We used a specific and sensitive radioenzymatic method to establish a reference interval for the concentration of serotonin in platelet-poor plasma in 98 healthy volunteers (49 men, 49 women). The interval was 0–11 nmol/L with a median of 2.8 nmol/L. No difference in concentration in relation to sex or age was observed. In a group of eight very old volunteers (ages 86–92 years), however, concentrations were increased. In addition, we monitored the plasma concentrations of serotonin in 20 healthy women (ages 26–45 years) through two menstrual cycles. Periovulatory and premenstrual concentrations were greater than the serotonin concentration at the start of menstruation.

Additional Keyphrases: reference interval · radioenzymatic assay · variation, source of · geriatric chemistry

Recent data on the concentration of serotonin (5-hydroxytryptamine, 5-HT) in platelet-poor plasma (PPP) are indicative of the existence of extraplatelet 5-HT in the low nanomolar range (1–6). The equilibrium between intra- and extraplatelet 5-HT is a function of platelet activity and the active reuptake of 5-HT by platelets at any given moment. Acute changes in this equilibrium are not reflected in whole-blood 5-HT because the intraplatelet concentration of 5-HT is by far greater. The intraplatelet 5-HT concentration has been taken as a marker of central serotonergic activity (7). Whether free plasma 5-HT may be used to estimate changes in central 5-HT activity remains to be clarified, but recent data suggest such a relationship (8). Sarrias et al. (9) found low plasma 5-HT in melancholic patients, compared with results for control subjects, and a significant decrease in platelet 5-HT on clomipramine treatment, whereas plasma 5-HT remained unchanged. This points to a distinct pool of plasma 5-HT independent of the platelet pool, and Sarrias et al. suggest the use of plasma 5-HT as a peripheral indicator of abnormal serotonergic function in melancholia and possibly in other diseases.

Moreover, the last decade has brought new insight into the cardiovascular effects of 5-HT, with the characterization of distinct receptors for 5-HT in the blood vessel wall (10). Serotonin seems to play a pivotal role in the regulation of coronary vascular tone and, locally, free 5-HT interacts with a still increasing range of vasoactive "hormones" in health and disease (11). In view of this, a reference interval for 5-HT would be of interest.

Earlier, we reported on the use of a specific and sensitive radioenzymatic assay to investigate platelet activation in different stressful situations. We found an increase of 5-HT in PPP during and after major surgery (12), after dynamic exercise (13), and after psychological stress (14).

We now report on a reference interval for 5-HT in PPP, established in 98 healthy volunteers. In addition, we determined the serotonin concentration in PPP in a group of very old, healthy volunteers. We also found that PPP serotonin increases during the menstrual cycle, as studied in 19 women without premenstrual syndrome.

Materials and Methods

Subjects

The reference group (49 men and 49 women) consisted of healthy volunteers from the laboratory staff, otherwise healthy patients scheduled for elective surgery, and healthy volunteers entering studies on physical and psychological stress. All were nonmedicated, had been fasting overnight, and had been resting in the supine position for ≥0.5 h before the blood sampling.

Eight healthy elderly men and women (ages 86–92 years, four men and four women) fasted overnight and rested in the supine position until the blood sampling. No medication was taken for 10 days before the blood sampling.

Twenty women (ages 26–45 years), not taking contraceptives or any other medication, recorded daily basal body temperature and menstrual flow pattern before the study period. During the following two menstrual cycles, blood samples were obtained on the first day of menstruation, during ovulation, and premenstrually, i.e., as close as possible to the next menstruation. Blood sampling was done after a light morning meal only, without tea or coffee, and after 15 min of rest in the sitting position.

All patients and volunteers had given informed consent according to the Helsinki Declaration II.

Procedures

Blood was drawn in the morning from a cubital vein without stasis, with an 18-gauge needle, and with free flow. The first 5 mL of blood was discarded, and 9 mL of blood was collected in a prechilled polypropylene tube containing 1 mL of a 0.12 mol/L solution of citrate. Platelet-rich plasma was prepared by centrifugation for 15 min at 180 × g and then further centrifuged in a second polypropylene tube for 20 min at 2000 × g to yield PPP. Without shaking the tube, we transferred about half of the volume from the intermediate part of the plasma (thus avoiding the small platelets in the
upper part) with a polyethylene pipette to a microvial. The absence of platelets was verified by cell counting and the PPP was kept at −20 °C until analysis.

The concentration of 5-HT was determined with a radioenzymatic method (15). The method involved acetylation of the sample with acetic acid anhydride in acetone and further methylation with S-adenosyl-L-[3H]methylmethionine and hydroxymethylene-O-methyl transferase. The [3H]melatonin formed was extracted with toluene, and the radioactivity determined after washing and re-extracting the toluene extract. The samples were analyzed in triplicate with internal calibrator. The detection limit was 0.9 nmol/L, and the inter- and intra-assay CVs were 11% and 5.4%, respectively. The accuracy was evaluated by adding to a PPP sample known amounts of 5-HT, up to 250 nmol/L; there was a linear correlation between y = counts/min and x = 5-HT concentration: y = 85.2x + 697 (r = 0.999) (15).

The results were evaluated by using nonparametric statistics (two-sided tests).

Results

Of the 98 PPP samples, 8 had serotonin concentrations below the detection limit of the assay (0.9 nmol/L); the highest concentration was 17.3 nmol/L. The distribution of concentrations was skewed (Figure 1), with a median of 2.8 nmol/L and an upper 0.975-fractile of 11.0 nmol/L. Of the 98 samples, there were no significant differences with respect to sex or age. The concentrations thus constitute a reference interval of 0−11 nmol/L. The individual concentrations for men and women divided into three age groups are given in Figure 2. The concentrations and the medians (6.2 nmol/L) for the eight elderly volunteers are also shown in Figure 2.

Of the 20 women followed through two menstrual cycles, results for one were excluded because of sampling error. Figure 3 depicts for the remaining 19 women the individual concentrations and medians at the three measurement points for each of the two cycles. The premenstrual concentration of 5-HT in both cycles was significantly greater than on the first day of menstruation (P ≤0.01 in cycle A and P ≤0.05 in cycle B). In cycle B, the concentration during ovulation was also significantly greater (P ≤0.05) than on the first day of menstruation.

Discussion

The reference interval for 5-HT in PPP agrees with the lower concentrations reported in the literature. With respect to sex differences, however, the results in the literature are conflicting. Picard et al. (2) found lower concentrations for women than for men (10.4 vs 15.4 nmol/L, each a mean of 10 subjects), whereas Ortiz et al. (6), in much larger groups (83 men, 92 women), found higher concentrations for women than for men. In our groups, we saw no significant difference in concentration due to sex, neither in the total reference group nor in any of the three age groups.
Within the reference group, we found no correlation with age. However, increased concentrations were found in the eight elderly volunteers. This is in accordance with an increased platelet activity in old people, as evidenced by an increased epinephrine-induced cell aggregation, a decrease in intraplatelet cyclic ADP content, and increased concentrations of β-thromboglobulin and thromboxane in plasma (16-18).

The increase in 5-HT concentration in PPP through two menstrual cycles agrees with a tendency towards an increase in blood found by Rapkin et al. (19). The higher concentrations found in the 19 women than in our reference interval may be ascribed to the difference around the blood sampling: a light morning meal and 15 min in the sitting position vs fasting overnight and 30 min in the supine position before blood sampling.

Our study thus confirms the existence in humans of 5-HT in the low nanomolar range in plasma. The variation of plasma concentrations of 5-HT during the menstrual cycle must be taken into account in future studies.

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References

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Nonimmunological Assay of Urinary Albumin Based on Laser-Induced Fluorescence

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We describe the first nonimmunological assay of albumin in urine with a detection limit of 1 mg/L. The method is simple, rapid, and accurate. It is based on the probe Albumin Blue 670, which becomes highly fluorescent on binding to albumin. An inexpensive diode laser was used as the light source for measurement of laser-induced fluorescence. The assay was coupled to a flow-injection analysis system capable of running 20 samples per hour.

The working range was 1–100 mg/L, which covered albumin concentrations found in nonpathological urine and in urine with slightly increased albumin. This range makes prediction of nephropathy possible at an early stage. Other serum proteins and hemoglobin do not interfere. The coefficients of variation were <4% and <7% within one day and from day to day, respectively. A correlation coefficient of 0.990 (n = 100) was obtained for comparison with the Behring nephelometric assay.

Additional Keyphrases: diabetes · flow-injection analysis · lasers · urine

Determination of albumin in urine has gained importance during the last few years because many diseases have been associated with an increased albumin excre-