5-Hydroxytryptamine Kinetics and Activation of Blood Platelets in Patients With Essential Hypertension

Natalia Fetkovska, Ruth Amstein, Fabrizia Ferracin, Martin Regenass, Fritz R. Bühler, and Alfred Pletscher

To investigate possible alterations in 5-hydroxytryptamine (5HT) kinetics and sensitivity of blood platelets in patients with essential hypertension, 45 essential hypertensive patients and 45 normotensive healthy subjects matched in pairs for age, sex, and smoking status were compared. There were 18 women and 27 men in each group, ranging from 30 to 73 years of age. Results of essential hypertensive patients differed in several ways from those of normotensive subjects. In essential hypertensive patients, maximal 5HT uptake velocity (Vmax) decreased with increasing blood pressure and age and was reduced the most in older men. Vmax was positively related to the EC50 of 5HT for inducing a shape change reaction. In essential hypertensive patients, both Vmax of 5HT uptake and the EC50 of 5HT for shape change showed positive correlations with the 5HT content in platelets; the former relation was different between the essential hypertensive and normotensive groups (F=5.53; p=0.02). These results indicate reduced uptake of 5HT by blood platelets and suggest enhanced 5HT plasma concentrations in local areas, especially vascular lesions in essential hypertensive patients. Increased periplatelet concentrations of 5HT may lead to preactivation of platelets and possibly stimulation of vascular smooth muscle via their 5HT2-receptors. These changes are likely to be involved in the pathogenesis of increased thromboembolic complications in essential hypertensive patients, particularly in older men. (Hypertension 1990;15:267-273)

Blood platelets have a specific uptake mechanism for 5-hydroxytryptamine (5HT) (serotonin) at the plasma membrane, intracellular amine storage organelles (dense bodies), and an amine metabolizing enzyme (monoamine oxidase) as well as 5HT2-receptors. Stimulation of the latter causes platelet activation, rise of intracellular messengers (e.g., inositoltrisphosphate and calcium) leading to shape change reaction and eventually aggregation. In normotensive (NT) healthy subjects, platelet activation by 5HT and 5HT kinetics has been shown to depend on age and sex.1 The observed age-dependent increase in platelet reactivity to 5HT in healthy men tallies with their higher cardiovascular thromboembolic complication rates. Enhanced 5HT-stimulated vasoconstriction, including that induced via endothelium-derived contracting factors,2-4 and increased platelet aggregation are likely to contribute to the development of high blood pressure as well as to the greater thromboembolic risk of essential hypertensive (EHT) patients.

So far, results of platelet studies in EHT patients are inconsistent. Usually, two or three functions were measured, but 5HT kinetics and platelet responses were not assessed in a comprehensive way. Thus, in EHT patients 5HT uptake5-8 and 5HT content in platelets were found to be decreased,7,9,10 and the release of 5HT was shown to be increased in some studies.10-12 Other investigations showed no statistically significant differences between EHT patients and NT subjects relative to 5HT uptake13,14 or 5HT content.5,6,11,13,15 Such discrepancies may be explained by differences in the patient populations (e.g., age, sex, and smoking habits). Moreover, results relating 5HT kinetics to platelet sensitivity in EHT patients are scant.

The present study investigates the possible role of the platelet 5HT system with regard to 5HT kinetics and 5HT sensitivity in the EHT and NT groups; the groups were strictly matched for age, sex, and smoking status. Special attention was paid to possible age- and sex-related influences.

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Received May 8, 1989; accepted in revised form September 21, 1989.
Methods

Subjects

The EHT patient group (World Health Organization functional class I and II) had a mean blood pressure of 165.5±18.9/106.4±11.0 mm Hg and consisted of 45 participants, 27 men and 18 women, who were from 30 to 73 years of age. Patients who had previously been treated with antihypertensive drugs received a placebo for a period of at least 4 weeks before study. Patients were matched in pairs according to sex, age, and smoking status. Patients that had been treated with antiplatelet agents during the previous year were matched in pairs according to sex, age, and smoking status with 45 NT (blood pressure ≤145/85 mm Hg) healthy volunteers. Because there were only five pairs of smokers, this aspect was not pursued in the analysis. Only subjects without a history of treatment with antiplatelet agents during the previous year were recruited. All subjects were asked to avoid products rich in tryptophan (e.g., nuts, tomatoes, bananas, chocolate, vanilla) for a minimum of 48 hours before study and to refrain from drugs for at least 2 weeks. All subjects gave their informed consent. Blood samples were taken at 8:00 AM on the same day, after the subjects had fasted overnight with the women between the 10th and 18th day of the menstrual cycle, if present. A 24-hour urine collection was made on the day before study for the determination of 5-hydroxyindoleacetic acid (5HIAA) excretion rates.

Processing of Samples

Blood from the antecubital vein was mixed with 3.8% sodium citrate (9:1) and immediately centrifuged (at room temperature for 10 minutes at 160g) to obtain platelet rich plasma (PRP). Part of the PRP was taken for studies of platelet aggregation and shape change reaction; the remainder was further centrifuged with Na2EDTA (final concentration 3 mM) at room temperature for another 10 minutes at 600g to obtain platelet poor plasma (PPP) and a platelet pellet. Part of the PPP was used to adjust the platelet number for the aggregation and shape change studies, and the remainder was centrifuged at 10,000g for 5 minutes to obtain platelet free plasma (PFP), which served as a reference in aggregation studies. The platelet pellet was resuspended in calcium-free tyrode buffer containing 3 mM EGTA and centrifuged at room temperature for 10 minutes at 600g. The sediment was resuspended to give a stock suspension of 108 platelets/ml, which was used to determine 5HT content, 5HT uptake, and 5HT release.

For the determination of β-thromboglobulin (β-TG), 4 ml blood was drawn into an ice-cold syringe containing 1 ml anticoagulant solution (A.C.D., EDTA, adenosine, prostaglandin E1). PPP and PFP were obtained as described above; the latter was stored at −70° C (up to a maximum of 3 months) for β-TG measurement.

Samples of 24-hour urine collection (collected from 7:00 AM onward into 10 ml 6N HCl) were passed through a purification filter containing silicagel RP C18.46 The eluate was injected into a high-performance liquid chromatography (HPLC) system for the determination of 5HIAA.

Measurements

Shape change reaction of platelets to 5HT (10−8 to 5×10−7 M) was measured as previously described.17 Results are expressed as the concentration inducing half of the maximal shape change response (EC50).

Platelet aggregation was performed according to Born and Cross with slight modifications.19 Thus, platelet number was adjusted to 2.5×109/ml with PPP, and tris-buffer was not added because of its interference with platelet function.20 Platelet response induced by 5 μM 5HT was expressed as the slope of aggregation (% change of light transmission/min).

To assess platelet turnover, β-TG in PFP was determined with a commercial radioimmunoassay kit (The Radiochemical Center, Amersham, UK) according to the method of Ludlam et al.21 To exclude artifacts caused by ex vivo platelet destruction, β-TG was not evaluated in those samples with platelet factor 4 (PF4) more than 10 ng/ml.22 PF4 was determined by radioimmunoassay (Abbott Labs., Chicago, Illinois).

[14C]5HT uptake was measured in samples of platelet stock suspension (50 μl) diluted with 450 μl buffer containing various concentrations of [14C]5HT (10−7 to 10−3 M). After 2 minutes of incubation at 37° C, the reaction was stopped by addition of 3 ml ice-cold buffer, and samples were immediately filtered under vacuum through Whatman GF/C glass fiber filters. The latter were then rinsed twice with 2×3 ml ice-cold buffer and transferred to plastic vials. The radioactivity on the filters was extracted with 10 ml scintillation fluid (Quickszint 212, Zinsser Analytic, Frankfurt, FRG) and counted by liquid scintillation spectrometry. Nonspecific uptake was determined by parallel incubation with 10 μM paroxetine. Maximal uptake velocities (Vmax) and affinity constants (Kd) were calculated as uptake parameters according to Michaelis-Menten kinetics.

5HT content in platelets was determined by a slight modification of the method of Honegger et al.23 A solvent delivery pump (LC 410, Kontron Analytic, Zürich, Switzerland) was used in conjunction with a cooled (2° C) automatic sampler (655A-40, Merck-Hitachi, Zürich, Switzerland). Pulseless solvent delivery was achieved with two membrane-type dampers (Portmann, Tharwil, Switzerland). The column system consisted of a New Guard RP-18 and a 22 cm analytical cartridge RP-18 Spheri-5 with 4.6 mm i.d. (G18-013 and OD-224, Brownlee Labs., Santa Clara, California). The electrochemical detector (656 with 641 VA Detector, Metrohm, Herisau, Switzerland) with a glassy carbon electrode was set at a potential of 0.8 V (vs. Ag/AgCl reference electrode) and a sensitivity of 5 nA. To cut off the first peak a Gynkotek Column Switching Module (Labomatik, Allschwil, Switzerland) was inserted between the column and the detector. This whole system was controlled by an integrator (HP 3390 A, Hewlett-Packard, Avondale, Pennsylvania) and an intermedi-
Statistical Analyses were obtained from commercial sources.

UK); and paroxetine was a gift from Hoffmann-La Roche AG (Basel, Switzerland). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, Missouri); [14C]5HT from Amersham Int. (Amersham, Bucks, England); 5HICA. After centrifugation, the supernatants were injected into the HPLC system. The minimum amounts of 5HT that could be measured were 20–40 pg, and the within-day variation was less than 5%.

Spontaneous release of 5HT was measured by using platelets suspended in calcium-free tyrode buffer (10^(-3) M) containing 5 μM paroxetine as 5HT uptake inhibitor. Platelets were incubated at 37°C for 5 or 60 minutes. After incubation, samples were cooled in ice and centrifuged in a Heraeus swing-out centrifuge (10 minutes, 4°C, 9,000g). Twenty microliters of supernatant was injected into the HPLC system. The minimum amounts of 5HT that could be measured were 20–40 pg, and the within-day variation was less than 5%.

TABLE 1. Comparison of Platelet 5-Hydroxytryptamine Kinetics and Platelet Function in Normotensive and Essential Hypertensive Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NT</th>
<th>EHT</th>
<th>Difference</th>
<th>Significance</th>
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<tbody>
<tr>
<td><strong>Platelet</strong></td>
<td></td>
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<tr>
<td>Shape change: 5HT (EC_{50} nM 5HT)</td>
<td>98.10±7.7</td>
<td>105.70±8.8</td>
<td>7.60±8.19</td>
<td>p=0.36</td>
</tr>
<tr>
<td>Aggregation: 5HT (Δ% transm/min)</td>
<td>9.40±0.9</td>
<td>10.94±1.0</td>
<td>1.5±1.22</td>
<td>p=0.20</td>
</tr>
<tr>
<td>5HT uptake: V_{max} (pmol/10^10 pl/min)</td>
<td>60.00±2.1</td>
<td>58.40±2.3</td>
<td>-1.61±2.13</td>
<td>p=0.45</td>
</tr>
<tr>
<td>5HT content (nmol 5HT/10^9 pl)</td>
<td>3.50±0.2</td>
<td>3.36±0.2</td>
<td>-0.14±0.232</td>
<td>p=0.54</td>
</tr>
<tr>
<td>5HT release (nmol 5HT from 10^9 pl/h)</td>
<td>0.14±0.03</td>
<td>0.16±0.02</td>
<td>0.013±0.036</td>
<td>p=0.72</td>
</tr>
<tr>
<td>n (×10^10/ml PRP)</td>
<td>3.16±0.11</td>
<td>3.09±0.14</td>
<td>-0.06±0.152</td>
<td>p=0.66</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
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</tr>
<tr>
<td>β-TG (ng/ml)</td>
<td>14.75±1.15</td>
<td>14.38±1.03</td>
<td>-0.368±1.378</td>
<td>p=0.79</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5HIAA (μmol/24 hrs)</td>
<td>13.72±0.75</td>
<td>13.52±0.76</td>
<td>-0.198±0.868</td>
<td>p=0.82</td>
</tr>
</tbody>
</table>

Values are mean±SEM. NT, normotensive subjects; EHT, essential hypertensive patients; 5HT, 5-hydroxytryptamine; EC_{50}, concentration inducing half the maximal shape change response; V_{max} maximal uptake velocity; PRP, platelet rich plasma; β-TG, β-thromboglobulin; 5HIAA, 5-hydroxyindoleacetic acid.

For univariate analyses, Student's test for paired samples (statistical siblings with respect to age, sex, and smoking status) and, as a more conservative approach, Student's t test for unpaired samples were applied; levels of significance 2 α=0.05. Results are expressed as mean±SEM.

For multivariate analyses, a linear regression model testing the equality of regression coefficients between NT subjects and EHT patients applied to the matched pairs to detect differences between NT subjects and EHT patients was used.

**Results**

There were no overall differences in platelets of EHT patients and NT subjects regarding 5HT-induced shape change and aggregation, 5HT uptake, 5HT content, and 5HT release, or in β-TG concentrations in PFP as well as in 24-hour urinary excretion of 5HIAA (Table 1) when univariate analyses were applied. Several relations were particular for platelets from EHT patients, and in multivariate analyses there were significant differences between EHT patients and NT subjects. They can be described as follows: V_{max} showed a positive correlation with the platelet 5HT content (n=45, r=0.588, p<0.001) (Figure 1) and this held true for both men (n=27, r=0.536, p<0.01) and women (n=18, r=0.622, p<0.01). Regression parameters of EHT patients and NT subjects for V_{max} in relation to 5HT content were different (F=5.53, df=1,42, p=0.02). Logistic regression showed an interaction between V_{max} and 5HT content only in EHT patients with either simultaneously high or low values of these parameters (p=0.03).

V_{max} of 5HT uptake was negatively correlated with both diastolic (n=45, r=-0.378, p<0.01) (Figure 2A) and mean blood pressure (n=45, r=-0.338, p<0.05), but K_{in} did not correlate with blood pressure.

In the whole patient group, V_{max} but not K_{in} decreased with age (n=45, r=0.309, p<0.05) (Figure 2B), and this was mainly due to an age-related decrease observed in men (n=27, r=0.526, p<0.005).
Scatterplots showing correlation between platelet 5-hydroxytryptamine (5HT) content and maximal uptake velocity ($V_{\text{max}}$) of 5HT in (•) essential hypertensive patients ($n=45$, $r=0.588$, $p<0.001$) and in (○) normotensive control subjects ($n=45$, $r=0.098$, $p=0.52$).

No such correlation was found for NT subjects ($n=45$, $r=0.052$, $p=0.73$).

$V_{\text{max}}$ was lower in EHT men older than 55 ($n=13$) than in those younger than 55 years of age ($n=14$; $48.76\pm3.34$ vs. $61.17\pm3.59$ pmol 5HT/10$^8$ platelets/min, $p<0.05$). In women, these age differences did not exist ($63.45\pm6.14$, $n=8$, vs. $62.84\pm4.56$ pmol 5HT/10$^8$ platelets/min, $n=10$). In men, differences were even more pronounced when, for example, age groups less than 50 and more than 60 years of age were compared. Thus, concentrations of 5HT (calculated from Michaelis-Menten equation) corresponding to mean $V_{\text{max}}$ values were $9.5\pm0.1\times10^{-7}$ M 5HT in EHT men older than 60 versus $3.8\pm0.1\times10^{-6}$ M 5HT in EHT men younger than 50 years ($p<0.001$) (Figure 3).

$V_{\text{max}}$ was positively correlated with the EC$_{50}$ of the 5HT-induced shape change reaction ($r=0.531$, $n=45$, $p<0.001$) (Figure 4). There was a difference between regression parameters of EHT patients and NT subjects for EC$_{50}$ of 5HT-induced shape change response in relation to $V_{\text{max}}$ ($F=7.43$, df=1,42, $p=0.009$).

The EC$_{50}$ of the 5HT-induced platelet shape change reaction showed a positive correlation with the platelet 5HT content ($n=45$, $r=0.700$, $p<0.001$) in EHT patients but not in NT subjects ($n=45$, $r=0.231$, $p=0.13$).

Discussion

There were no overall differences in platelet 5HT kinetics and 5HT sensitivity between the EHT and NT groups when univariate analyses were applied. Also, 5HT kinetics and 5HT dynamics depended on age and sex in platelets of EHT patients, similar to those of NT subjects. However, in the EHT group multivariate analyses showed particular features that were not present in the NT group. The most striking alterations present in platelets of EHT patients were a decrease of $V_{\text{max}}$ with higher blood pressure and age, especially in men, a dependency of the 5HT content of platelets on $V_{\text{max}}$, and a rise in platelet 5HT sensitivity with decreasing $V_{\text{max}}$.

Reduced $V_{\text{max}}$ of 5HT uptake indicates a deranged 5HT uptake mechanism, for example due to a reduction in the number of functional uptake sites (transporter molecules). In previous experiments, it has been claimed that an endogenous digitalislike plasma factor decreases 5HT uptake of platelets, yet a
FIGURE 3. As an example, graph showing 5-hydroxytryptamine (5HT) uptake in hypertensive (EHT) men younger than 50 years (n=9) and older than 60 years (n=10) is presented. Curves (calculated as mean±SEM from Michaelis-Menten equation for each group) represent the values of uptake velocities corresponding to different 5HT concentrations in medium. Uptake velocities (Vmax) differ significantly between the two groups at 5HT concentrations above $8 \times 10^{-7}$ M 5HT. Vmax, maximal uptake velocity in EHT men <50 years of age; Vmax, maximal uptake velocity in EHT men >60 years of age; Vp, uptake velocity at physiological 5HT concentrations; CΔ, mean 5HT concentration corresponding to maximal uptake in EHT men <50 years of age; CΔ, mean 5HT concentration corresponding to maximal uptake in EHT men >60 years of age. 5HT concentrations at which maximal uptake is reached differ significantly between the two groups *p<0.05, **p<0.02, ***p<0.001. Mean±SEM.

FIGURE 4. Scatterplots showing correlation between 5-hydroxytryptamine (5HT)-induced platelet shape change reaction and maximal uptake velocity (Vmax) in patients with essential hypertension (●) (n=45; r=0.531; p<0.001) and in normotensive control subjects (○) (n=45; r=0.143; p=0.35).

major influence of such a factor was unlikely as our experiments were performed in tyrode buffer. The small difference in platelet 5HT content does not appear to be a confounding factor as even complete depletion of platelet 5HT by reserpine does not affect Vmax.25 The present experiments rather indicate that the decrease of Vmax of 5HT with increasing blood pressure and age was due to alterations of the platelet plasma membrane.26 Thus, impaired Vmax of 5HT uptake may lead to an altered relation between platelet 5HT content and periplatelet 5HT plasma concentration. This is supported by the positive correlation between Vmax and the platelet 5HT content of EHT patients in our study as well as investigations with vervet monkeys where platelet 5HT content was found to be a function of the number of platelet 5HT uptake sites.27 The in vivo periplatelet 5HT plasma concentrations cannot be reliably measured, but it can be assumed that this concentration increases when Vmax is reduced (e.g., in older patients with high blood pressure). Elevated periplatelet 5HT concentrations, especially in local areas with vascular pathology in which intermittent release of 5HT from aggregating platelets occurs,28 may lead to platelet preactivation via 5HT stimulation of 5HT2-receptors at the plasma membrane. This may induce an increase in intracellular messengers (e.g., phosphatidylinositol metabolites and cytosolic free calcium concentration) causing sensitization of platelets to aggregating agents. 5HT concentrations as low as $10^{-9}$ M cause a marked amplification of the aggregatory effect of epinephrine.29 In EHT patients, platelet preactivation is also indicated by the direct relation found between cytosolic free calcium and blood pressure.30 The observed positive correlations between EC50 of 5HT for inducing a shape change reaction on the one hand and Vmax of 5HT uptake and 5HT content of platelets on the other hand confirm that the activation state of blood platelets increases with diminishing Vmax possibly as a conse-
quence of the previously mentioned pathogenetic mechanism. Taken together, a decrease of V_{max} associated with higher blood pressure, especially in older age, may be a determining factor for sensitizing platelets to aggregating agents.

There is no simple explanation for the group difference in V_{max} of 5HT uptake and platelet 5HT content between the EHT and NT groups. We hypothesize that in EHT patients the plasma concentrations of 5HT in vascular areas with endothelial damage or arteriosclerotic lesions may be relatively high owing to enhanced 5HT release from aggregating platelets. This has been documented in experiments with dogs in which high local concentrations of 5HT (about 20 times above normal) have been found in constricted areas of coronary arteries with endothelial damage. In other studies with dogs, plasma 5HT concentrations in the coronary sinus were found to exceed 2 × 10^{-6} M during acute thrombus formation in the coronary artery. These 5HT plasma concentrations are well within the range that corresponds to V_{max} of platelet 5HT uptake in EHT patients, especially when V_{max} is decreased (Figure 3). That part of 5HT taken up by platelets during their passage through such vascular areas would then mainly (or only) depend on V_{max} and the observed correlation between V_{max} and platelet 5HT content may be the consequence. Elevated plasma concentrations of 5HT in local vascular areas of EHT patients, especially in men of older age, could lead to platelet activation and vasoconstriction, and this may be further enhanced by damaged endothelium. Such local 5HT uptake inhibition does not appear to be reflected in intergroup differences of systemic 5HT turnover as 5HIAA excretion rates did not differ. Moreover, the similarity of plasma β-TG indicates that there were no differences in overall platelet turnover between the EHT and NT groups.

Platelets of healthy normotensive subjects are exposed to plasma 5HT concentrations that are far below those corresponding to V_{max} of 5HT uptake. Under these circumstances, the plasma 5HT concentration is probably a more important determinant of platelet 5HT content than V_{max}, and thus a correlation between V_{max} and platelet 5HT content may not become apparent.

In conclusion, impaired platelet 5HT uptake and increased local periplatelet plasma concentrations of 5HT in EHT patients may lead to platelet activation via stimulation of 5HT_{2}-receptors, and this could be involved in the pathophysiology of thromboembolic complications. The preferential decrease of V_{max} in hypertensive men of older age is in agreement with the particular high rate of cardiovascular complications in this patient group. Conceivably, enhanced plasma 5HT concentrations also contribute to elevated blood pressure via activation of vascular smooth muscle 5HT_{2}-receptors.

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**KEY WORDS** • essential hypertension • serotonin • kinetics • platelets • age • sex differences
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doi: 10.1161/01.HYP.15.3.267

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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