young prematures less than 32 weeks, prematures 33–36 weeks, 37–42 weeks (full term), and older infants up to 6 months. HVA and 5-HIAA levels in the subjects are tabulated in Table 1; values from all premature infants are combined. HVA levels increased progressively with increasing maturity ($p < 0.05$; ANOVA). Although mean HVA values of girls are slightly higher than those of boys in each age group, these differences are not statistically significant. In contrast to HVA, 5-HIAA levels are similar in the three age groups ($p > 0.5$; ANOVA), and there are no significant sex differences. Mean 5-HIAA values in all three groups of infants are considerably higher than values in older children (Seifert et al., 1980).

We also studied ventricular fluid from two subjects. HVA concentrations were 487 ng/ml in the first and 712 ng/ml in the second, both more than three times as high as lumbar CSF values in infants. HIAA concentrations were 181 ng/ml and 284 ng/ml, both about twice as high as in lumbar CSF. The second patient's ventricular tap was performed because obstruction of CSF flow out of the ventricular system (hydrocephalus) developed on day 49 of life. A lumbar CSF obtained the next day contained markedly reduced metabolite levels: HVA, 18 ng/ml, and 5-HIAA, 40 ng/ml. The HVA concentration was 9% of that obtained in the infant two weeks before CSF obstruction (202 ng/ml) and the 5-HIAA concentration was 32% of the previous value (124 ng/ml).

TABLE 1. *Homovanillic acid and 5-hydroxyindoleacetic acid levels in cerebrospinal fluid*

Compares premature and term neonates and older infants	n	5-HIAA (ng/ml)	HVA (ng/ml)
Group 1. Premature neonates	38	96 ± 10	106 ± 10
Group 2. Term neonates	19	104 ± 7	129 ± 11
Group 3. Infants	13	85 ± 7	159 ± 9*

Premature neonates range in gestational age from 28 to 36 weeks, term neonates from 37 to 42 weeks. Infants were 1–6 months of age. Values are means ± SEM.

* $p < 0.05$ using analysis of variance to compare all HVA values in the three groups.

In Fig. 1, mean HVA/HIAA ratios are plotted for three groups of neonates and the older infants. The mean HVA/HIAA ratio in the most immature infants (28–32 weeks gestation) is 1.0 ± 0.12 (mean ± SEM), whereas in the most mature group (1 week–6 months old) it is 1.98 ± 0.17 ($p < 0.01$, comparing these two groups by Student's *t* test).

DISCUSSION

Measurement of CSF monoamine metabolite concentrations currently provides the most accessible measure of functional activity of central dopaminergic and sero-

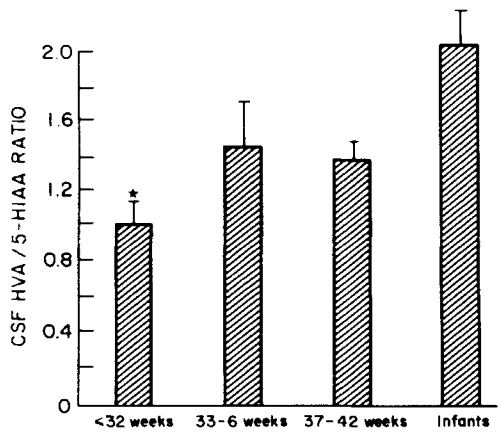

FIG. 1. The ratio of HVA to 5-HIAA values for each subject was calculated. Comparison of the HVA/5-HIAA ratios (mean ± SEM) in neonates, grouped according to gestational age [32 weeks or less (n = 12), 33–36 weeks (n = 26), 37 weeks or more (n = 19)], and infants 1–6 months old (n = 13); ★ indicates $p < 0.01$, comparing the most immature neonates with the infants using Student's *t* test.

tonergic neurons. The brain is the major site of HVA synthesis (Papeshi et al., 1971; Garelis et al., 1973), and although up to 30% of 5-HIAA is produced in the spinal cord, the neurons of origin are in the brainstem (Loewy and McKellar, 1981). Our observations on an infant with obstruction of CSF flow out of the brain are consistent with these estimates. The hydrocephalus was associated with accumulation of metabolites in the lateral ventricle and reduced lumbar concentrations, HVA more than 5-HIAA. The results agree with reports on older human subjects (Post et al., 1973; Sjostrom et al., 1975) and experimental animals (Gordon et al., 1975).

Concentrations of monoamine neurotransmitter metabolites in CSF are determined by the rates of synthesis, release, and degradation of the parent compounds as well as the rate and efficiency of elimination of metabolites from the brain and spinal fluid. Therefore, interpretation of our data must consider possible developmental changes in each of these factors. As reported by others (Andersson and Roos, 1969; Seifert et al., 1980), values for infants 1–6 months of age are considerably higher than those of older children and adults. This may reflect a relatively high monoamine turnover at this age and/or changes in metabolite clearance from the brain. Comparing premature and term neonates, Bhat et al. (1983) found no difference in CSF HVA levels, but mean values were lower in the prematures, the variation was large, and relatively few subjects were included. In immature rodents, the turnover of serotonin and dopamine, measured in terms of brain or CSF HVA and 5-HIAA, is relatively high compared with adult levels (Keller et al., 1973; Shaywitz et al., 1983).

Experimental work in animals suggests that the mode of metabolite clearance from CSF may change considerably during early development. Atack et al. (1974) showed that the major route of 5-HIAA efflux from brain to blood in newborn rats is through CSF pathways to the choroid plexus. At 30 days, more than 75% exits directly from brain to blood, presumably across the blood-brain capillary endothelial barrier via a probenecid-blockable organic acid transport system (Kartzinel et al., 1976; Elghozi et al., 1983). Therefore, one reason for high HVA and 5-HIAA in infants may be that the CSF pool represents a larger share of the metabolites formed at this age. Another hypothesis suggested to explain the inverse relationship between HVA and 5-HIAA levels and age is that the surface area for reabsorption of CSF by bulk flow is reduced in young, smaller children so that higher concentrations are present in lumbar CSF (Seifert et al., 1980). Although a gradient of HVA levels has been described in the lumbar sac of adults (Jakupcevic et al., 1977), this is unlikely to be a factor in explaining the differences in infants since such small volumes were removed. Further, since all infants who had lumbar punctures were recumbent and relatively inactive, differences in amount of mixing of CSF are unlikely to account for the age-related changes.

Our data indicate that HVA concentrations increase over the period from the last trimester of pregnancy into early infancy, while 5-HIAA levels are relatively stable. Thus, the HVA/5-HIAA ratio changes markedly over this time period. Since both metabolites are synthesized and cleared by very similar mechanisms, it is unlikely that either changes in transmitter degradative enzymes (Baker et al., 1974) or transport out of brain explains this differential effect. The data suggest that the maturation of human dopaminergic neuronal activity lags behind that of serotonin neurons in the perinatal period. This could be related to differences in neuronal development, to regulatory alterations in other contiguous neuronal circuits, or to changes in transmitter substrate availability (Yogman and Zeisel, 1983). Differences in the developmental patterns for individual neuronal groups have been described in experimental animals (Johnston and Coyle, 1981), and it is likely that rates of biochemical differentiation also vary according to neuronal type in man. Dopamine and serotonin are present in human fetal brain as early as 3 to 4 months gestation (Nobin et al., 1973), but regional brain levels of serotonin and dopamine have not been measured in infant human brain.

There is considerable variation in metabolite levels among infants of the same gestational age. Factors such as intrauterine or perinatal stress, nutritional status, and genetic makeup may contribute to the regulation of rates of neuronal maturation. Thus, although developmental trends are clear, identification of pathological alterations in individual patients will require study of a larger number of patients or marked deviations from normal.

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