Decreased Uptake of $^3$H-Serotonin and Endogenous Content of Serotonin in Blood Platelets in Hypertensive Patients

LINDA A. KAMAL, KIM HANH LE QUAN-BUI, AND PHILIPPE MEYER

SUMMARY The uptake and content of serotonin in blood platelets were studied in patients with essential hypertension and in five families in which at least one member was hypertensive. Blood was obtained from male and female normotensive volunteers and hypertensive patients who were free of medication. Lineweaver-Burk plots of $^3$H-serotonin uptake from both control subjects and hypertensive patients were linear, which suggested simple Michaelis-Menten uptake kinetics. The maximal uptake velocity ($V_{\text{max}}$) in hypertensive patients was significantly lower than in control subjects (control = 41.7 ± 3.3 pmol/min/10⁸ platelets, $n = 17$; hypertensive = 26.6 ± 3.0 pmol/min/10⁸ platelets, $n = 16$; $p < 0.005$). The affinity constant ($K_m$) was slightly but significantly lower in hypertensive patients (control = 0.70 ± 0.08 μM; hypertensive = 0.46 ± 0.08 μM; $p < 0.05$). The serotonin content in blood platelets determined by high pressure liquid chromatography with electrochemical detection was significantly lower in hypertensive patients (control = 165.0 ± 12.9 nmol/10⁸ platelets, $n = 29$; hypertensive = 105.9 ± 10.4 nmol/10⁸ platelets, $n = 27$; $p < 0.001$). In the five families investigated, the lowered serotonin content was observed in some normotensive members. The reduced number of carriers of serotonin uptake and the slight decrease in the affinity constant observed in platelets of patients with essential hypertension suggest that serotonin metabolism is altered in essential hypertension and that blood platelets may be a useful model in studying the serotonergic modifications at the molecular level. (Hypertension 6: 568-573, 1984)

KEY WORDS • hypertension • serotonin • blood platelets • uptake mechanisms

There is increasing experimental evidence relating serotonin and blood pressure regulation. In the peripheral vessels, the vasoconstrictor effects of serotonin result in increased vascular resistance. In addition, serotonin can also accentuate the vasoconstricting effects of other substances.¹ Serotonin is present in the heart and blood vessels.² Blockade of serotonergic receptors in these structures by ketanserin antagonizes the vasoconstricting effects of serotonin and reduces blood pressure in hypertensive animals and humans.¹ In the central nervous system, the role of serotonin in regulating the blood pressure is less clear. Although serotonergic neurons are located in areas of the brain involved in cardiovascular regulation,³ pharmacological manipulation of these brain areas by either direct injections of serotonin⁴ or by intravenous injections of serotonergic agonists, antagonists, and inhibitors of synthesis⁶⁻¹⁰ has demonstrated that serotonergic neurons can both inhibit and facilitate central sympathetic activity.

The human blood platelet has been extensively studied as a model for central serotonin neurons.¹¹,¹² The mechanisms involved in the transport, storage, and release of serotonin in the blood platelet share similarities with those of central serotonergic neurons.¹³,¹⁴ The accessibility of the human blood platelet has made it an ideal model for central serotonin nerve functioning and has been studied in various neurological and psychiatric diseases.¹⁵ There is experimental evidence suggesting that in essential hypertension, mechanisms involved in the transport of serotonin in blood platelets may be modified.¹⁶,¹⁷

The aim of the present investigation was to study the uptake of $^3$H-serotonin and the endogenous serotonin level in blood platelets in normotensive and hypertensive subjects. In addition, since several studies have suggested that essential hypertension may be genetically transmitted,¹⁸⁻²⁰ the endogenous serotonin levels were measured in families in which at least one member was considered to have essential hypertension.

Material and Methods

The hypertensive subjects chosen for our study were considered as having essential hypertension based on family history and physical examination. The 16 sub-
jests (six men and 10 women) ranged in age from 22 to 45 years, with a mean age of 40.1 ± 3.1 years. All subjects had unrestricted dietary sodium intake. Blood pressure was recorded with a mercury manometer with the subjects seated. All had sustained hypertension, with a systolic blood pressure greater than 160 mm Hg and a diastolic blood pressure greater than 95 mm Hg. The diagnosis of essential hypertension was established after careful clinical investigation, which included intravenous pyelogram. None of the patients had plasma creatinine levels above 100 μmol/liter or marked alterations such as hemorrhage or papilledema of the retina. Daily urinary sodium excretion varied between 100 and 205 mmol/24 hr. Plasma renin activity ranged between 0.4 and 2.3 ng/ml/hr (normal values: 1.5 ± 0.5 ng/ml/hr).

Subjects were eliminated from the study if they presented cardiac or renal insufficiencies, or if they were taking contraceptives or aspirin. All subjects were free of antihypertensive medication for at least 2 weeks before the time of blood sampling.

The families studied were those of volunteers in which one member was diagnosed hypertensive, either a parent, spouse, child, or sibling. This group included 18 normotensive subjects (13 females and five males) and ranged in age from 12 to 64 years, with a mean age of 30.0 ± 3.9 years. The average blood pressure of these individuals was systolic 123.1 ± 6.3 and diastolic 73.4 ± 4.0 mm Hg.

Control subjects were members of our medical staff and consisted of 11 men and six women aged 19 to 42 years. Control subjects were considered normotensive on the basis of having no prior personal or family history of hypertension. The average blood pressure was systolic 124 ± 4.5 and diastolic 78 ± 2.0 mm Hg.

For the uptake study, approximately 20 ml of venous blood was drawn from each subject by a 19-gauge needle to which plastic tubing was attached. The blood (20 ml) was collected directly into plastic tubes containing 2 ml of anticoagulant (citric acid 2.73%, trisodium citrate 4.48%, and glucose 2%). After the blood was gently mixed with the anticoagulant, it was transferred to 5 ml plastic tubes and immediately centrifuged at 300 × g for 20 minutes. The pellet was separated from the supernatant and resuspended in 1 ml of 0.4 M perchloric acid and 10⁻³ M ascorbic acid. Samples were either stored at −20°C or analyzed directly. Before analysis, samples were recentrifuged at 5000 g, and the supernatant was removed.

The endogenous platelet serotonin level was determined by high performance liquid chromatography (HPLC) with electrochemical detection as previously described by Le Quan-Bui et al. The HPLC system (Waters, Milford, Massachusetts) consisted of a pump F6000A, an injection valve U6 K with a loop of variable volume, a precolumn filled with 37 to 50 μm particles (Bondapak C18/Corsil), and a reverse phase column (300 mm length × 3.9 mm i.d.) prepacked with 10 μm particles of octadecyl bound silica (μ Bondapak C18). The outlet of the column was connected to a thin layer TL-4 flow cell. The pump was set at a flow rate of 0.7 ml/min. The mobile phase consisted of 0.1 M citric acid and 0.1 M monohydrogen phosphate buffer (3 vol/2 vol) containing 8% to 12% methanol, which was filtered on Millipore organic filters under vacuum.

The electrochemical detection system consisted of three electrodes. The working electrode was packed with CP-S carbon paste. The reference electrode was a silver wire recovered by a precipitate of silver chloride, and the compartment of reference was filled with 3 M NaCl solution. The auxiliary electrode was a platinum tube. The applied potential of the working electrode versus the reference was set at +650 mV. The potential was kept constant by a PRG-DEL electronic controller (Tacular, Lyon, France). The chromatograms were registered with a recorder Servotrace (Sefram, Paris, France). Quantities as low as 50 femtomoles of serotonin injected into the column could be detected with a signal-to-noise ratio of 5/1. Samples of 10 μl were injected into the column with a Hamilton
serotonin. The difference between two consecutive injections did not vary more than 2.5% for seven samples tested. Serotonin was identified by comparison with the retention time of standards and quantified by its peak height (Figure 1). The specificity of the technique has been previously described.\textsuperscript{21}

Serotonin loss due to platelets still suspended in plasma after centrifugation represented between 5 and 11% of the total serotonin level in the samples.

**Results**

The uptake of \(^3\)H-serotonin was linear, with a platelet count of from 0.9 to \(5 \times 10^8\) platelets in the serotonin concentration ranges studied for 30 seconds. Lineweaver-Burk plots of uptake of \(^3\)H-serotonin in the platelets of control and hypertensive subjects were found to be linear, which suggested simple Michaelis-Menten uptake kinetics. Figure 2 shows a typical Lineweaver-Burk plot for control and hypertensive subjects. The \(V_{\text{max}}\) was found to be significantly lower \((p < 0.005)\) in hypertensive subjects (Table 1).

In preliminary studies, we determined that no significant difference in \(V_{\text{max}}\) existed between the male and female subjects of each experimental group. However, there was a tendency toward lower \(V_{\text{max}}\) values in male subjects in both control and hypertensive groups. The \(K_m\) was found to be lower in hypertensive subjects \((p < 0.05)\) (Table 1). Endogenous serotonin levels in hypertensive subjects were also significantly lower than in control subjects \((p < 0.001)\) (Table 2).

We studied the endogenous serotonin levels in families having at least one hypertensive member. Figure 3 shows the incidence of hypertension in the five families over several generations. Nearly all of the family members diagnosed as hypertensive had the lowest endogenous platelet serotonin levels (Figure 4). In two

**Table 1.** Maximal Velocity \((V_{\text{max}})\) and Affinity Constant \((K_m)\) of Serotonin Uptake by Platelets of Normotensive Controls and Hypertensive Patients

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>(V_{\text{max}}) ((\text{pmol/min/10}^8) platelets)</th>
<th>(K_m) ((10^{-6} \text{ M}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17</td>
<td>41.7 \pm 3.3</td>
<td>0.70 \pm 0.08</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>16</td>
<td>26.6 \pm 3.0*</td>
<td>0.46 \pm 0.08*</td>
</tr>
</tbody>
</table>

The values represent means \(\pm\) SEM.

*\(p < 0.005\) when compared to control subjects.

**Table 2.** Serotonin Content in Blood Platelets of Normotensive and Hypertensive Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>Serotonin levels ((\text{nmol/10}^{11}) platelets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29</td>
<td>165.0 \pm 12.9</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>27</td>
<td>105.9 \pm 10.4*</td>
</tr>
</tbody>
</table>

The values represent means \(\pm\) SEM.

*\(p < 0.001\) when compared to control subjects.
families, several family members, although normotensive, had serotonin levels below the mean control value (Figure 4). Repeated measurements of both uptake of $^3$H-serotonin and endogenous serotonin level in several individuals gave reproducible results over time.

**Discussion**

The present results indicate that subjects with essential hypertension show modifications in the mechanisms involved in the active uptake of $^3$H-serotonin in blood platelets. The $V_{\text{max}}$ was significantly reduced and the $K_m$ of the carrier for serotonin was enhanced in individuals with essential hypertension compared to control subjects. The endogenous serotonin content was also found to be lower in subjects with essential hypertension. The decrease in $V_{\text{max}}$ established that the low serotonin uptake in hypertensive patients was not due to the dilution of labeled serotonin by serotonin leaked out from hypertensive platelets, and suggests that either the number or the turnover of carriers is lower in these subjects. The slight increase in $K_m$ may have been due to a structural alteration in the binding site of the serotonin molecule to the carrier.
In the periphery, the reduced uptake of serotonin in blood platelets could result in an increase in its concentration at sites such as the heart and blood vessel walls, where receptors of serotonin are located. Stimulation of these receptors by increased levels of serotonin would result in an enhancement of vascular resistance, which could increase blood pressure. In support of this view, the peripheral serotonin antagonist ketanserin is effective in the treatment of hypertension and may act by blocking the vasoconstrictor effects of serotonin.

The reduced uptake of H-serotonin in blood platelets may also reflect a defect in the serotonergic neuronal uptake mechanism in the central nervous system. The uptake of serotonin in human platelets appears to be similar in kinetic and pharmacological properties with that in animal brain slices and synaptosomes. In spontaneously hypertensive rats, the uptake of 14C-serotonin was compared in platelets and synaptosomes; a decrease in Vmax and no change in Km were reported in the platelets, but no changes were found in either Vmax or Km in the synaptosomes. The difference in results from the two experimental preparations may be due to the fact that synaptosomes from whole brain were used, which could mask any changes in the uptake mechanism of serotonin neurons located in discrete brain areas that are more directly involved in blood pressure regulation.

The exact physiological role of serotonin with respect to blood pressure regulation in the central nervous system remains controversial. Some authors have shown that serotonin injected intracerebroventricularly can increase blood pressure. Others have found that serotonin decreases blood pressure. Precursors of serotonin, such as 5-hydroxytryptophan or L-tryptophan, and serotonin agonists and antagonists have also been shown to both increase and decrease blood pressure in rats and dogs. Therefore, there is a strong likelihood that central serotonin neurons are involved in blood pressure regulation, since pharmacological manipulation of these neurons causes changes in blood pressure. The variability in results obtained thus far may be due to the lack of standardized experimental conditions.

Our findings are compatible, in general, with those of Ahtee et al. and Bhargava et al. for the uptake of 3H-serotonin and endogenous serotonin content in platelets in hypertensive subjects. However, these authors found either no modification of 3H-serotonin uptake at low concentrations of serotonin, very slight decreases, or a content of endogenous serotonin unchanged in hypertensive subjects. The differences may be due to the choice of experimental conditions. The uptake of serotonin is reported to consist of a high-affinity, energy-dependent, saturable component and a passive nonsaturable component. Long incubation times and high concentrations of substrate tend to favor passive diffusion and may mask differences in active uptake. It has been reported that the active uptake of serotonin at 37°C may be linear only up to 2 minutes. Therefore, discrepancies in the results may be due to the incubation times chosen, since we used 30 seconds and these authors used either 20 minutes or 30 minutes. It is possible that the experimental conditions used by these authors enhanced the contribution of passive diffusion to the high affinity uptake of serotonin.

It has been suggested that essential hypertension may be at least partially determined by genetic factors. With one or both parents hypertensive, the possibility of hypertension in the offspring is 9 to 15 times greater than in families with no history of hypertension. In addition, the increase in efflux of norepinephrine from platelets in individuals from hypertensive families is visible before the onset of hypertension. The Na+,K+-cotransport system in erythrocytes is functionally deficient in patients with essential hypertension and in some of their family members. To determine whether the reduced endogenous serotonin content in platelets is a primary or a secondary compensatory factor associated with hypertension, we measured this parameter in individuals from hypertensive families. The serotonin platelet content was observed to vary from family to family independently of hypertension. This might be related to variations in dietary habits concerning tryptophan intake, or to some genetic differences of serotonin metabolism. When each family is compared as a single unit, however, in nearly all cases the family hypertensive member had the lowest endogenous serotonin level.

Our results are in agreement with those suggesting a hypertensive effect of serotonin in the peripheral and central nervous system. However, our results do not allow us to conclude whether serotonin plays a primary role in blood pressure regulation. Experimental evidence suggests that serotonin does not play a primary role, since despite depletion of serotonin with parachlorophenylalanine or with neurotoxic indolethylamines, blood pressure remains normal.

The binding of the transport carrier to a serotonin molecule depends on the concentration of sodium and chloride ions in the external medium. An association exists between the uptake of serotonin and of sodium into the platelet and the efflux of potassium. Thus, there is a close relationship between the uptake of serotonin and the balance of plasmatic sodium and potassium ions; changes in this balance could modify the uptake of serotonin into platelets. The carrier system for serotonin, which goes against the concentration gradient, is maintained by a Na+,K+-pump, which depends on the activity of Na+,K+-ATPase. In animal and human studies, there is evidence suggesting that a circulating substance may be associated with hypertension. Recently, it was reported that a factor isolated from human plasma inhibits Na+,K+-ATPase, and it has been recently reported that the uptake of H-serotonin in human blood platelets is reduced in the presence of an endogenous inhibitor-enriched fraction of Na+,K+-ATPase. Therefore, the decrease in H-serotonin uptake in blood platelets may not reflect a primary dysfunction in central serotonin neurons, but a consequence of the effects of circulating endogenous Na+,K+-ATPase inhibitors.
In conclusion, the present study suggests that the mechanisms involved in the transport of \(^3\text{H}\)-serotonin in human blood platelets are modified in essential hypertension. Endogenous serotonin levels in platelets are reduced in essential hypertension and in some individuals from hypertensive families, which may represent a genetic marker in the disease. The reduced uptake of serotonin and low endogenous serotonin levels in platelets may reflect a dysfunctioning in central serotonergic neurons involved in blood pressure regulation, which may be primary or secondary to other biological modifications in essential hypertension.

References

14. DeWardener HE, MacGregor GA. Dahl’s hypothesis that a saline diet may be responsible for a sustained rise in arterial pressure: its possible role in essential hypertension. Kidney Int 1980;18:1–9
29. Vanvenrooij JJ, Van Nuenen JM. General pharmacological profile of ketanserin (R 41 468), a selective 5-HT\(_1\) receptor antagonist. In: De Clerck F, Vanhuette PM, eds. 5-hydroxytryptamine in peripheral reactions 1982:193–197
Decreased uptake of 3H-serotonin and endogenous content of serotonin in blood platelets in hypertensive patients.
L A Kamal, K H Le Quan-Bui and P Meyer

Hypertension. 1984;6:568-573
doi: 10.1161/01.HYP.6.4.568

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1984 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/6/4/568

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/