Evaluation of Plasma Catecholamines in Humans
Correlation of Resting Levels with Cardiac Responses to Beta-Blocking and Sympatholytic Drugs

JOLLY THOMAS, M.D., FETNAT M. FOUD, M.D., ROBERT C. TARAZI, M.D., AND EMMANUEL L. BRAVO, M.D.

SUMMARY The changes in systolic time intervals (STI) following reduction of adrenergic activity was used to validate supine resting plasma catecholamines (CATs) as an index of sympathetic activity. Blockade of sympathetic activity was achieved by two means in two groups: propranolol (10 mg i.v.) and clonidine (0.3 mg p.o.). The diminished sympathetic effect was evidenced by slowing (p < 0.01) of heart rate with both drugs and the reduction (p < 0.01) of blood pressure with clonidine. There was no correlation in our study between resting plasma CATs (norepinephrine alone or total), and changes in heart rate and pre-ejection period (PEP). Moreover, to avoid changes in PEP that could be related to differences in blood pressure levels (clonidine-reduced blood pressure while propranolol did not), the changes in PEP were corrected for the change of mean arterial pressure (MAP) in the same patients (ΔPEP/ΔMAP and %ΔPEP/%ΔMAP). No correlation could be found, still, between resting supine plasma CATs and these ratios. The difficulty in demonstrating a correlation between resting plasma CATs and the immediate cardiac response to adrenolytic agents can be explained by the number of factors influencing plasma levels. Circulating plasma CATs represent the spillover from adrenergic nerve endings, and, therefore, their level would depend on several factors including sympathetic nervous system activity, rate of reuptake, and rate of degradation.

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KEY WORDS • systolic time interval • catecholamines • adrenergic blockade • propranolol • clonidine

THERE is still an active controversy regarding the value of supine resting plasma catecholamines (CATs) as a quantitative measure of sympathetic activity. \(^1\) \(^2\) Norepinephrine (NE) released from sympathetic nerve endings is subjected to a number of local influences including reuptake and enzymatic degradation, so that only a small and variable proportion reaches the systemic circulation. \(^3\) Various methods have been used to validate the determination of plasma NE as an index of sympathetic activity. These included correlation with systolic time intervals (STI) at rest, \(^4\) or response of plasma CATs to neurogenic stimuli such as head-up tilt or cold pressor test. \(^1\) \(^5\) \(^6\) However, response to a stimulus does not necessarily reflect the adrenergic activity at rest; and a correlation between resting plasma CATs and STI at rest \(^7\) does not necessarily reflect the adrenergic influences on these intervals, because STIs at rest are also influenced by cardiac status and other factors. \(^8\) On the other hand, the change in STI following interference with, rather than stimulation of, adrenergic activity has been taken as an indication of adrenergic influence on these intervals. \(^9\)

A study of the relationship between this measure of adrenergic activity and circulating plasma CAT levels has not been previously reported, to our knowledge. The correlation between resting plasma CATs and the change in STI produced by clonidine or by propranolol was therefore used as a test of the quantitative value of resting plasma CAT levels. These drugs were chosen in order to interfere at different levels with adrenergic influences on the heart. Moreover, clonidine lowers blood pressure while intravenous propranolol does not, so that a differentiation could be made between the response to the two maneuvers.

Material and Methods
Twenty subjects participated in the study. Thirteen had essential hypertension, five had borderline hypertension, and two were normal volunteers. All subjects were not receiving medications at the time of the study. Only two of the hypertensive patients had been

From the Research Division, The Cleveland Clinic Foundation, Cleveland, Ohio. Supported by Grant HL-6835 from National Institutes of Health. Address for reprints: Fetnat M. Fouad, M.D., Research Division, Cleveland Clinic Foundation, Cleveland, Ohio 44106. Received July 26, 1982; revision accepted July 4, 1983.
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treated previously, but they had discontinued their antihypertensive medications (vasodilators, propranolol, or methyldopa) at least 2 weeks prior to the study. Each subject gave informed consent after full explanation of the procedure as well as of the mode of action and possible side-effects of the drugs used in the study.

Protocol
All tests were performed in the morning after an overnight fast and following 30 minutes of supine rest. To avoid possible changes in plasma CATs induced by anxiety or pain, the intravenous needle for blood sampling and injection was positioned at the beginning of the test before the 30 minutes of rest started; similarly, all transducers, carotid pulse recording piece, and ECG were positioned, tested, and fixed before the test. Two study protocols were used in order to differentiate between the effects of various adrenolytics on STI; clonidine has been shown to reduce blood pressure and plasma CATs by central mechanisms whereas i.v. propranolol is a peripheral beta-receptor blocker.

Protocol A: Clonidine
Clonidine was used in 12 subjects (fig. 1). After obtaining control blood pressure, heart rate, plasma CATs, and STI, a single oral dose of clonidine (0.3 mg) was administered as previously described. Blood pressure and heart rate were monitored every hour for 2 hours. STI and plasma CATs were obtained 1 hour following clonidine, since previous studies had shown that plasma CAT changes reach a plateau at that time.

Protocol B: Propranolol
Propranolol 10 mg i.v. (at a rate of 1 mg/min) was used in eight subjects (fig. 2). After obtaining control blood pressure, heart rate, plasma CATs, and STI, propranolol was injected slowly intravenously (1 mg/min). Blood pressure and heart rate were monitored every 5 minutes for 20 minutes at which time STIs were recorded and blood sampling for plasma CATs was obtained. This 20-minute interval was chosen because previous studies had shown that resting hemodynamics (heart rate and cardiac output) reached a steady level at that point.

Recording of Systolic Time Intervals
STIs were measured from a simultaneously recorded electrocardiogram (Lead II), indirect carotid pulse tracing, and external phonocardiogram on a photographic paper (speed of 100 mm/sec). Carotid pulse tracing was obtained from a transducer with a flat response, which was held steadily over the common carotid artery while the subject was lying down flat with the neck comfortably slightly extended. Heart sounds were recorded using a Sanborn dynamic microphone positioned on the precordial area giving the clearest inscription of the high frequency vibrations of S1 and the two components of S2. Each recording period extended for at least 15 cardiac cycles.

Systolic time intervals were measured using a computer program based on digitization of phonocardiographic, electrocardiographic, and carotid tracings. The following intervals were averaged from at least 10 cardiac cycles: left ventricular ejection time (LVET), Q-S1, R-R intervals, preejection period (PEP), and PEP/LVET ratio. When required, LVET was corrected for heart rate according to the formula:

\[ \text{LVETc (msec)} = \text{LVET} + 1.7 \times \text{HR for men; } \text{LVET} + 1.6 \times \text{HR for women.} \]

Analytical Procedures
Plasma CATs were measured by radioenzymatic assay according to the method described by Peuler and Johnson and fractionated into NE and epinephrine (E)
as described previously. The values established for supine resting total plasma CATs in our laboratory for normal volunteers and for essential hypertensive patients are shown in table 1.

Results

Baseline Characteristics

There was no statistically significant difference in the baseline characteristics of the 12 patients who received clonidine and the eight patients who received propranolol. Data regarding mean arterial pressure (MAP), heart rate (HR), plasma CATs, PEP, and LVET corrected for HR (LVETc) are summarized in table 2.

The effect of age on plasma CAT levels is well recognized. Except for two of 20 patients who were under 30 years, and two who were over 60 years, the majority were between 34 and 59 years old, that is, in an interval where the slope of the correlation of plasma CATs with age is not very acute. Moreover, among the patients investigated there was no relationship between the basal CAT levels and age — neither for the entire group (n = 20, r = -0.003) nor for each study group separately (r = 0.165, p = ns, n = 12 for the clonidine study, and r = -0.224, p = ns, n = 8 for the propranolol study). Further, the lack of correlation observed in subsequent analyses between either plasma CATs or plasma NE and STI was not due to any single outlying point.

Response to Reduction of Adrenergic Effects

Protocol A: Clonidine

Following clonidine (0.3 mg p.o.), there was a significant reduction in HR (84 ± 4 to 74 ± 3 bpm, p < 0.01), MAP (115 ± 5 to 98 ± 5.7 mm Hg, p < 0.01) and a prolongation of PEP (111 ± 9 to 129 ± 7 msec, p < 0.01) (table 3). Plasma NE decreased significantly (311 ± 37 to 198 ± 38 ng/liter, p < 0.025), but total plasma CAT changes (368 ± 45 to 243 ± 43) did not attain statistical significance. LVETc was not altered by clonidine (406 ± 7 to 408 ± 7 msec, p = ns).

Protocol B: Propranolol

Following i.v. propranolol, there was a significant reduction in HR (78 ± 5 to 63 ± 4 bpm, p < 0.01) and prolongation of PEP (103 ± 5 to 115 ± 4 msec, p < 0.01) (table 4). However, plasma NE, plasma CATs, MAP, and LVETc did not change significantly (304 ± 48 to 230 ± 13 ng/liter, 375 ± 43 to 306 ± 25, 110 ± 4 to 113 ± 4 mm Hg and 417 ± 6 to 415 ± 4.9 msec, respectively, (p = ns for all).

Table 1. Supine Resting Total Plasma Catecholamine Values

<table>
<thead>
<tr>
<th>Subject</th>
<th>Total plasma CATs (ng/liter)</th>
<th>Plasma NE (ng/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Mean 260 ± 123–545</td>
<td>Range 98–436</td>
</tr>
<tr>
<td>Essential hypertensive</td>
<td>Mean 522 ± 141–1183</td>
<td>Range 113–946</td>
</tr>
</tbody>
</table>

These values were obtained in our laboratory from a different series of patients and normal volunteers who are not included in this study.

Table 2. Baseline Characteristics of the Two Study Groups

<table>
<thead>
<tr>
<th>Subject</th>
<th>Protocol A</th>
<th>Protocol B</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>115 ± 5.3</td>
<td>110 ± 4.0</td>
<td>ns</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>84 ± 3.8</td>
<td>78 ± 4.8</td>
<td>ns</td>
</tr>
<tr>
<td>CATs (ng/liter)</td>
<td>311 ± 37.3</td>
<td>304 ± 48.5</td>
<td>ns</td>
</tr>
<tr>
<td>PEP (msec)</td>
<td>111 ± 9.3</td>
<td>103 ± 4.9</td>
<td>ns</td>
</tr>
<tr>
<td>LVETc (msec)</td>
<td>406 ± 7</td>
<td>417 ± 6.4</td>
<td>ns</td>
</tr>
</tbody>
</table>

Protocol A = patients who received one oral dose of clonidine (0.3 mg) Protocol B = patients who received i.v. propranolol (10 mg).

Table 3. Baseline Plasma Catecholamines and Response of Blood Pressure and STI to Clonidine (0.3 mg p.o.)

<table>
<thead>
<tr>
<th>No.</th>
<th>Baseline plasma NE (ng/liter)</th>
<th>∆MAP (mm Hg)</th>
<th>∆HR (bpm)</th>
<th>∆PEP (msec)</th>
<th>∆LVETc (msec)</th>
<th>∆PEP/∆MAP</th>
<th>∆NE (ng/liter)</th>
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<tr>
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<td>20</td>
<td>9</td>
<td>18</td>
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<td>0.65</td>
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<tr>
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<td>29</td>
<td>14</td>
<td>16</td>
<td>11</td>
<td>0.58</td>
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<tr>
<td>3</td>
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<td>14</td>
<td>22</td>
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<td>4.04</td>
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<tr>
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<td>418</td>
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<td>18</td>
<td>12</td>
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<td>0.34</td>
<td>-311</td>
</tr>
<tr>
<td>6</td>
<td>306</td>
<td>11</td>
<td>2</td>
<td>22</td>
<td>33</td>
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<td>10</td>
<td>12</td>
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<td>1.6</td>
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</tr>
<tr>
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<td>301</td>
<td>10</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>1.2</td>
<td>-67</td>
</tr>
<tr>
<td>12</td>
<td>545</td>
<td>28</td>
<td>36</td>
<td>43</td>
<td>10</td>
<td>2.8</td>
<td>-128</td>
</tr>
</tbody>
</table>

Abbreviations are as in table 2.
ADRENERGIC BLOCKADE AND RESTING PLASMA CATECHOLAMINES

As previously reported for propranolol, other beta blockers, and central alpha agonists there was a significant \( p < 0.05 \) correlation in both therapeutic groups between the change in HR and initial HR \( (r = 0.86 \) with propranolol, \( r = 0.72 \) with clonidine) and between changes in PEP and initial PEP \( (r = 0.69 \) with propranolol and \( r = 0.73 \) with clonidine), suggesting a significant adrenergic influence on these indices even at rest. These correlations were also significant when the changes in PEP and HR were calculated as a percentage of their respective initial values.

Correlation between Changes in STI and Resting Plasma CATs

**Group A**

Supine resting plasma NE did not correlate significantly with changes in either PEP (fig. 3) or changes in LVET \( (r = 0.31 \) and \( r = 0.16 \) respectively). Since changes in blood pressure (BP) induced by clonidine could have influenced PEP responses, PEP was also corrected for the change in MAP. Changes in both PEP and MAP were calculated as a percentage of control (pre-clonidine) values and expressed as the ratio of \( \Delta \text{PEP} / \Delta \text{MAP} \). There was also no significant correlation between this value and resting plasma NE, nor between \( \% \Delta \text{PEP} / \% \Delta \text{MAP} \) and resting plasma NE (fig. 4). Essentially, the same lack of correlation was found when ASTIs were analyzed in relation to total plasma CATs.

**Group B**

There was no significant correlation (fig. 5) between supine resting plasma NE or total plasma CATs and prolongation of PEP induced by beta blockade \( (r = 0.45, \) and \( r = 0.51, p > 0.05, \) respectively). The lack of statistical significance despite the relatively high \( r \) value might be related to the small number \( (n = 8) \) of

### Table 4. Baseline Plasma Catecholamines and Response of Blood Pressure and STI to IV Propranolol (10 mg)

<table>
<thead>
<tr>
<th>No.</th>
<th>Baseline plasma NE (ng/liter)</th>
<th>( \Delta \text{MAP} ) (mm Hg)</th>
<th>( \Delta \text{HR} ) (bpm)</th>
<th>( \Delta \text{PEP} ) (msec)</th>
<th>( \Delta \text{LVETc} ) (msec)</th>
<th>( \Delta \text{NE} ) (ng/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>361</td>
<td>2</td>
<td>22</td>
<td>16</td>
<td>9</td>
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</tr>
<tr>
<td>2</td>
<td>429</td>
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<td>9</td>
<td>5</td>
<td>-263</td>
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<tr>
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</tr>
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<td>171</td>
<td>3</td>
<td>9</td>
<td>3</td>
<td>4</td>
<td>+47</td>
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</table>

Abbreviations are as in table 2.
Discussion

Systolic time intervals (STI) have been used for a long time to assess adrenergic influences on the heart. Many investigators have documented both in animals and in man the shortening of STI following epinephrine or isoproterenol infusion. Conversely, acute intravenous propranolol lengthened PEP in normal volunteers and hypertensive patients.

A previous study has reported the interrelationship between STI measured at rest and supine resting plasma CATs. However STI at rest might be influenced by many factors other than adrenergic activity. On the other hand, STI changes following interference with adrenergic drive would conceptually give an accurate index of the effect of adrenergic influences on the heart. The correlation of these changes with circulating plasma CATs measured before adrenergic blockade would therefore help determine the relation between circulating resting plasma CATs and cardioadrenergic drive. Based on these considerations, we have used the change in STI following reduction of adrenergic activity by drugs to evaluate supine resting plasma CATs as an index of sympathetic activity at rest. Blockade of sympathetic activity was therefore achieved by two means: propranolol (10 mg i.v.) and clonidine (0.3 mg p.o.). These drugs, which interfere with adrenergic influences on the heart at different levels of the sympathetic nervous system, were chosen because of their different acute effects on BP and on circulating plasma CATs. Clonidine reduces blood pressure while intravenous propranolol does not; moreover, clonidine has been demonstrated to decrease circulating plasma CATs in non-pheochromocytoma hypertensive patients and in normal subjects, whereas i.v. propranolol does not. The diminished sympathetic effect was evidenced by slowing of HR with both drugs and the reduction of BP with clonidine. Moreover, the higher the initial level of adrenergic sympathetic influence (the shorter the PEP and the higher the HR), the greater were the changes induced by these agents (both propranolol and clonidine), as evidenced by more marked slowing of HR, and prolongation of PEP.

Previous studies using adrenolytic agents have shown that BP response to ganglion blockade, to clonidine, or to alpha blockade in combination with beta blockers correlated with resting plasma CATs. This, however, was not our experience in this group of patients; among our 12 patients who received clonidine, there was no correlation between resting supine initial plasma CATs and the change in MAP induced by the drug. These discrepancies may reflect in part the complexity of factors determining the arterial pressure in different patients. We believe, therefore, that analysis using simpler indices such as STI might be more suitable to assess the value of resting plasma CATs.

Again in our study, there was no correlation between resting plasma NE or total plasma CATs and the changes induced by either clonidine or propranolol in either HR or PEP (figs. 3 and 5). With regard to PEP, to avoid changes that could be related to differences in BP response (clonidine reduced BP while propranolol did not), the changes in PEP were corrected for the change in MAP in the same patient (% ΔPEP/% ΔMAP). Still, no correlation could be found between resting supine plasma CATs and this ratio, nor between resting supine plasma CATs and the ratio of the absolute change in PEP to the absolute change in MAP (ΔPEP/ΔMAP).

The lack of correlation between ΔSTI and basal plasma CATs was strongly established by the very low r value found in the 12 patients studied. However, in the propranolol-treated group, the r value for the correlation between resting plasma NE and ΔPEP reached 0.45 (p > 0.05 for n = 8). It is possible that a larger number of patients would have helped this correlation attain statistical significance. This possibility is strengthened by the results of Esler et al. who reported a very similar r value (r = 0.52, p < 0.02 for n = 20) for the same correlation (NE and ΔPEP). However, the low order of correlation (r² = 25%) and the dependence of statistical significance on the number of patients investigated suggest that plasma CATs are not a precise measure of adrenergic activity, at least as judged by this approach.

The biological significance of the measurement of HR and PEP was demonstrated by their significant reduction in response to adrenolytic agents. The poor correlation between these changes and resting plasma CATs suggests that the latter could not be used as a
quantitative index of adrenergic activity at rest. The difficulty in demonstrating a correlation between resting plasma CATs and the immediate cardiac response to adrenergic agents can be explained by the number of factors influencing plasma levels. Circulating plasma CATs represent the spillover from adrenergic nerve ending, and therefore their level would depend on several factors including sympathetic nervous system activity, rate of reuptake, and rate of degradation. This must not be taken to mean that the change in plasma CATs either in response to stimulation (standing or cold pressor test) or to suppression is meaningless; this change represents the effectiveness of the stimulus. Our results apply to the significance of resting plasma CATs only.

References

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