Lack of circadian rhythm of plasma concentrations of vasoactive intestinal peptide in patients with orthotopic heart transplants

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Abstract

Objective—To study the circadian pattern of plasma concentrations of vasoactive intestinal peptide (VIP) in patients with orthotopic heart transplants. Circulating VIP is known to have neural and immunological sources.

Patients and methods—13 patients with orthotopic heart transplants were studied 12–53 months (mean 31.8 months) after operation. All were haemodynamically compensated and had no histological evidence of rejection. They were being treated with cyclosporin, azathioprine, and prednisone. Ten healthy individuals were studied as controls. Circulating VIP was assayed six times within a 24 h period. Time qualified data were analysed by ANOVA and the cosinor method. Student’s t test for unpaired data and Bingham’s test for cosinor-derived parameters were used for statistical comparisons.

Results—Plasma concentrations of VIP were lower in the patients with orthotopic heart transplants than in the controls (p < 0.001). ANOVA and the cosinor method respectively showed a statistically significant within-day variability and circadian rhythm in the controls but not in the patients with heart transplants.

Discussion—The low plasma concentrations of VIP in the patients with heart transplants could be the result of the lack of contribution by the cardiac VIPergic fibres, a reduction of VIP release by the pharmacologically suppressed immune system, the inhibitory effects of cyclosporin on neural function and humoral secretions, and the effects of negative feedback on VIP release of high concentrations of atrial natriuretic peptide. The lack of the circadian rhythm suggests a structural disorder, which should be further investigated.

Vasoactive intestinal peptide (VIP) is widely distributed throughout the central and peripheral nervous system where it acts as a neurotransmitter or neuromodulator or both. The cardiovascular system of various mammalian species is innervated by VIP fibres, which are more dense in the sinoatrial node, atrioventricular node, atrial myocardium, and epicardial coronary arteries and less dense in the ventricular myocardium. The physiological role of endo-genous VIP in the cardiovascular system has not been clearly established. In adult animals and humans intravenous VIP causes vasodilatation of the systemic and coronary arteries and has positive inotropic and chronotropic effects.

VIP is assumed to be a neurotransmitter rather than a bloodborne hormone and its circulating concentrations probably represent an overflow of the neuronal release. Plasma concentrations of VIP were increased in severe hepatic failure and cardiac failure but unchanged in patients with circulatory shock or myocardial infarction.

Patients with heart transplants show abnormal circulating concentrations or circadian rhythmicity or both of the hormones responsible for the regulation of the cardiovascular system.

In the present study we investigated the hypothesis that, like other vasoactive peptides, VIP release is also impaired. We used a chronobiological protocol for this study because plasma concentrations of VIP show a circadian rhythm.

Patients and methods

We studied 13 patients with orthotopic heart transplants (10 men and 3 women, aged 27–56 years) who were haemodynamically compensated. They all had transplantation because of refractory congestive heart failure (New York Heart Association grade IV) caused by primary dilated cardiomyopathy. The patients and controls gave their informed consent. Patients with transplants were enrolled 12–53 months (mean 31.8 months) after operation when there was no histological evidence of rejection in the endomyocardial biopsy specimens. Extensive physical examination and instrumental and laboratory investigations showed normal cardiac function. Patients with transplants were treated with cyclosporin (3–5 mg/kg/24 h, three times a day in equal doses at 0800, 1600, and 2400), azathioprine (2 mg/kg/24 h, three times a day in equal doses at 0800, 1600, and 2400), prednisone (0.2–0.3 mg/kg/24 h three times a day in equal doses at 0800, 1600, and 2400). Each subject was synchronised to a light-dark cycle (light on 0700 and light off 2300) and to meal times (breakfast 0830, lunch 1230, dinner 1830). Intakes of calories (25 kcal/kg), sodium (120–140 mEq/24 h), and potassium (50–70 mEq/24 h) were normal. The per-
Cosinor-derived parameters of vasoactive intestinal peptide (VIP) circadian rhythm in controls and patients with orthotopic heart transplants

<table>
<thead>
<tr>
<th>Group</th>
<th>p</th>
<th>Mean (SE)</th>
<th>Amplitude (95% CI)</th>
<th>Acrophase (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>&lt;0.01</td>
<td>42.70</td>
<td>11.3* (1.20)</td>
<td>18-20</td>
</tr>
<tr>
<td>Patients</td>
<td>NS</td>
<td>9-08</td>
<td>0.95* (1.85)</td>
<td>2-40</td>
</tr>
</tbody>
</table>

*p < 0.001 controls vs patients (Bingham’s test).

The percentage distribution of calories among the three meals was 10%, 55%, and 35%, respectively for breakfast, lunch, and dinner. A control group of 10 clinically healthy subjects with a similar age and sex distribution were investigated according to the same protocol and meal times.

VIP concentrations were measured in plasma samples of systemic venous blood taken from an indwelling non-thrombogenic catheter in the antecubital vein at 0600, 0800, 1200, 1800, 2000 and 2400. Patients and controls had been in bed for eight hours before the first blood sample was drawn. Venous blood was drained into a Vacutainer containing aprotinin and was immediately centrifuged to separate the plasma which was stored at −40°C until assay. VIP was radioimmunoassayed according to a procedure described by Fahrenkrug.28

STATISTICAL ANALYSIS

Individual plasma VIP concentrations for each time point were summed and the mean and standard error were calculated. We used a one way ANOVA to detect the effect of time on the within-day variability of plasma VIP concentrations.

Then we used the single cosinor method29 to examine the evidence of circadian rhythm in each subject and quantify its parameters. Individual rhythmometric estimates were summarised by means of population mean cosinor30 to statistically validate the group-related circadian rhythm and measure its parameters. The cosinor method is a periodic regression analysis for fitting a cosine function, \( Y_t = M + A \cos(\omega t + \phi) \), to raw data by the least squares method. In the formula \( M \) is mesor (midline estimating statistic of rhythm); \( A \) is amplitude, the extent of sinusoidal oscillation from \( M \); and \( \phi \) is acrophase, the timing of the oscillatory crest related to local midnight.

For between-group comparisons we used Student’s \( t \) test for the time-qualified mean values and Bingham’s test for the rhythmometric mean estimates.31

Results

The figure shows the mean plasma VIP concentrations in the patients and controls. Plasma VIP concentrations changed in both groups. One-way ANOVA showed that within-day variability was statistically significant in the controls (\( p < 0.01 \)) but not in the transplant patients (\( p > 0.05 \)). The mean plasma VIP 24 h concentration was significantly lower (\( p < 0.001 \)) in the heart transplant patients than in the controls (8.89 pg/ml vs 47.28 pg/ml) (\( t \) test).

The table shows the rhythmometric parameters estimated by the population mean cosinor. The circadian pattern of plasma VIP concentrations was statistically significant in the controls but not in the transplant patients. In the controls circulating VIP reached a peak late in the afternoon. Mesor and amplitude were significantly lower (\( p < 0.001 \)) in the transplant patients than in the controls (Bingham’s test).

Discussion

We found that plasma VIP concentrations were much lower in the transplant patients than in the controls and also that the normal circadian pattern was abolished in the transplant patients. It is difficult to explain these results because little is known about circulating VIP concentrations in disease. Plasma VIP concentration was within the normal range in patients with liver transplants.32 The decrease in circulating VIP in elderly people was ascribed to the age-related involution of VIPergic neurons.27 The finding that VIPergic fibres are less dense in transplanted hearts suggests that the low concentrations of VIP in patients with heart transplants could be caused partly by the lack of a cardiac source in such patients. But VIP is also synthesised and secreted by lymphocytes36–38 so that the pharmacological suppression of the immune system in transplant patients could account for their low plasma VIP concentrations. In addition cyclosporine itself interferes both with neural function39 and hormone secretion.39–43

It is also possible that humoral factors had an indirect effect. VIP acts as a natriuretic factor44 and inhibits the release of atrial natriuretic peptide (ANP) by the heart.45 There is convincing evidence that ANP is increased in patients with heart transplants.46 So it could be that high circulating concentrations of ANP inhibit the release of VIP in transplant patients. Such an interaction suggests a negative feedback between VIP and ANP. Another hypothesis is suggested by the normal peak of plasma VIP concentration late in
the afternoon. Though the within-day variability may be caused by an inherently endogenous rhythm, it is also possible that VIP release is synchronised by meals,48 with the elevation of energetic demands being a pivotal role in the physiological 24 h periodicity of circulating VIP. The lack of the circadian rhythm could be a sign that gut-derived VIP release was inhibited by the immunosuppressive therapy.

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