Determination of Reference Values for Serotonin Concentration in Platelets of Healthy Newborns, Children, Adults, and Elderly Subjects by HPLC with Electrochemical Detection

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We adapted a high-performance liquid chromatographic method with electrochemical detection (Clin Chim Acta 1984;139:1-12) to the determination of platelet-serotonin. We used this method to determine platelet serotonin reference values in a healthy population, measuring platelet serotonin concentration in the following subjects: 31 newborns (16 girls, 15 boys); 41 children (11 girls, 30 boys), ages 20 months to 15 years; 56 adults (26 women, 30 men), ages 20 to 58 years; and 20 elderly subjects (16 women, four men), ages 65 to 94 years. There was no significant difference in platelet serotonin concentration between sexes in each age group. However, significant changes (P < 0.001) were observed between the newborns (mean ± SD: 1.67 ± 0.74 nmol/10^8 platelets) and the children (4.09 ± 1.04) or the adults (3.81 ± 0.87). Moreover, the platelet serotonin concentration in the elderly subjects (2.57 ± 1.12) was significantly (P < 0.001) lower than in the adults and children and significantly higher (P < 0.01) than in the newborns. Such age-related differences must be taken into consideration when data from neurological or psychiatric patients and control subjects are compared.

Additional Keyphrases: age-related effects \* neurotransmitter \* reference interval

Studies over the past 15 years have demonstrated that blood platelets can serve as a peripheral model for the central serotonin (5-hydroxytryptamine, 5-HT) presynaptic nerve terminals because the platelets accumulate, store, and release serotonin in a manner analogous to the central serotonergic synaptosomes (1, 2).

Because blood platelets can be easily obtained, the concentration of serotonin in platelets has been extensively measured as an indirect index of central serotonergic function in various psychiatric or neurological diseases, in basal conditions (3, 4), and during treatment with various drugs known for interfering with the central uptake or release of serotonin (5).

However, interpretation of the above studies can be difficult because of the lack of reference values for platelet serotonin concentration at various ages. The aim of this work was, therefore, to determine these values in a healthy population of newborns, children, adults, and elderly subjects of both sexes.

Materials and Methods

Instrumentation

Platelets in whole blood and in platelet-rich plasma (PRP) were counted with an HPC 52 platelets counter (HyceI, Rennes, France).

The chromatographic system is described in Gagnieu et al. (6); however, we used a sample processor WISP 712, instead of a U6K injector (both from Waters Associates, Milford, MA).

Reagents

The mobile phase consisted of sodium acetate buffer (0.15 mol/L, pH 4.0), containing 150 mL of methanol and 0.5 mmol of EDTA per liter. The buffer was filtered through a 0.45-μm (pore size) membrane filter (Millipore, Bedford, MA) and degassed.

The acetic acid and sodium acetate used to prepare the mobile phase were both Normapur grade, from Prolabo (Paris, France); chromatographic-grade methanol was from Merck (Darmstadt, F.R.G.).

A reference stock solution (400 μmol/L) of 5-HT (Sigma Chemical Co., St. Louis, MO) was prepared in distilled de-ionized water and stored at 4°C for as long as six months.

On the day of the experiment, a standard solution of 5-HT, 1 μmol/L, was prepared by diluting the stock solution in distilled water.

Samples

Sample collection: Except for the newborns who had their umbilical cord blood withdrawn at the time of the birth, blood was collected by venipuncture, between 0900 and

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1000 h, into plastic tubes containing 12 mg of EDTA as anticoagulant. Samples were kept at room temperature and centrifuged to obtain PRP as soon as possible (always within 2 h after the puncture). No special diet was observed by the patients, and all were free of drugs known to interfere with 5-HT metabolism or determination.

Sample preparation: After the platelet count in whole blood, the samples were centrifuged for 15 min at 120 × g at room temperature (in a Jouan CT 411 centrifuge) to obtain PRP. We removed 1.250 mL of PRP from each tube. We counted the platelets in 0.05 mL of this and centrifuged the remaining 1.2 mL for 20 min at 1100 × g at room temperature to obtain the platelet pellet, which was frozen at −70 °C. These pellets can be kept for at least three weeks under these conditions without any alteration of 5-HT content.

At the time of the experiment, frozen samples were thawed at room temperature and 1 mL of distilled water was added to each tube plus 100 μL of 70 g/L perchloric acid solution to deproteinize the samples. We then added, without delay, 20 μL of 5 mol/L NaOH solution to adjust the pH to 5.0 because 5-HT is not stable in a more acidic phase. We centrifuged these samples (1100 × g, 20 min) at 4 °C, and poured off the clear supernates into storage tubes for frozen storage for at least 1 h before analysis.

Measurement of 5-HT

To determine 5-HT by HPLC, we injected 20 μL of clear supernate (see above), a new sample every 10 min. The flow rate was 1.2 mL/min, the working potential 0.75 V, and the detector sensitivity 20 nA/V. The recorder chart speed was 5.0 mm/min. Under these conditions, retention time for 5-HT was about 3.25 min. All samples were injected twice into the HPLC system, and a 1 μmol/L standard solution (processed the same as the PRP samples with HClO4 and NaOH) was injected every 10 samples.

Results
Determination of 5-HT

Figure 1 shows typical chromatograms for a standard solution and a platelet sample processed as described above (i.e., including two injections per sample). The chromatographic peaks of the samples were quantified by peak-height measurement and compared with the peak height for the standard solution. To verify the identity of the serotonin peak, we compared the retention times of the peak found for platelet samples with the peak given by the standard solution. Further, we mixed equal volumes of standard solution and various platelet samples: the chromatograms of the mixtures always showed a single peak with a retention time identical to the retention time of 5-HT from the standard solution. Moreover, the peak heights were modified as expected from the proportions of the two original relative concentrations.

To study the possible interference of compounds related to monoamine metabolism and possibly present in platelets, we successively added each of these compounds to the standard solution; this allowed the determination of their retention times (Figure 2). Thus, under our conditions, it appears unlikely that a significant interference would occur between 5-HT and any of the tested compounds.

The main characteristics of the method used for 5-HT determination are as follows: The response varied linearly with sample concentrations ranging from 0.05 to 8.00 μmol/L. The detection limit at 20 nA/V was 0.01 μmol/L (i.e., for a mean platelet count of 270 × 10^9/μL, this was 0.03 nmol/10^9 platelets). The within-day precision was determined by injecting 20 times a standard solution (mean ± SD: 0.990 ± 0.008 μmol/L, CV = 0.80%), and 13 times a platelet sample (1.55 ± 0.02 μmol/L, CV = 1.24%). The day-to-day precision was obtained by injecting daily (a) a freshly prepared standard solution for 13 days (0.990 ± 0.020 μmol/L, CV = 2.02%), and (b) platelet samples prepared on the same day from the same blood, frozen at −70 °C and thawed just before the assays, for 10 days (1.19 ± 0.035 μmol/L, CV = 2.80%). The accuracy of the method was tested as follows: In each series of determinations, we injected into the HPLC system two platelet samples with a serotonin concentration ("low" and "high") known from a previous series of experiments; we then re-calculated the values of these two samples by comparison with the standard solution of the current experiment. The results obtained for these control platelet samples never differed from the theoretical values by more than 2% ("high" sample) or 3% ("low" sample).

Determination of Reference Values for Platelet Serotonin

In newborns, we determined platelet serotonin concentrations in cord blood at birth for 16 girls and 15 boys who were healthy according to the standard criteria used in the Maternity Unit (Pr. Thoulon, Hôtel-Dieu Hospital, Lyon): between 37 and 42 weeks of gestation, and normal height, weight, and neurological examination. The data, which showed no sex-related difference, were significantly lower for the newborns than for the children, adults, and elderly subjects (Figure 3).
The data for children were obtained from 11 girls and 30 boys, ages 20 months to 15 years (mean 9.2 years). These subjects were hospitalized (Neurology Unit, Dr. de Villard, Neurological Hospital, Lyon), but were not suffering from any psychotic disorder and had normal intellectual development. As in the newborn group, there was no sex-related difference. The values obtained for this group were significantly (P < 0.001) higher than those obtained for the newborns and were similar to those of the adults (Figure 3).

The adults group was represented by 56 apparently healthy subjects (26 women and 30 men), ages 20 to 58 years (mean 28.9 years)—hospital staff, technicians, and students.

The elderly group comprised 20 nonhospitalized subjects who came to the Lyon Charpennes Hospital (Gerontology Unit, Dr. Chapuis) for a physical examination and who were free of neurological or psychiatric disease. This group consisted of 16 women and four men, ages 65 to 94 years (mean 81.3 years), none of whom were taking treatment known to interfere with 5-HT metabolism.

Platelet counts were performed on all the blood samples. There was no significant difference between the various age groups, except for the children.

Reference ranges suggested for 5-HT in platelets are as follows (mean ± SD):

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>5-HT concn, nmol/10⁶ platelets</th>
<th>Measured platelets, 10⁶/μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborns</td>
<td>31</td>
<td>1.67 ± 0.74</td>
<td>267 ± 70</td>
</tr>
<tr>
<td>Children</td>
<td>41</td>
<td>4.09 ± 1.04</td>
<td>322 ± 90*</td>
</tr>
<tr>
<td>Adults</td>
<td>56</td>
<td>3.81 ± 0.87</td>
<td>286 ± 79</td>
</tr>
<tr>
<td>Elderly</td>
<td>20</td>
<td>2.57 ± 1.12</td>
<td>243 ± 55</td>
</tr>
</tbody>
</table>

* Significantly different (P < 0.01) from all other groups.

Discussion

The technique used to determine 5-HT was derived from a method previously developed in our laboratory for determining acidic monoamine metabolites in human cerebrospinal fluid (6). We made only two changes in the original technique: the molarity of the acetate buffer in the mobile phase was decreased by 10-fold to avoid crystallization in the circuitry, and the polarity of the mobile phase was increased by using 150 mL of methanol per liter (instead of ethanol, 15 mL/L) to better resolve the 5-HT peak from the solvent front.

In making this study to determine the reference values for platelet serotonin concentration at various ages in healthy subjects, at least two sources of bias had to be avoided. First, we had to consider the possibility of diurnal or seasonal rhythms. A diurnal rhythm for serotonin in blood and (or) platelets has been found by some authors (3, 4) but not by others (7, 8); a recent study (9) involving HPLC, a technique more specific than the nonchromatographic methods previously used, found diurnal change in the concentration of 5-HT in blood. Nevertheless, except for newborns, the blood sampling in our study was always performed at the same time each day, between 0900 and 1000 h. Badcock et al. (9) did suggest there might be small seasonal variations when winter values were compared with summer values. However, our study was performed between November and May, a period that makes unlikely a significant influence of this possible seasonal rhythm. Another source of bias in this type of study is the possible influence of diet. In the present study, the patients followed no special diet because previous reports have demonstrated no "meal effect" (9, 10) on the concentrations of 5-HT in blood.

A general result of importance in our study is the lack of male–female differences for platelet 5-HT concentration over a large number of individuals at various ages. This is at variance with some reports (11, 12) but agrees with most of them (9, 13, 14). The practical consequence of this finding is that, for forthcoming studies, patients of either sex can be pooled within the same age group.

Among the results obtained for the various groups, platelet 5-HT was lowest for the newborns. Because it is...
widely accepted that cord blood is a source of pure fetal blood and is not contaminated by maternal blood (15), our results are likely to reflect a low concentration of platelet 5-HT in the newborns. The values we observed representing less than half the values observed for adults, are in agreement with the findings of Tu and Wong (16), who reported that the mean concentration of fetal 5-HT was about half that of the maternal value. Such a difference may be caused by a very active 5-HT catabolism in utero, as suggested by the high concentration of its metabolite 5-hydroxyindoleacetic acid in amniotic fluid (16).

The results obtained for the children are not significantly different from those of the adults, and both are in agreement with the values reported in the literature (9, 14, 17, 18).

The elderly subjects have concentrations of 5-HT in platelets significantly lower than those found for children and adults. Such a decrease with age has previously been reported (18, 19) and may originate partly, at least for women, from hormonal suppression; Guicheney (18) found platelet 5-HT contents to be correlated with plasma estrogen concentrations.

In conclusion, the differences we report for 5-HT concentration in platelets as a function of age should be taken into account before interpreting pathophysiological or pharmacological changes observed in patients of various ages.

References