Sodium Depletion Increases Platelet and Plasma Catecholamines in Hypertensive Men

SVERRE ERIK KJELDSEN, ARNE WESTHEIM, KNUD LANDE, KNUD GJESDAL, PAUL LEREN, ERIK ENGER, AND IVAR KONRAD EIDE

SUMMARY The catecholamine content in blood platelets is considerably higher than that in plasma, and platelet catecholamines must be taken up from plasma, since blood platelets lack enzymes for catecholamine synthesis. However, it is unknown whether platelets take up and store catecholamines during physiological in vivo increments in plasma catecholamines. Previously untreated 50-year-old men \((n = 17)\) with mild to moderate essential hypertension were given a low sodium diet for 2 weeks. Urinary excretion of sodium decreased from \(201 \pm 11\) \((SE)\) to \(24 \pm 5\) and \(19 \pm 4\) mmol/24 hr after 1 and 2 weeks, respectively. During the first week, the blood platelet concentration of norepinephrine increased from \(27.2 \pm 2.9\) to \(39.6 \pm 4.7\) pg/mg \((p<0.005)\) and venous plasma norepinephrine increased from \(254 \pm 22\) to \(347 \pm 28\) pg/ml \((p<0.001)\). The platelet concentration of dopamine increased from \(3.7 \pm 0.4\) to \(5.6 \pm 0.5\) pg/ml \((p<0.005)\), and venous plasma dopamine increased from \(26 \pm 4\) to \(41 \pm 5\) pg/ml \((p<0.05)\). During the second week, both plasma and platelet norepinephrine and dopamine remained elevated. Platelet epinephrine showed a small increase from baseline to the second week \((p<0.05)\), but no concomitant increase in plasma epinephrine occurred. Thus, sodium depletion increases both platelet and plasma catecholamines and blood platelets may take up catecholamines in vivo. Platelet catecholamine content may be an integrated measure of plasma catecholamine concentrations during variations caused by sodium depletion. (Hypertension 11: 477-482, 1988)

KEY WORDS • blood pressure • electrolytes • epinephrine • norepinephrine • sympathetic tone • thrombocytes

SOME of the knowledge about platelet 5-hydroxytryptamine (5-HT) and catecholamine content has been summarized by Da Prada and Picotti.1 The platelet levels of these monoamines are much higher than corresponding plasma concentrations. However, the molar contents of norepinephrine, dopamine, and epinephrine are several thousand times lower than the molar content of 5-HT. Platelet catecholamines are essentially free (unconjugated), and the subcellular localization in the platelets seems to be the 5-HT organelles (dense granules). Since platelets lack enzymes for catecholamine synthesis, it may be that the catecholamines can be actively transported and taken up through the platelet 5-HT carrier mechanism, although at a relatively low receptor/cARRIER affinity.

Unfortunately, the uptake mechanism for the catecholamines is not yet understood. Zweifler and Julius2 found that platelet catecholamines were useful in the diagnosis of pheochromocytoma since patients with pheochromocytoma had markedly elevated platelet norepinephrine and epinephrine concentrations. Platelet catecholamine levels returned to normal more slowly than did plasma concentrations after tumor removal, thus providing a long-term index of circulating catecholamine levels.2 Except for pheochromocytoma, only limited data exist on platelet catecholamines during various physiological conditions or diseases in humans. Rosen et al.3 recently reported that platelets concentrate epinephrine during increases in plasma epinephrine levels in humans following intravenous infusion of this catecholamine. However, Smith et al.4 found no changes in platelet catecholamines after 10 minutes of exercise despite marked increments in plasma catecholamines. Probably a longer period of time with high plasma catecholamines would have been necessary to increase platelet concentrations.

Recently, we reported a 44% increase in plasma norepinephrine and a doubled plasma dopamine con-
centration while plasma epinephrine concentration remained unchanged during sodium depletion in healthy young men. The present study attempted to test the hypothesis that similar increments in plasma norepinephrine and dopamine levels for days or weeks augment the platelet uptake of catecholamines. Both sodium restriction and potassium supplementation are used for nonpharmacological treatment of high blood pressure.5-10 Patients with mild to moderate essential hypertension underwent 1 week of sodium depletion alone and a second week of sodium depletion in combination with potassium supplementation. Plasma catecholamines were measured in peripheral venous blood and, in 10 patients, also in arterial blood since plasma catecholamines are subject to considerable local metabolism in most organs.9

Subjects and Methods

Seventeen men, all 50 years old with untreated, sustained World Health Organization Class I essential hypertension were recruited from the Oslo Study of Cardiovascular Diseases.12 Ten years earlier, they had had systolic blood pressure below 150 mm Hg and diastolic blood pressure above 95 mm Hg. At the time of the present study, each subject had stable blood pressure above 150/100 mm Hg. They had normal ocular fundi and electrocardiograms, and kidney function was normal as estimated by creatinine clearance and urinalysis. They were outpatients maintaining a daily work routine. All were familiar with clinical examination, blood pressure measurement, and blood sampling. None was addicted to alcohol or taking any drug. Prior to dietary intervention they underwent three clinical examinations with blood pressure measurements at 4-week intervals. These recordings followed 10 minutes of supine rest and averaged 167 ± 4/110 ± 2, 168 ± 4/111 ± 3, and 165 ± 4/107 ± 2 mm Hg, respectively.

Protocol

Informed consent was obtained from each subject, and the study was approved by the ethics committee of Ullevaal Hospital. A baseline examination took place immediately before intervention, 4 weeks after the last pretreatment examination. During the dietary intervention they had two examinations: The first took place immediately before intervention, 4 weeks after the last pretreatment examination and the second after completion of the second week. These examinations were performed between 800 and 1000 following an overnight fast with and without intake of fluid, food, nicotine, or caffeine after 2400. They abstained from alcohol during the whole study period.

The subjects were interviewed by professional dieticians with respect to the various dietary components. After the baseline examination, the participants consumed a diet containing approximately 20 mmol Na+ and 100 mmol K+ (K+ not added) per day for 1 week until the next examination. Care was taken not to change the intake of other food items. The diet was individually composed to maintain total calory intake. During the second week of the low sodium diet, potas-
sium tablets were given in an amount of 150 mmol K+ per day. At the end of the second week, the final examination and blood sampling took place.

Arterial blood was collected from the left brachial artery and venous blood from the right median basilic vein through short Teflon catheters (Venflon, 19G and 18G, respectively; Viggo AB, Hälsingborg, Sweden). The catheters were introduced with the subjects under local anesthesia without epinephrine (Xylocaine, Astra). The subjects then rested supine for 30 minutes in a quiet room in the presence of the examining physician only. Talking was avoided. Systolic and fifth phase diastolic blood pressures were then measured with a mercury sphygmomanometer, and pulse rate was counted for 1 minute. Blood was collected into polypropylene syringes, and the first 1 to 2 ml was discarded. Venous blood was used for assay of platelet and plasma catecholamines and serum electrolytes, and arterial blood was used for measuring plasma catecholamines. Then, after subjects had spent 30 minutes in the erect position walking slowly around in the corridor, measurements of blood pressure and heart rate and blood sampling for plasma catecholamines were repeated.

Blood samples for catecholamine assays were immediately mixed with glutathione and EGTA and placed on melting ice. They were centrifuged at 4°C, and plasma and preparations for platelet catecholamines were stored at −70°C. A 24-hour urine specimen was collected for determination of electrolyte excretion before each examination.

Analytical Methods

The platelet preparation was derived from 10 ml of blood as described by Zweifler and Julius.2 Platelet-rich plasma was obtained by centrifugation at 200 g for 15 minutes at 4°C and then transferred to preweighed borosilicate tubes, which were centrifuged at 2500 g for 20 minutes, also at 4°C. Platelet pellet weights were obtained by reweighing the tubes after platelet-poor plasma had been removed by pipetting followed by inverting the tubes on a filter paper for 15 minutes. In our hands, a coefficient of variation of 12 to 19% (median, 16%) was calculated for repeated determinations of platelet pellet weights in blood from five healthy persons. The platelets were then homogenized in ice-cold 0.03% perchloric acid with a glass hand homogenizer. After removal of precipitated protein by centrifugation at 2500 g for 5 minutes at 4°C, the supernatant from this homogenate was pipetted into plastic tubes and stored at −70°C until assay.

Catecholamines in plasma and extracts of platelet pellets were measured radioenzymatically13 as previously reported for plasma samples.14,15 The intra-assay coefficients of variation for platelet catecholamines were measured in healthy persons (n = 12) and were 6% for norepinephrine, 17% for epinephrine, and 11% for dopamine. The average platelet catecholamine values in normal subjects of the same age and sex as in the present study (n = 16) were 23.4 ± 2.7 pg/mg for norepinephrine, 2.0 ± 0.2 pg/mg for epinephrine,
and 6.5 ± 0.9 pg/mg for dopamine. Electrolytes were assayed on an autoanalyzer (SMA 12/60, Technicon Instruments, Tarrytown, NY, USA). All assays were examiner-blind.

Statistical Methods
Data are presented as means ± SE. Differences were examined with the two-tailed Student's t-test for paired comparison and considered statistically significant at a p level below 0.05.

Results
Platelet and Plasma Norepinephrine
During the first week with sodium depletion and unchanged potassium intake, platelet norepinephrine increased by 45% from 27.2 ± 2.9 to 39.6 ± 4.7 pg/mg, venous plasma norepinephrine by 38% supine (from 254 ± 22 to 347 ± 28 pg/ml) and 43% standing, and arterial plasma norepinephrine by 25% supine and 40% standing (Figure 1). After the second week, when sodium depletion was combined with potassium supplementation, these variables were still considerably higher than baseline: 48% for platelet norepinephrine (40.6 ± 3.7 pg/mg), 30% for supine and 28% for standing venous plasma norepinephrine, and 32% for supine and 33% for standing arterial plasma norepinephrine.

Platelet and Plasma Dopamine
The platelet content of dopamine increased from 3.7 ± 0.4 to 5.6 ± 0.5 pg/mg during the first week with sodium depletion, while supine venous plasma dopamine increased from 26 ± 4 to 41 ± 5 pg/ml and standing, from 31 ± 4 to 57 ± 6 pg/ml (Figure 2). After the second week when dietary potassium was added, platelet dopamine averaged 5.6 ± 0.6 pg/mg, and supine venous plasma dopamine averaged 40 ± 4 pg/ml and standing, 43 ± 5 pg/ml. Supine arterial dopamine remained essentially unchanged, while standing arterial dopamine increased from 26 ± 4 pg/ml at baseline to 49 ± 8 pg/ml after the first week and 38 ± 6 pg/ml after the second week.

Platelet and Plasma Epinephrine
Platelet epinephrine changed from 1.65 ± 0.15 to 1.81 ± 0.17 pg/mg (p = NS) during the first week with sodium depletion (Figure 3). After the second week with both sodium depletion and potassium supplementation, platelet epinephrine had increased to 2.16 ± 0.21 pg/mg. The difference between platelet epinephrine at baseline and after the second week...
FIGURE 3. Platelet, venous, and arterial epinephrine at baseline, at low sodium and normal potassium intake (Week 1), and at low sodium and high potassium intake (Week 2). Dotted bars indicate platelet concentrations; open bars indicate supine and hatched bars standing plasma concentrations (n = 17 except for arterial samples, where n = 10). Symbol indicates significant difference (p<0.05) compared with baseline.

(A0.51 ± 0.27 pg/mg) reached statistical significance (p<0.05). Plasma epinephrine remained unchanged throughout.

Platelet to Plasma Catecholamine Gradients

A gradient was calculated for each subject using the following formula: platelet catecholamine content (pg/mg) x 1000/plasma catecholamine concentration (pg/ml). We assumed the relative weight of plasma and platelets to be equal. The gradient for norepinephrine averaged 119 ± 15 at baseline, 112 ± 15 after the first week, and 133 ± 17 after the second week. Corresponding results for dopamine were 219 ± 39, 158 ± 23, and 167 ± 23, and for epinephrine were 51 ± 8, 45 ± 5, and 52 ± 5. None of these changes in platelet to plasma gradients was statistically significant.

Platelet Weight, Blood Pressure, Heart Rate, and Body Weight

Only small and insignificant changes were seen in platelet weight during the 2-week study (Table 1). Supine blood pressure decreased by 14 ± 3/7 ± 2 mm Hg and standing blood pressure by 12 ± 3/6 ± 3 mm Hg during sodium depletion alone. During the second week, supine blood pressure was reduced by 19 ± 3/13 ± 2 mm Hg and standing blood pressure was reduced by 19 ± 3/13 ± 3 mm Hg compared with baseline. The difference in blood pressure between low sodium diet and sodium restriction combined with potassium supplementation was significant (p<0.05) only for diastolic blood pressure in the standing position. Supine heart rate remained unchanged, while standing heart rate increased by 6 ± 1 beats/min during the first week and 3 ± 1 beats/min during the second compared with baseline. Body weight decreased during the first period by 2.2 ± 0.2 kg and further by 0.6 ± 0.1 kg in the second week (see Table 1).

Serum and Urine Electrolytes

Serum Na⁺ decreased during the first week, while serum K⁺ increased during both the first and second weeks (see Table 1). Urinary sodium excretion was reduced by 177 ± 13 to 24 ± 5 mmol/24 hr during the first week and then remained essentially unchanged through the second week. Urinary potassium excretion increased by 114 ± 8 mmol/24 hr through the second week but remained unchanged during mere sodium depletion (see Table 1). Thus, the urinary Na⁺/K⁺ ratio changed from baseline 2:1 to 1:4 and 1:11 during the two intervention programs.

Discussion

In the present study, sodium depletion elevated both platelet and plasma norepinephrine and dopamine whereas further potassium supplementation had no detectable additional effect. The platelets lack enzymes for catecholamine synthesis. Therefore, we assume that the increased platelet catecholamines during sodium depletion were caused by uptake from plasma stimulated by the higher plasma catecholamine concentrations, by sodium depletion per se, or by both. The results indicate that the platelet catecholamine contents under certain circumstances (e.g., sodium depletion) reflect the plasma concentrations and present an integrated measure of variations in plasma catecholamines.

The platelet concentrations of norepinephrine and dopamine were more than 100 times higher than the plasma concentrations. Because of catecholamine leakage caused by the unavoidable rupture of platelets during pellet preparation, it is possible that the true concentration gradients for the catecholamines are even higher, especially during sodium depletion, since an increased platelet content of norepinephrine may be related to increased platelet aggregability. Also, plasma adsorbed to the platelets through preparation may increase platelet pellet weight and dilute the catecholamine concentrations. On the other hand, release of even all the platelet content of catecholamines in vivo would not be detectable for epinephrine and dopamine and would, at most, increase the plasma concentrations of norepinephrine by 10% because of the low total platelet volume compared with plasma volume. Increased plasma norepinephrine during short-term sodium depletion has been well documented. Watson et al. reported increased synaptic norepi-
PLATELET CATECHOLAMINES/Kjeldsen et al.

TABLE 1. Platelet Pellet Weight (per 10 ml Blood), Blood Pressure, Heart Rate, Body Weight, and Serum and Urine Electrolytes During Dietary Intervention in 17 Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Week 1 (low Na⁺, normal K⁺)</th>
<th>Week 2 (low Na⁺, high K⁺)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet pellet weight (mg)</td>
<td>25.9 ± 2.8</td>
<td>22.7 ± 1.9</td>
<td>27.1 ± 3.8</td>
</tr>
<tr>
<td>Supine systolic BP (mm Hg)</td>
<td>157 ± 4</td>
<td>143 ± 3*</td>
<td>138 ± 2†</td>
</tr>
<tr>
<td>Supine diastolic BP (mm Hg)</td>
<td>110 ± 2</td>
<td>103 ± 2*</td>
<td>97 ± 2†</td>
</tr>
<tr>
<td>Standing systolic BP (mm Hg)</td>
<td>155 ± 3</td>
<td>143 ± 4*</td>
<td>136 ± 3†</td>
</tr>
<tr>
<td>Standing diastolic BP (mm Hg)</td>
<td>113 ± 3</td>
<td>107 ± 3</td>
<td>100 ± 1†</td>
</tr>
<tr>
<td>Supine HR (beats/min)</td>
<td>61 ± 2</td>
<td>63 ± 3</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>Standing HR (beats/min)</td>
<td>75 ± 3</td>
<td>81 ± 4†</td>
<td>78 ± 3*</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>95.7 ± 2.5</td>
<td>93.5 ± 2.5†</td>
<td>92.9 ± 2.5†</td>
</tr>
<tr>
<td>Serum Na⁺ (mmol/L)</td>
<td>140.7 ± 0.3</td>
<td>137.7 ± 0.4†</td>
<td>137.5 ± 0.5†</td>
</tr>
<tr>
<td>Serum K⁺ (mmol/L)</td>
<td>3.81 ± 0.06</td>
<td>4.05 ± 0.08‡</td>
<td>4.51 ± 0.10†</td>
</tr>
<tr>
<td>Urine Na⁺ (mmol/24 hr)</td>
<td>201 ± 11</td>
<td>24 ± 5†</td>
<td>19 ± 4†</td>
</tr>
<tr>
<td>Urine K⁺ (mmol/24 hr)</td>
<td>101 ± 6</td>
<td>89 ± 7</td>
<td>215 ± 8†</td>
</tr>
</tbody>
</table>

Data are means ± SE. BP = blood pressure; HR = heart rate.

* p<0.05, † p<0.001, ‡ p<0.01, compared with baseline values.

nephrine release and subsequent spillover to blood, while Lineraes et al.18 recently suggested a decreased plasma clearance rate of norepinephrine as the most likely explanation. The initially high plasma norepinephrine concentration seen during sodium depletion declines with time.19 Therefore, the exact level of plasma norepinephrine and the lag time necessary for platelet uptake in vivo remain unknown. The pheochromocytoma patients of Zweifler and Julius2 had plasma concentrations far above those measured in the present study and platelet catecholamine contents were proportionally higher. The physiological increases caused by bicycle exercise in the study of Smith et al.4 were only maintained for 10 minutes and did not change the platelet catecholamine content. However, Da Prada and Picotti1 induced rather large rises in platelet catecholamines, especially norepinephrine and dopamine, and dopamine has the chemical structure closest to 5-HT, and it has been suggested that the platelet 5-HT carrier mechanism and organelles exhibit a higher affinity for dopamine than for the other two catecholamines.1

The platelet content of epinephrine is approximately 5% that of norepinephrine, approximately the same as in the sympathetic nerve terminals.22 Also, platelet epinephrine increased slightly throughout the present study, but was not accompanied by an increase in either venous or arterial plasma epinephrine levels. Maybe electrolyte changes per se facilitate platelet catecholamine uptake, or perhaps a common uptake mechanism for the catecholamines, such as the 5-HT carrier,1 is activated. On the other hand, plasma epinephrine concentration fluctuates slightly above the threshold for platelet uptake3 but appeared unchanged even during the strictly standardized conditions for blood sampling in the present study.

The present subjects had stable blood pressure during the run-in period, and they experienced a substantial fall in blood pressure during the dietary intervention. A blood pressure-lowering effect of sodium depletion has been well documented6-8 and probably is the main explanation. However, some fall in blood pressure caused by adjustment to the study conditions cannot be ruled out. The weight loss probably can be ascribed to loss of water in the sodium-depleted state,23 especially when the fall in body weight is related to the relatively high initial weight in the present subjects.

In conclusion, concomitant rises in platelet and plasma catecholamines, especially norepinephrine and dopamine, were seen during sodium depletion in mild to moderate essential hypertensive subjects. These results support the contention that blood platelets may take up catecholamines in vivo and suggest that platelet catecholamine levels may serve as an integrated measure of variations in plasma catecholamines during
sodium depletion. The effect of increased platelet catecholamines remains incompletely understood.

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