Twenty-four-hour rhythms of plasma catecholamines and their relation to cardiovascular parameters in healthy young men

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Abstract
Diurnal and ultradian rhythms of plasma norepinephrine and epinephrine and their role in the regulation of cardiovascular parameters were investigated over 24 h of recumbency in a group of five men. Catecholamines were measured at 10 min intervals, and blood pressure and heart rate were recorded continuously. Norepinephrine and epinephrine rapidly fluctuated in each subject, with no obvious diurnal rhythm. Spectral analysis suggested two ultradian rhythms with periods of around 12 h and 50–100 min for both catecholamines. The pulse detection programs PULSAR and CLUSTER revealed 20–30 pulses/24 h for norepinephrine and epinephrine, with a significant correlation between the two rhythms ($r = 0.63–0.80$, $P < 0.001$). Neither the frequency nor the amplitude of these rapid fluctuations differed between day and night. Arousal in the morning caused a small increase in plasma catecholamines and getting out of bed a large increase. Thus changes in posture and activity are the main influences on the diurnal variations of plasma catecholamines reported previously, while the ultradian rhythms of sympathoadrenomedullary activity appear to be of intrinsic origin. Blood pressure and heart rate exhibited a diurnal rhythm with a nightly decrease. Arousal and rising from bed increased blood pressure and heart rate significantly. Although the amplitude of the rapid fluctuations of plasma catecholamines at times exceeded those caused by postural changes in the morning, when both plasma norepinephrine and epinephrine levels correlated highly with all cardiovascular parameters, correlations were not significant during recumbency. Thus the intrinsic ultradian fluctuations of plasma catecholamines appear not to be involved in the control of cardiovascular parameters during recumbency, and the increase in blood pressure and heart rate in the morning appears to be controlled by direct sympathetic neural input to the heart and vasculature in response to changes in activity and posture rather than by an endogenous surge of plasma catecholamines.

European Journal of Endocrinology (1997) 137 675–683

Introduction
Norepinephrine and epinephrine are the predominant mediators of sympathoadrenomedullary regulation and play key roles in the regulation of cardiovascular function, airway reactivity, energy metabolism and many other processes. Norepinephrine is released from postganglionic sympathetic nerves (1), and both norepinephrine and epinephrine are secreted from the adrenal medulla (1). Diurnal changes in plasma and urinary catecholamines have been reported, with the lowest levels occurring at night (2–8). In addition, ultradian variations in plasma norepinephrine have been suggested to occur in humans (9) and the rhesus monkey (10). These short-term fluctuations in plasma norepinephrine, however, were only investigated over short periods of time, and it is therefore not known whether they persist throughout the day and whether they are subject to diurnal changes, as has been reported from ultradian rhythms in other hormonal systems (11–13). Several lines of evidence suggest that diurnal changes in plasma catecholamines underly the diurnal pattern of the onset of unfavorable cardiovascular events such as myocardial infarction (14–16). It is therefore important to understand the mechanisms underlying the diurnal changes in plasma catecholamines for the design and timing of drug regimes to prevent or reduce cardiovascular events during this vulnerable period of the day. However, it is still not known whether the diurnal changes in plasma catecholamines arise from endogenous rhythms or whether they fluctuate as a function of posture and activity (3, 5, 7). Furthermore, the reported short-term fluctuations in plasma norepinephrine are of considerably higher amplitude than the diurnal changes, and they may well be equally important for the regulation of cardiovascular functions under both physiologic and pathophysiologic conditions. To investigate further diurnal and ultradian rhythms of plasma catecholamines and their role in the regulation of
cardiovascular parameters, we determined the plasma concentrations of norepinephrine and epinephrine at 10 min intervals and measured blood pressure and heart rate continuously by means of the Portapres system in supine subjects over 24 h. In a second experimental situation the effect of arousal and postural changes on plasma catecholamines and on cardiovascular parameters in the early morning hours were studied in more detail.

**Subjects and methods**

**Subjects**

Five normotensive healthy young men, aged 24–26 years, were studied. All subjects had a similar lifestyle and a regular sleep–wake cycle. Screening of the subjects included a medical history, a physical examination, routine laboratory testing and an electrocardiogram. Informed consent was obtained from each subject, and the study was approved by the local ethical committee.

**Sample collection**

Each subject was studied on two occasions separated by 3 to 6 weeks. No alcohol, smoking or coffee was allowed 12 h before and during the study period. In a first experimental set-up an indwelling central venous catheter was put in place and the Portapres system (TNO, Amsterdam, Netherlands), which allows continuous indirect non-invasive measurement of beat-by-beat blood pressure and heart rate (17, 18), was installed. Although not directly tested in our study group, Portapres measurements of blood pressure correspond closely to intra-arterial measurements, both at rest and during physiologic changes in blood pressure (17). Each subject was fitted with two Portapres cuffs appropriate for the size of the fingers tested. During the study period the Portapres system switched from one finger to the other every 30 min for the subject’s comfort. The blood pressure values determined by the Portapres system were confirmed by single controls using conventional blood pressure measurements. After this set-up the subjects rested in a supine position for at least 2 h before the commencement of blood sampling and until the end of the study. Sampling was started at 1800 h, and 2·7 ml blood were drawn every 10 min for the subsequent 24 h through an extension tube. The total volume of blood taken was approximately 400 ml/24 h. The catheter was kept open by a slow infusion of saline (1 litre/24 h). Blood samples were collected into prechilled EGTA-containing tubes, plasma was separated immediately in a refrigerated centrifuge, and stored in aliquots at −70 °C until analysis. Meals were provided at 0800, 1200 and 1730 h. Sleep was allowed from 2300 to 0700 h. During this period the lights were switched off. In a second experimental set-up, an indwelling venous catheter was put in place, and non-invasive monitoring of heart rate and blood pressure using the Portapres system was started at 2100 h. At 2300 h the lights were switched off and the subjects were allowed to sleep. A 2·7 ml sample of blood was taken at 2 min intervals starting at 0530 h the following morning while the subjects were asleep and ended at 0715 h. At 0600 h the subjects were aroused by an alarm clock and the lights were switched on. At 0645 h the subjects got up and stood erect until the end of the study.

**Assays**

Plasma epinephrine and norepinephrine were measured in duplicate by a single isotope COMT radioenzymatic assay (19) combined with separation of the labeled metabolites by reverse-phase HPLC before scintillation counting. The lower detection limit of this method was 5·6 pg/ml (epinephrine) and 7·8 pg/ml (norepinephrine). In our laboratory the intra-assay and interassay coefficients of variation at the relevant plasma levels were 5·2 and 8·8% for epinephrine and 4·6 and 7·2% for norepinephrine respectively. Parts of the 24 h rhythms were repeated independently and superimposable data were obtained. All measurements were performed within 8–12 weeks of sampling.

**Analysis of rhythms and statistics**

The 24 h rhythms were analyzed by calculating the power spectrum of the time series (MATLAB, The MATHWORKS Inc.) and by using the heuristic pulse-detection programs CLUSTER (20) and PULSAR (21). Two points were chosen for scanning for significant increases and decreases in the hormone concentration time series by the CLUSTER program using a pooled t statistic. The threshold for the t values was defined as 2·0 for an upstroke and 2·0 for a downstroke. For the PULSAR program, the G parameters used for pulse detection were G(1)=4·40, G(2)=2·60, G(3)=1·92, G(4)=1·46, G(5)=1·13. We used a 4 h sliding window to compute the moving average of the individual time series. Data are presented as the mean±S.D., or values of individual subjects are given. Statistical analysis was performed by the two-tailed paired or unpaired Student’s t-test and Fisher’s r to z transformation for statistical significance of correlation coefficients using StatView software. The level of significance was taken as P<0·05.

**Results**

**Twenty-four-hour rhythms of plasma norepinephrine and epinephrine**

Plasma concentrations of norepinephrine and epinephrine fluctuated widely throughout the 24 h in each subject.
while no obvious diurnal rhythm related to the light–dark cycle could be observed (Figs 1 and 4, Table 1). This was true for the group means of norepinephrine and epinephrine, while in some individuals the means for norepinephrine or epinephrine during darkness were significantly higher or lower than the corresponding means during the light period (Table 1). Spectral analysis for both catecholamines revealed an increased relative power at about 12 h and a second peak at around 60 min and 50–100 min for norepinephrine and epinephrine respectively (Fig. 2). To determine the peaks and troughs for the individual 24 h rhythms, the data of each subject were smoothed by moving average analysis using a time window of 4 h. The smoothed curves of the five subjects are given in Fig. 3. Consistent with a slow ultradian rhythm (period about 12 h) the smoothed curves show more than one (usually two) troughs and peaks (Fig. 3). However, the peaks and troughs of the individual rhythms varied widely over the 24 h with no evidence of synchronization among subjects or a clear relation to the light–dark cycle (Fig. 3). Short-term fluctuations in both norepinephrine and epinephrine were observed in all subjects. In agreement with the faster ultradian rhythm of the spectral analysis (period 50–100 min), the pulse-detection programs PULSAR and CLUSTER revealed 20–30 pulses/24 h for norepinephrine and epinephrine (Fig. 4, Table 2). The frequency of these fluctuations was almost identical for both catecholamines (Fig. 4, Table 2), and in each subject the fluctuations in norepinephrine and epinephrine coincided with a correlation coefficient at zero phase shift of \( r=0.63–0.80 \) \((P<0.001)\). These rapid fluctuations persisted throughout the 24 h, without any significant changes in frequency or amplitude between the light and dark period, suggesting that these short-term fluctuations in sympathoadrenomedullary activity are not subject to diurnal variations (Table 2).

**Twenty-four-hour measurements of cardiovascular parameters and correlations with plasma catecholamines**

During the 24 h, systolic and diastolic blood pressure and heart rate were continuously recorded by means of the Portapres system and averaged over 2 min intervals. In contrast with plasma norepinephrine and epinephrine, both blood pressure and heart rate clearly showed a diurnal variation with the lowest values occurring during darkness in all subjects (Fig. 1). During the daytime (0700 h–2300 h), mean values for heart rate, systolic and diastolic blood pressure were 76 ± 4 beats/min, 137 ± 6 mmHg and 70 ± 4 mmHg; they were 66 ± 3 beats/min, 121 ± 5 mmHg and 61 ± 4 mmHg during the night (2300 h–0700 h). Differences in the means for systolic and diastolic blood pressure as well as heart rate between the light and dark period were highly significant, both individually and for the group \((P<0.001)\) for each parameter. A highly significant correlation between mean heart rate and mean systolic and diastolic blood pressure pooled at 2 min intervals was found at zero phase shift \((r=0.80 \text{ and } 0.52, \; P<0.001)\). In contrast, plasma catecholamine levels pooled at 10 min intervals did not correlate...
Table 1 Means ± S.D. levels of plasma norepinephrine and epinephrine n.s., not significant in the five subjects and of the pooled levels during the light and the dark period.

<table>
<thead>
<tr>
<th></th>
<th>Light (0700–2300 h)</th>
<th>Dark (2300–0700 h)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td></td>
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<tr>
<td>S1</td>
<td>251 ± 74</td>
<td>217 ± 73</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>S2</td>
<td>160 ± 40</td>
<td>196 ± 21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S3</td>
<td>154 ± 57</td>
<td>203 ± 58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S4</td>
<td>128 ± 41</td>
<td>144 ± 40</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>S5</td>
<td>378 ± 116</td>
<td>349 ± 139</td>
<td>n.s.</td>
</tr>
<tr>
<td>Group mean</td>
<td>199 ± 35</td>
<td>211 ± 36</td>
<td>n.s.</td>
</tr>
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| Epinephrine (pg.ml) |                     |                    |         |
| S1                 | 75 ± 19             | 64 ± 13            | <0.001  |
| S2                 | 50 ± 16             | 58 ± 11            | <0.01   |
| S3                 | 66 ± 22             | 70 ± 18            | n.s.    |
| S4                 | 36 ± 12             | 37 ± 9             | n.s.    |
| S5                 | 92 ± 23             | 96 ± 29            | n.s.    |
| Group mean         | 58 ± 10             | 58 ± 7             | n.s.    |

Figure 2 Mean of the power spectra of individual 24 h plasma catecholamine rhythms. A, norepinephrine; B, epinephrine.
with the cardiovascular parameters pooled at 2 min intervals, either at zero phase shift or after various time lags ranging from −8 min to +8 min during 24 h of recumbency (not shown).

**Effect of arousal and posture on plasma catecholamines and cardiovascular parameters**

To define more clearly the role of changes in activity and posture as potential causes for diurnal changes in plasma catecholamine concentrations, we investigated the effects of arousal and orthostasis on plasma catecholamines and cardiovascular parameters. Norepinephrine and epinephrine were determined every 2 min, and heart rate, systolic and diastolic blood pressure were recorded continuously and averaged at 1 min intervals. Arousal caused a small but significant increase in plasma norepinephrine and epinephrine, systolic and diastolic blood pressure, and heart rate, whereas rising from bed and standing upright caused an abrupt and large increase in plasma catecholamines, blood pressure and heart rate (Table 3). Under these conditions, the pooled means for norepinephrine and epinephrine correlated highly significantly with the pooled means for systolic blood pressure ($r=0.94$ and $r=0.91$ respectively, $P<0.001$), diastolic blood pressure ($r=0.92$ and $r=0.87$ respectively, $P<0.001$), and heart rate ($r=0.94$ and $r=0.93$ respectively, $P<0.001$) at zero phase shift.
Discussion

In the present study we determined 24 h rhythms of plasma norepinephrine and epinephrine measured at 10 min intervals in healthy supine subjects. No evidence for an endogenous diurnal rhythm of plasma catecholamines with a nightly decrease was found. This is supported by several findings. First, the group mean for norepinephrine and epinephrine was not different between day and night, and in some subjects the plasma catecholamine concentrations at night were higher than during the day. Secondly, spectral analysis of the 24 h rhythms of norepinephrine and epinephrine revealed no diurnal rhythm. Thirdly, the peaks and troughs of the individual 24 h rhythms smoothed by moving average analysis varied widely between subjects with no clear relation to the light–dark cycle. Fourthly, the short-term fluctuations in both catecholamines showed no significant changes in the frequency or amplitude between the light and dark period. This contrasts with previous reports in which diurnal rhythms of plasma norepinephrine or epinephrine have been described (3, 5–8). In contrast with our study in which catecholamines were determined in
central venous blood, most groups measured the plasma concentrations of norepinephrine or epinephrine in venous forearm blood. In venous forearm blood, however, norepinephrine in particular is disproportionately influenced by forearm sympathetic activity (1) which might exhibit a profound day to night variation because of changes in forearm activity, even in otherwise strictly supine subjects (6, 8). Changes in body position as little as 25° in the angle of recumbency result in significant differences in catecholamine secretion (22). In several studies describing diurnal changes in plasma catecholamines, posture was either uncontrolled (3) or changed during the 24 h sampling period (5–7). Thus the diurnal changes in plasma catecholamines reported by others (3, 5–8) could be explained by variations in regional sympathetic activity and body position. This, together with the morning increase in plasma catecholamines caused by arousal and rising from bed, indicates that predominantly external factors rather than a true endogenous pacemaker underlies the reported diurnal changes in plasma catecholamines (3, 5–8). This view is supported by recent findings demonstrating no endogenous increase in muscle sympathetic nerve activity at rest in the early morning hours (23).

Plasma norepinephrine and epinephrine fluctuated widely during the 24 h. Spectral analysis of the 24 h profiles suggested the presence of two ultradian rhythms for both norepinephrine and epinephrine with a period of about 12 h and between 50 and 100 min. Consistent with a slow ultradian rhythm with a frequency of about one cycle/12 h, the moving averages of the 24 h rhythms showed two significant troughs and peaks in four of the five subjects. The pulse detection programs PULSAR and CLUSTER found about 20–30 significant pulses for norepinephrine and epinephrine per 24 h profile, which fits a faster ultradian rhythm with a frequency of around one cycle/60 min. There was a high correlation between the two catecholamines at zero phase shift in the individual subjects. As circulating plasma norepinephrine and epinephrine originate from different sources and reflect different aspects of sympathetic nervous system activity, this suggests a coordinated and common cause for the ultradian rhythms. In animal studies, pulsatile release of norepinephrine and epinephrine has been demonstrated in the hypothalamus, which exhibited two ultradian rhythms with periods of about 12 h and 90–100 min (24). As in our study, both norepinephrine and epinephrine fluctuated almost in phase. This points to centrally located oscillators in the brain underlying the ultradian rhythms of plasma norepinephrine and epinephrine. Alternatively, the sympathetic nervous system including the adrenal medulla could be perceived as a functional network with intrinsic oscillatory features that causes episodic bursts of norepinephrine and epinephrine secretion.

In accordance with previous results from supine subjects, heart rate and blood pressure decreased at night and increased with arousal in the morning. This occurred in all subjects and is consistent with a diurnal rhythm of cardiovascular parameters coupled to the sleep–wake cycle (6, 8). Since it has been shown that the nocturnal decrease in blood pressure and heart rate is due to sleep in subjects kept completely recumbent (25, 26), this indicates that sleep occurred in our subjects even though no sleep recordings were performed. It further demonstrates that consistent diurnal rhythms could be reliably observed in our study despite the small number of subjects. Despite the fact that the amplitude of the fast ultradian rhythms of norepinephrine and epinephrine sometimes exceeded those caused by a postural change in the morning, when levels of both catecholamines correlated highly with all cardiovascular parameters, correlations were not significant during recumbency, indicating that other factors, such as an increased parasympathetic tone at night, may underly the diurnal rhythms of blood pressure and heart rate in supine subjects (27, 28). The discrepancy could be explained by changing contributions of individual organs to circulating plasma catecholamines, especially circulating norepinephrine, under different conditions (1, 29, 30). While the increase in plasma norepinephrine during orthostatic stress may predominantly arise from an increased neural sympathetic input...
to the cardiovascular system, the contribution of the cardiovascular system to fluctuations in plasma norepinephrine during recumbency might be minimal (29, 30). Hence significant correlations between plasma catecholamines, especially norepinephrine, and cardiovascular parameters are expected during orthostatic stress but not during recumbency. This, however, implies that it is not the circulating plasma catecholamines that predominantly control cardiovascular parameters, and care has to be taken when correlations between plasma catecholamines and cardiovascular parameters are calculated and causal relationships are being suggested. The physiologic role, however, of the endogenously occurring short-term fluctuations of plasma catecholamines remains to be established. They have to be taken into account when plasma catecholamines are used as indicators of sympathetic activity.

Several studies have reported an increased occurrence of unfavorable cardiovascular events in the morning (14–16, 31–33), and an increase in plasma catecholamines has been suggested as one precipitating factor amongst others (14–16). Our findings argue against an intrinsic catecholaminergic surge causing a prominent increase in heart rate and blood pressure in the morning, but rather indicate that postural changes trigger an increase in heart rate and blood pressure thereby increasing cardiac oxygen demand. This fits very well with studies in which the time of onset of symptoms of myocardial infarction could be closely related to awakening (33) and to a change in posture in the morning (16). If our observations can be transferred to patients suffering from ischemic heart disease, then this could have implications for the design and timing of therapeutic regimens.

References


