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THE METABOLISM OF 5-HYDROXYTRYPTAMINE BY BLOOD PLATELETS FROM CHILDREN WITH MONGOLISM

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Abstract—The experiments were made to determine whether our previously reported finding of decreased 5-hydroxytryptamine binding by blood platelets from children with Down's syndrome (mongolism) involved changes in platelet monoamine oxidase Platelets from normal and mongol children were incubated with 5-hydroxytryptamine. The efflux of the 5-hydroxytryptamine metabolites 5-hydroxyindolylacetic acid and 5-hydroxytryptophol was measured during a 2-hr incubation. There were highly significant reductions in the efflux of 5-hydroxytryptamine and 5-hydroxyindolylacetic acid from mongol platelets. When 5-hydroxytryptamine loaded platelets were incubated with reserpine, 5-hydroxytryptamine release was greatly augmented in both groups, but there was no increased efflux of 5-hydroxyindolylacetic acid. We conclude that mongol platelets form abnormally low amounts of 5-hydroxyindolylacetic acid *in vitro*. However, the decreased 5-hydroxytryptamine binding reported before cannot be related to any defects of platelet monoamine oxidase until the enzyme has been characterized, since there is the possibility that the enzyme in mongoloid cells is not homogeneous but abnormally heterogeneous.

PREVIOUSLY we have described several defects in the binding of 5-hydroxytryptamine (5-HT) by blood platelets from children with Down's syndrome,[1,2] the primary defect being a deficiency of ATP, the 5-HT binding material, which results in a reduced concentration of 5-HT in platelets of mongol children. It was also conceivable that the decreased 5-HT binding we observed might result from an increased activity of monoamine oxidase, because after enhanced metabolism, less of the 5-HT transported into the cell would be available for incorporation into the storage organelles. Thus, an analysis of the monoamine oxidase activity in the platelets of these children was warranted.

Since the monoamine oxidase of plasma would complicate the analyses of 5-HT metabolism by platelets, all experiments were carried out using platelets suspended in Krebs solution.

METHODS

The experiments involved six mongol children of either sex, Karyotyped as Trisomy 21, aged 5–12 years. The control subjects were siblings in two instances and normal schoolchildren matched for age, sex and body weight in the other four cases. The experimental subjects were requested to eliminate tryptophan-containing foods (list supplied)

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from their diets for 3 days prior to blood sampling; otherwise there were no dietary restrictions. No haematological abnormalities were noted other than those described in Results and in an earlier paper.[2]

Our technique for studying the uptake and binding of 5-HT by platelets have been described in earlier papers.[1–3] 5-Hydroxyindolylacetic acid (5-HIAA) was extracted from platelets and Krebs solution[4] by the method of Udenfriend *et al.*[5] and assayed spectrophotofluorimetrically at excitation and emission wavelengths (uncorrected) of 295 and 340 nm. 5-HT, 5-HIAA and 5-hydroxytryptophol (5-HTOL) were also identified using the paper chromatographic method of Bartholini *et al.*[6] Samples were spotted on Whatman No. 1 chromatography paper and run overnight in a propanol/NH_3 1 N (5 : 1) solvent system, dried sprayed with 4 N HCl and developed under ultraviolet light.

RESULTS

Platelets loaded with 5-HT were resuspended in Ca^{2+}-free Krebs solution and the losses of 5-hydroxyindoles (5-HT, 5-HTOL and 5-HIAA) were measured after incubation for 2 hr at 37°C. The appearance of 5-HIAA and 5-HTOL in the incubation mechanism were taken as evidence of 5-HT metabolism. Table 1 shows that considerable amounts of 5-hydroxyindoles were lost from normal platelets during this period, the efflux being over double the loss occurring from mongoloid cells. However, when the loss was expressed as a percentage of the total 5-HT content of the loaded platelets (column 2, Table 1), the efflux was the same in both groups. Thus, the loss of 5-HT from normal platelets[1] seems to be augmented when the platelets are suspended in a saline medium. The amount of 5-HIAA (column 5, Table 1) and the percentage of 5-HIAA in relation to the total output of 5-hydroxyindoles (column 6, Table 1) into Krebs solution was much greater in the normal platelets. This observation suggested that there was a reduction in the activity of platelet monoamine oxidase in mongols.

At this point it was considered that the effects of reserpine would be of interest. The main action of reserpine appears to be to prevent the storage of catecholamines and indolylalkylamines in tissues, the amines appearing extracellularly in the form of deaminated metabolites.[7] We thought that we might be able to obtain further information on the activity of monoamine oxidase by seeing the action of reserpine on the efflux of 5-HT, and formation of 5-HIAA by platelets incubated with 4·1 nmoles/ml reserpine for 2 hr.

The results are given in Table 1. Reserpine caused a net loss of about 35 per cent 5-HT from both mongol and normal platelets (column 4), efflux being largely in the form of unmetabolized 5-HT. The total amounts of 5-HIAA formed were not increased, but due to the greatly augmented loss of 5-HT, the percentage of total 5-hydroxyindoles represented by 5-HIAA was reduced.

For each type of experiment described above we identified 5-HT and 5-HIAA in the efflux medium using paper chromatography (see Methods). No significant amounts of 5-HTOL were found.

DISCUSSION

Human platelets isolated from adults are known to possess an active monoamine oxidase.[8–10] We have tested platelets from normal children and from children with Down's syndrome and have also found an active enzyme in each case, the latter

TABLE 1. 5-HT EFFLUX AND METABOLISM IN PLATELETS FROM NORMAL AND MONGOL CHILDREN

Experiment	5-HT load released (%)	nmoles/10^{11} platelets			5-HIAA expressed as % of total hydroxyindoles released
		Total 5-hydroxyindoles released	5-HT released	5-HIAA formed	
Efflux in Krebs solution:					
Control	12·1 ± 1·1	260·1 ± 24·0	147·5 ± 11·3	112·6 ± 13·2	43·3 ± 5·0
Mongol	13·2 ± 1·4	110·6 ± 12·1	94·0 ± 14·4	16·6 ± 1·5	15·0 ± 1·4
Efflux in Krebs solution after reserpine:					
Control	44·4 ± 2·7	952·0 ± 58·0	851 ± 118	101·0 ± 9·4	10·6 ± 1·0
Mongol	49·3 ± 3·9	413·6 ± 33·0	393 ± 54	20·6 ± 2·9	5·0 ± 0·7

The experiments were made with normal or mongol platelets which had been loaded with 5-HT by incubation with 10^{-5} M for 90 min. Platelet 5-HT content at the end of incubation was: normal, 2144 ± 189; mongol, 839 ± 76 nmoles/10^{11} platelets. 5-HIAA was assayed in the incubation medium 2 hr after resuspension in fresh Krebs solution or Krebs solution containing 4·1 nmoles/ml reserpine. All values are the mean ± standard error of the mean of 6 observations from 6 mongol and 6 normal children.

possessing lower than normal activity. The only other report on the metabolism of 5-HT by mongol platelets was by Benson and Southgate[11] who found diminished monoamine oxidase activity, and concluded that low platelet 5-HT was not due to an increased rate of 5-HT metabolism. There are other pieces of evidence indicating that abnormal 5-HT metabolism does occur in the disease. Jerome *et al.*[12] found reduced urinary excretion of 5-HIAA in mongols, and Jerome[13] and Tissot *et al.*[14] confirmed this finding. More recently, Airaksinen[15] reported increased 5-HIAA excretion. O'Brien and Groshek[16] gave a tryptophan load to mongol subjects and saw a diminished excretion of 5-HIAA and seven other tryptophan metabolites in the urine. These earlier studies cannot be taken as proof of any specific defect in 5-HT metabolism, because they would equally well be due to a shunt in the metabolic pathway of tryptophan away from tryptamine derivatives in the direction of kynurenine and other metabolites.

On the basis of the results of our ealier work,[1,2] and the current experiments, mongol platelets have the following abnormalities: Defective 5-HT uptake and abnormal binding due to a deficiency of ATP, resulting in efflux of 5-HT from platelets at a high rate, and low platelet monoamine oxidase activity.

With respect to the significance of these results in relation to the etiology of mongolism there is at present no evidence of any brain defects in relation to 5-HT synthesis or metabolism. Nevertheless, as the platelet may serve as a model for serotonergic and dopaminergic neurones[2,17–19] there is the possibility that there may be defective 5-HT synthesis and metabolism in brain neurones in the disease. We must, however, exercise caution in this regard since normal platelet MAO is electrophoretically homogeneous and has different substrate specificities from the heterogeneous isoenzymes found in human brain.[20] It may well be, however, that the mongol platelet MAO is indeed not homogeneous and has different substrate specificities from the normal enzyme (this alone could explain the results we describe in this paper). Other tissue MAOs may be similarly abnormal. All of these points require further work to obtain a definitive answer.

Note added in proof. Airaksinen and Airaksinen[21] have recently shown that tetrabenazine also does not affect the efflux of 5-HT from platelets of adults with Down's syndrome.

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