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PERIPHERAL AND CENTRAL 5-HYDROXYTRYPTAMINE IN TRISOMY 21

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Summary

Peripheral and central metabolism of 5-hydroxytryptamine was studied in 16 patients with trisomy 21 and compared to that in 4 karyotypically normal mentally retarded children. Serum 5-hydroxytryptamine was markedly decreased in the trisomics whereas cerebrospinal fluid levels of 5-hydroxytryptamine and 5-hydroxyindole acetic acid were increased in the same groups. These results are discussed with respect to regulatory mechanisms of 5-HT metabolism.

Data concerning 5-hydroxytryptamine (5-HT) metabolism in trisomic 21 patients are generally conflicting. A majority of studies are related to 5-HT in blood platelets (1, 2, 3) and general agreement exists as to 5-HT being decreased in the platelets of patients with trisomy 21. Nevertheless, different hypothetical mechanisms have been proposed to explain this phenomenon, such as modification of monoamine oxidase activity (4), or variations of the uptake of this amine in platelets (5, 6).

Aside from studies by Partington and Lott et al. (7, 8), few results are now available about the central metabolism of 5-HT in trisomic 21 patients. Urinary 5-hydroxyindole acetic acid (5-HIAA) has sometimes been detected (9), but the urinary content of the acidic catabolite of 5-HT can reflect both the peripheral and central metabolism of 5-HT. Nevertheless, since the previous therapeutic trials of 5-HT amino acid precursors, i.e. tryptophan and 5-hydroxytryptophan (9, 10), and their relative success in reversing hypotonia in infants with trisomy 21, there is some evidence that the metabolic pathway of 5-HT is disturbed in these patients.

In order to determine whether or not different steps of 5-HT biosynthesis are modified in patients with trisomy 21, a complete study of endogenous 5-HT, precursor and catabolite was performed at both peripheral and central level. Detection of the concentrations of free tryptophan, albumin and non esterified free fatty acids in serum was also made to determine if the lower content of 5-HT in platelets of trisomic 21 patients was correlated with modifications of tryptophan metabolism. Additionally, free tryptophan content in serum may be taken as an index of the activity of central serotoninergic systems (11, 12).

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Patients and Methods

Blood and cerebrospinal fluid (CSF) samples were collected from fourteen trisomic 21 patients (age ranging from 7 to 17 years, mean : 12.35 ± 0.70) and from four karyotypically normal mentally retarded children (age ranging from 14 to 16 years, mean : 14.24 ± 0.62) in San-Salvadour Hospital (Hyères, France). There was only one female in the control group and four in the trisomic group. None of the subjects presented acute illness or supplementary clinically evident disorders and all had been without any drug treatment for ten days prior to testing. All patients received a similar diet. Blood and lumbar CSF samples were both made at 8 a.m., and were immediately placed at 4°C for biochemical analysis.

TRP, 5-HT and 5-HIAA determinations in blood samples : Blood was allowed to clot at 4°C and serum was obtained by cold centrifugation at 2000 x g for 30 minutes. Aliquots of 500 µl were mixed with 6 ml of an ethanol-water solution (74 : 16 v/v) containing 0.05% EDTA and 0.05% ascorbic acid. 5-HT, total TRP and 5-HIAA were then successively separated by ion exchange chromatography on Amberlite CG-50, Dowex AGW x 4 and adsorption on Sephadex G 10 (13, 14). 5-HT and 5-HIAA were estimated spectrofluorimetrically by the orthophtaldialdehyde method of Curzon and Green (15). Free tryptophan was estimated as previously described by Bourgoin et al. (16, 17). After ultrafiltration in an Amicon dialysis cone (CF 50) made by centrifugation at 800 x g for 30 minutes, TRP was separated in the ultrafiltrate and the corresponding whole serum (Dowex AGW x 4) and measured using the spectrofluorimetric technique of Denckla and Dewey (18).

Total proteins, albumin and non esterified free fatty acids (NEFA) in serum : Total proteins were estimated with bovine serum albumin as standard according to the method previously developed by Lowry et al. (19). Serum albumin was detected by bromocresol green binding (20) using the appropriate Sigma kit. NEFA determination was performed according to Falholt et al. (21) with palmitic acid as standard.

5-HT and 5-HIAA determinations in CSF samples : 5-HT was determined in 1 ml of lumbar CSF using the method of Boireau et al. (22). Briefly, 5-HT was separated from precursors and catabolites and salts discarded on a Sephadex G 10 column eluted with formic acid (0.5 M). Following concentration of the acid solution (under vacuum), 5-HT was dissolved in 50 µl of 0.2 M sodium phosphate buffer pH 7.9, and the radioenzymatic assay of Saavedra et al. (23) was performed. [^{3}H]melatonin formed, extracted into toluene, was further purified by thin layer chromatography on silica gel plates. The sensitivity of the whole procedure was about 10 pg ([^{3}H]melatonin radioactivity was two times the blank with this minimal amount of detectable 5-HT).

5-HIAA in CSF was estimated using a slight modification of the method of Giacalone and Valzelli (24) previously described for brain tissue. 1 ml of CSF containing 50 µl of 5% ascorbic acid and 25 µl of 3N HCl was added to 1 ml butyl acetate. The tubes were shaken for 5 minutes and then centrifuged for 3 minutes. The organic phase was transferred in 1 ml of 0.1N HCl. After shaking and centrifugation, the organic phase was transferred again in 0.5 ml of 0.1 M phosphate buffer pH 7. The organic phase was discarded and 400 µl of the aqueous phase was treated for spectrofluorimetric determination of 5-HIAA (15).

All values detected for TRP, 5-HT, 5-HIAA were corrected for recovery.

Statistical calculations : The Mann Whitney U test was applied and SEM were calculated according to standard statistical procedures (25).