Unconjugated Hyperepinephrinemia: A Hallmark of Hypertension Imitating Pheochromocytoma?

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SUMMARY Hypertensive patients with elevated and hyperresponsive plasma norepinephrine and epinephrine (NE + E) associated with low conjugated NE + E were previously identified by determination of the sum of NE + E. Because of their excessive E but not NE responses to glucagon and also hypertension corresponding to E excess, we explored whether an elevated unconjugated E resulting from a selective E conjugation defect could be obscured by the sum of NE + E. We found that nine patients with elevated E (reflected by the normal 4:1 ratio of plasma NE to E reversed in favor of E), had, when compared to 31 patients with plasma NE exceeding E: 1) lower plasma conjugated E (mean 0.03 vs 0.27 ng/ml, p < 0.01), lower degree of E conjugation (8 vs 51%, p < 0.01), and a higher maximum systolic (p < 0.05) and higher pulse rates (p < 0.04), but no differences in the unconjugated and conjugated proportions of plasma NE; and 2) an absence of conjugated E throughout the circulation and relative preponderance of E over NE at sampling points close to the peripheral venous blood (p < 0.05). The absolutely and relatively decreased plasma conjugated E in patients with E exceeding NE (without difference in conjugated NE) is a preliminary indication that a selective sulfoconjugating defect of E results in plasma E higher than NE in accordance with the hyper-β-adrenergic features of their hypertension. Epinephrine, a circulating hormone, is more dependent on conjugation for its systemic Inactivation than NE, a local neurotransmitter. Determinations of basal free and conjugated E reflect better this defect than those measuring the sum of NE and E.

(Hypertension 3 (suppl II): II-129-H-133, 1981)

KEY WORDS • hypertension • epinephrine conjugation defect • pseudopheochromocytoma

In the past, the determination of plasma epinephrine (E) has been difficult because of its low concentration and the lack of sensitive methods for its measurement. Plasma catecholamines (CA) were therefore mainly expressed as the sum of norepinephrine and epinephrine (NE + E) or only NE. More recent radioenzymatic methods detected elevated E in some patients with essential hypertension (EH), which was attributed to its increased adrenomedullary release. High conjugated NE has been demonstrated in plasma. We observed that E is also highly (80%) conjugated. We found low conjugated NE + E in EH patients imitating pheochromocytoma who also responded to several stimuli (such as glucagon) by a higher increase in unconjugated NE + E than those with normal conjugated NE + E. Since NE and E were not separately measured, we were unable to determine whether defective conjugation of NE or E had the greater effect. With E separated from NE, hyperresponsiveness to glucagon concerned predominantly E. Under normal conditions the ratio of plasma NE to E is approximately 4:1, but this ratio is not necessarily maintained in conjugated NE and E, the latter being more conjugated than NE. Quantitative limits for the increase of E are difficult to establish. However, patients with increased plasma E not due to stress have E concentrations usually equal to or higher than NE. Therefore, we sampled blood, peripheral and at several sites of the circulation, and separately determined conjugated E and NE in patients with E ≥ NE and those with NE exceeding E (thus closer to "normal") to determine whether: 1) lower concentration of conjugated E can be found in patients with plasma E ≥ NE in the peripheral blood and throughout the circulation and whether NE is equally affected; 2) conjugated E is low, and to what degree such a conjugation defect of E can be related to this "asymmetric" hypersympathotonia and to its frequent association with the labile hyperadrenergic character of hypertension in these patients.
Methods

Patients

Forty patients with essential hypertension (18 men, 22 women, aged 17–56 years; nine of them included in the previous study; all except three of them hospitalized) were screened for secondary forms of hypertension. Twelve normotensive subjects (two men and 10 women, aged 21–59) served as controls. All patients had normal plasma creatinine, electrolytes, thyroid functions, and rapid sequence intravenous pyelogram. Most of the patients presented clinically as borderline hypertension (defined as arterial pressure repeatedly higher than 140/90 mm Hg, but often decreasing below this limit with rest) had very variable blood pressure (BP). A high proportion of patients (23 of 40) presented with periods of raised BP accompanied by palpitations, anxiety, sweating, and other symptoms resembling pheochromocytoma. These patients were subjected to adrenal CAT scan, renal arteriography, glucagon plasma catecholamine (CA) tests, and urinary CA and vanillyl mandelic acid (VMA) determinations, all with negative results.

Experimental Procedures

Drug treatment of hypertension had been discontinued for at least 1 week before the study, and longer-acting drugs such as rauwolfia, guanethidine, and α-methyl dopa for at least 1 month. None of the women had taken oral contraceptives for at least 3 months prior to the study. Blood pressure and pulse rates were recorded 8 to 10 times a day: in the recumbent and upright positions in the hospitalized patients. All subjects fasted overnight (at least 12 hours), were forbidden to smoke and drink coffee; and on the morning of the sampling spent at least 30 minutes recumbent. Saline was infused by intravenous drip with heparin for 20 minutes before blood was drawn for CA determinations.

In six patients having more baseline E than NE, the clinical suspicion of pheochromocytoma was such that they were subjected to catheterization and sampling from the suprarenal portion of the inferior vena cava (IVC), the peripheral artery, the superior vena cava, and the infrarenal portion of the IVC. From all these sites a sample was obtained for determination of NE and E and their conjugated fraction. The results were compared with those from three hypertensive patients who had baseline NE exceeding E in the peripheral blood.

Plasma NE and E were determined in duplicate with internal standards by the radioenzymatic method using thin-layer chromatography (TLC) to differentiate between NE and E. Conjugated CA were hydrolyzed by lyophilization in dilute perchloric acid as described before and the liberated free CA were measured to give a value of total (unconjugated + conjugated) CA. Subtraction of the unconjugated from the total values provide the concentrations of the conjugated CA. In some earlier studies we used the radioenzymatic method of Coyle and Henry13 which measures the sum of NE and E obtained by the da Prada method.11 Duplicate values differing by more than 20% were measured again. The data were statistically analyzed by Student’s t-tests and in cases in which the data were not normally distributed we used nonparametric tests such as the Wilcoxon rank sum test and the median tests.

Results

Values obtained in healthy subjects (table 1) showed a higher degree of E than NE conjugation, in agreement with most recent studies using either enzymatic or acid hydrolysis and a degree of NE conjugation comparable to that previously found. The analysis of hypertensive patients was based on distinguishing nine patients, all of them hospitalized, who had a baseline concentration of E of over 0.10 ng/ml, equal to or higher than that of NE, from the remaining 31 patients who had the usual ratio of NE:E with NE predominating.

### Table 1

<table>
<thead>
<tr>
<th>Hydrolysis method</th>
<th>Unconj NE (ng/ml)</th>
<th>Conj NE (ng/ml)</th>
<th>Conj (%)</th>
<th>Unconj E (ng/ml)</th>
<th>Conj E (ng/ml)</th>
<th>Conj (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid hydrolysis</td>
<td>0.3</td>
<td>0.7</td>
<td>70</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Haggeneral, 1963</td>
<td></td>
<td></td>
<td></td>
<td>0.04 ± 0.02</td>
<td>0.32 ± 0.1</td>
<td>88</td>
</tr>
<tr>
<td>Acid lyophilization</td>
<td>0.23 ± 0.05</td>
<td>0.57 ± 0.2</td>
<td>71</td>
<td>0.05 ± 0.01</td>
<td>0.30 ± 0.1</td>
<td>89</td>
</tr>
<tr>
<td>Buu &amp; Kuchel, 1977</td>
<td></td>
<td></td>
<td></td>
<td>0.08 ± 0.07</td>
<td>0.45 ± 0.1</td>
<td>80</td>
</tr>
<tr>
<td>Acid hydrolysis</td>
<td>0.5 ± 0.1</td>
<td>1.3 ± 0.4</td>
<td>71</td>
<td>0.08 ± 0.07</td>
<td>0.45 ± 0.1</td>
<td>80</td>
</tr>
<tr>
<td>Da Prada, 1980</td>
<td></td>
<td></td>
<td></td>
<td>0.33 ± 0.04</td>
<td>1.0 ± 0.2</td>
<td>68</td>
</tr>
<tr>
<td>Acid hydrolysis + heating</td>
<td>0.67±0.04</td>
<td>1.29±0.04</td>
<td>66±1</td>
<td>0.08±0.04</td>
<td>0.44±0.04</td>
<td>83±1</td>
</tr>
<tr>
<td>Nagel, Schumann, 1980</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Haggeneral did not use a radioenzymatic method, but a less sensitive fluorometric assay, which did not detect unconjugated E and conjugated E (last three columns)

†Values expressed as means of three plasma pools after subtracting the free from total NE and E values respectively.
The higher E in EH patients with E ≥ NE was accompanied by a lower conjugated E (p < 0.01) than found in EH patients with NE > E (fig. 1). There was no difference in age or sex between both groups. Patients with E ≥ NE had absolutely and relatively less conjugated E than those with NE > E (table 2). There were no differences in NE and its conjugated fraction between both groups. Patients with E ≥ NE had higher maximum systolic and pulse pressures and pulse rates than those with NE > E. Almost all patients with E ≥ NE but only half of the patients with NE > E were suspected on clinical grounds to harbour a pheochromocytoma. Two thirds of patients with E ≥ NE but only one third of those with NE > E would be classified as having low conjugated NE + E according to the previous criterion of conjugated NE + E below 0.23 ng/ml.

The selective catheterization data summarized on figure 2 show that patients with E ≥ NE had absent conjugated E at all sites of sampling, in contrast to patients with NE > E (p < 0.002). At sites of sampling close to the peripheral venous blood (i.e., the superior vena cava and infrarenal inferior vena cava) the patients with E ≥ NE had an E predominance over NE when compared to patients with NE > E as indicated by their lower NE/E ratios. We did not observe differences in the degree of NE conjugation between both groups at any site of sampling.

Discussion

The main difficulty in evaluating plasma CA, E in particular, is the known experience that any emotional input stimulates the appearance of E in the plasma, which may result in false readings of an apparently high plasma E. We tried to minimize this effect by a very careful sampling with the venous catheter installed sufficiently long so that the patient did not perceive any pain or anxiety at the moment of the sampling. This way of sampling probably explains the generally lower baseline values of free NE and E in our control subjects compared with those in other recent studies in which the usual sampling technique was used. It can thus be assumed that an as close as possible truly "basal" state of sympathetic activity has been achieved by these precautions.

The relatively high proportion of EH patients with E ≥ NE is probably due to the referral to our specialized center of patients who have very labile hypertension and are suspected of its secondary origin. Although such cohorts are not representative of EH, this bias underlined the association of high E with low conjugated E in patients with E ≥ NE who were previously shown and confirmed here to have the most typical features of hyperadrenergic EH, an increased heart rate and systolic BP resulting, with close to normal diastolic BP, in an elevated pulse pressure.
TABLE 2. Clinical and Laboratory Data (mean ± SE) in Patients with a Normal NE > E Ratio and Those with E ≥ NE

<table>
<thead>
<tr>
<th>Data</th>
<th>Plasma NE &gt; E (n = 31)</th>
<th>Plasma E ≥ NE (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, sex</td>
<td>32 ± 2 yrs, 14 M, 17 F</td>
<td>39 ± 4 2, 4M, 5F</td>
</tr>
<tr>
<td>Unconjugated:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma NE (ng/ml)</td>
<td>0.22 ± 0.03</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>Plasma E (ng/ml)</td>
<td>0.06 ± 0.02</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>Conjugated:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma NE (ng/ml)</td>
<td>0.29 ± 0.06</td>
<td>0.24 ± 0.09</td>
</tr>
<tr>
<td>Plasma E (ng/ml)</td>
<td>0.27 ± 0.11</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Conjugated NE (%)</td>
<td>44 ± 6</td>
<td>49 ± 12</td>
</tr>
<tr>
<td>Conjugated E (%)</td>
<td>51 ± 8</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Blood pressure:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest (mm Hg)</td>
<td>137 ± 4</td>
<td>137 ± 4</td>
</tr>
<tr>
<td>Highest (mm Hg)</td>
<td>82 ± 3</td>
<td>78 ± 4</td>
</tr>
<tr>
<td>Highest pulse pressure (mm Hg)</td>
<td>160 ± 5</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Maximum pulse rate (b/min)</td>
<td>100 ± 4</td>
<td>96 ± 8</td>
</tr>
</tbody>
</table>

Patients suspected clinically of pheochromocytoma | 15/31 | 8/9 |

Patients classified as having low conjugated NE + E (< 0.23 ng/ml) | 11/31 | 6/9 |

**Figure 2.** Plasma E (○) and conjugated E (●) at several sites of sampling in six hypertensive patients whose plasma E is equal to or exceeds NE and three patients where NE > E. IVC = inferior vena cava. The NE/E ratio at the respective site of sampling is at the bottom of each column.
It is known that 90% to 95% of all E that is secreted enters into the arterial blood and is removed by several passes through the circulation before being cleared. Very little arterial E gets into venous blood so that increases of venous E are harder to see. The lower degree of NE and E conjugation in hypertensive than control subjects is in accordance with our previous result suggesting that E, although becoming conjugated, is deconjugated before reaching the peripheral venous blood. 14, 16 The finding in our subset of patients, of higher venous E than arterial E but higher conjugated dopamine than control subjects, suggests a delay of the noradrenergic system and may be due to a selective conjugation defect of E.

There are evident differences in the biological roles for E and NE, the first being a circulating hormone more exposed to sulfocojugation and a systemic inactivation, the second a local neurotransmitter of which only the proportion leaking into the circulation (or during infusion of NE) has an access to sulfocojugation and is thus dealt with as a hormone. 16

It is not yet clear whether sulfocojugation is rate limiting for the plasma clearance of E. This preliminary result suggests, however, that this "hormonal" character of E secretion and its higher dependence on conjugation, combined with affinity of E to platelet phenolsulfotransferase lower than NE, 17 may translate a conjugation defect more easily into unconjugated hyperepinephrinemia than hypernorepinephrinemia. The absence of conjugated E over several sites of regional sampling in patients with E > NE shows that this anomaly is present throughout the circulation. This speaks against the possibility that E, although becoming conjugated, is deconjugated before reaching the peripheral venous blood. The lower degree of NE and E conjugation in hypertensive than control subjects is in accordance with our most recent observation (unpublished) that patients with essential hypertension have lower conjugated NE + E but higher conjugated dopamine than control subjects.

We are presently investigating which of the mechanisms of unconjugated hyperepinephrinemia, high E release overburdening the conjugation system or a primarily decreased E conjugation, predominates. High E is not present in all patients, as previously suggested. 6 A continuous excessive release of E from morphologically normal adrenal medulla is doubtfull but probably the adrenal medulla releases E episodically in response to stress. 18 We have observed that plasma E correlated positively with pulse pressure and pulse rates, the latter also negatively correlated with the degree of E conjugation. 20 The higher pulse rate and systolic BP in patients with E > NE is in accordance with hemodynamic actions of E found at levels close to 0.1 ng/ml encountered in all these patients but only in the minority of patients with NE > E. In contrast, the NE levels necessary to increase BP (above 1.5 ng/ml) 18 have not been observed in any of our EH patients. The physiological range of the threshold plasma E concentrations having hemodynamic actions, including a decrease in diastolic BP (0.2 ng/ml), 21 suggests that mild E surges easily encountered with minor stresses may be, particularly in E > NE patients, of hemodynamic importance and occur without being reflected in 24 hour integrated concentrations of E, which have been found to be normal in patients with mild essential hypertension. 22

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Unconjugated hyperepinephrinemia: a hallmark of hypertension imitating pheochromocytoma?
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doi: 10.1161/01.HYP.3.6_Pt_2.II-129

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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