CIRCADIAN FLUCTUATION OF PLASMA EPINEPHRINE IN SUPINE HUMANS

Oliver G. Cameron,*§ George C. Curtis,* Thomas Zelnik,* Daisy McCann,†
Thomas Roth,‡ Kenneth Guire§ and Marla Huber-Smith‖

*Department of Psychiatry, University of Michigan; †Departments of Biochemistry and Internal Medicine, University of Michigan, and University Medical Affiliates; ‡Department of Psychiatry, University of Michigan, at Henry Ford Hospital; §School of Public Health, and Statistical Research Laboratory, University of Michigan; and ‖University Medical Affiliates

SUMMARY

Previous studies have demonstrated circadian fluctuations of systemic catecholamines in man. However, methodological differences and conflicting results with epinephrine are apparent. In the present study, plasma and urinary epinephrine and norepinephrine and plasma cortisol were studied in healthy young adult males over 24 hr with 20 min plasma sampling and EEG monitoring of sleep. Plasma epinephrine did not have a circadian variation in supine subjects. Urinary epinephrine levels and small urinary circadian variations were increased by normal posture and activity. Sleep and sleep stage were not associated with different plasma epinephrine levels, and no ultradian fluctuation was observed. Levels of norepinephrine and cortisol were normal. Based on all studies to date, it appears that basal plasma epinephrine has either a very small amplitude or no circadian rhythm, but that changes in posture and activity or the rest/activity cycle may modify this pattern.

INTRODUCTION

CIRCADIAN rhythmic fluctuations of physiological functions are ubiquitous from single-celled organisms to man. Among these are the rhythms of the systemic catecholamines epinephrine and norepinephrine. Control of catecholamines is of particular psychoendocrinological interest because of the changes in levels associated with stress and anxiety (Frankenhauser, 1971; Rose, 1980; Ward *et al.*, 1983).

Urinary catecholamine levels in man fluctuate over 24 hr, with highest levels during the day (Karki, 1956; Froberg *et al.*, 1972; Townshend & Smith, 1973; Linsell *et al.*, 1985). However, only two studies used healthy subjects who maintained supine posture throughout. One study (Faucheux *et al.*, 1976) demonstrated a maximum level of both epinephrine and norepinephrine between 0830 hr and 2030 hr. In the second study (Ratge *et al.*, 1982), highest levels for both catecholamines were at 0800 hr – 1000 hr, with lowest levels at 0200 hr – 0400 hr.

Circadian fluctuations of plasma catecholamines also have been studied (Turton & Deegan, 1974; Prinz *et al.*, 1979, 1984; Barnes *et al.*, 1980; Mullen *et al.*, 1981; Linsell *et al.*, 1985). Four studies that used healthy subjects who maintained a supine posture considered norepinephrine (Aronow *et al.*, 1978; Stene *et al.*, 1980; Winer *et al.*, 1980;

¶Correspondence to: Dr Oliver G. Cameron, Department of Psychiatry, University of Michigan Hospitals, Ann Arbor, MI 48109, U.S.A.

Ratge *et al.*, 1982). Significant variations were observed, with the peak in the morning and the trough during the later part of sleep.

Unlike norepinephrine, the presence of a circadian rhythm of basal plasma epinephrine remains unresolved. In a detailed study (Linsell *et al.*, 1985), a significant rhythm was reported in healthy subjects. However, supine posture was assumed for only a few minutes before each sample, and increases in plasma epinephrine associated with upright posture have been observed (Lake *et al.*, 1984; Lake & Ziegler, 1985). Furthermore, in three studies in which supine posture was maintained, the results were inconclusive. In one study (Aronow *et al.*, 1978), only one of five subjects showed a significant fluctuation. In the second study (Ratge *et al.*, 1982), a significant but small fluctuation (– 20% to + 20% of the mean value) was observed, with the peak at mid-day and the trough at mid-sleep. In the third (Barnes *et al.*, 1980), the peak was approximately 200% of the trough; however, only two specimens, at 0400 hr and 2000 hr, were obtained.

Parameters of plasma fluctuations have been studied. One study reported an ultradian (less than 24 hr) plasma norepinephrine fluctuation (Levin *et al.*, 1979), but two others did not (Mullen *et al.*, 1981; Linsell *et al.*, 1985); epinephrine did not appear to have an ultradian rhythm (Linsell *et al.*, 1985). Several studies found no association between sleep stages and plasma norepinephrine levels (Brezinova & Carruthers, 1974; Prinz *et al.*, 1979, 1984; Stene *et al.*, 1980; Mullen *et al.*, 1981; Linsell *et al.*, 1985), and a similar lack of association between sleep stages and plasma epinephrine has been reported (Brezinova & Carruthers, 1974; Prinz *et al.*, 1979, 1984; Linsell *et al.*, 1985). However, only one of these studies involved healthy supine subjects (Stene *et al.*, 1980), and this study did not examine epinephrine. Finally, covariation of the two catecholamines has been reported (Turton & Deegan, 1974; Ratge *et al.*, 1982; Linsell *et al.*, 1985).

Since the existence of a circadian fluctuation of resting plasma epinephrine remains ambiguous, epinephrine levels were determined frequently in healthy subjects who remained strictly supine throughout the study. Furthermore, neither the relationship between epinephrine levels and sleep nor the presence of ultradian fluctuations have been studied in supine subjects. In order to document normal circadian fluctuations in these subjects, plasma norepinephrine and cortisol (Weitzman *et al.*, 1971; Gallagher *et al.*, 1973) levels also were studied. Finally, as a further comparison with prior research, urinary levels were studied while subjects remained supine and during normal ambulant activity.

METHODS

Subjects

The subjects were four healthy drug-free men between the ages of 21 and 35. They neither smoked nor excessively used caffeinated beverages. All were on a normal night-time-sleep, day-time-awake circadian cycle prior to and during the study. The study was approved by the institutional human research review committee, and all subjects gave informed consent.

Procedure

Two experiments were performed. In Experiment I, frequent sampling of plasma catecholamines for 24 hr was done, together with EEG monitoring of sleep. In order to promote habituation to the experimental situation, the subjects slept with EEG leads in place in the sleep laboratory on the night before the study. On the day before, the subjects avoided strenuous exercise. The starting time was counterbalanced, with two subjects starting in the morning (one at 0800 hr and one at 1200 hr) and two in the evening (both at 2000 hr). The subjects had indwelling intravenous catheters for blood sampling, which ran into an adjoining room. They assumed a supine

posture in bed at least 1 hr before the study started; thereafter, they strictly maintained a supine posture throughout the experiment (the subjects had their heads raised 30° to eat, and they used a bedpan). EEG electrodes were in place throughout the study; recording of sleep EEG was done throughout the lights-out period. Lights were out from 2300 hr to 0700 hr and on at other times; sleep was not permitted when lights were on. Meals were served at 0800 hr, 1200 hr and 1800 hr on the study day. The diet avoided caffeinated beverages and other foods or beverages known to be high in substances which affect catecholamine levels; salt intake was *ad libitum* (Robertson *et al.*, 1979; Stene *et al.*, 1980). Seven ml blood specimens were obtained every 20 min for 24 hr. In order to minimize specimen dilution or volume or red cell depletion through the duration of the study, the indwelling catheter was flushed of saline before each specimen was drawn; all flush blood was re-infused; and the volume was replaced with normal saline. The specimens were assayed for plasma epinephrine, norepinephrine, and cortisol, as described below.

In Experiment II, urinary creatinine and unconjugated epinephrine and norepinephrine were measured twice for 24 hr. Three of the four subjects participated. Dietary control was the same as in Experiment I. During one of the 24 hr periods, the subjects maintained normal daily activities but avoided strenuous exercise. Urine was collected in four 4-hr aliquots and one 8-hr aliquot (0800 – 1200 hr, 1200 – 1600 hr, 1600 – 2000 hr, 2000 – 2400 hr and 2400 – 0800 hr). During the other 24-hr period, urine was collected in two 12-hr aliquots, 0800 – 2000 hr and 2000 – 0800 hr. The subjects remained supine for at least 6 hr before and throughout this period. Urine specimens were assayed for creatinine and unconjugated epinephrine and norepinephrine only; cortisol was not measured.

The sleep EEG data included the standard central (C3) and occipital (OZ) electroencephalograms, electrooculogram, submental electromyogram, and electrocardiogram recorded with a V5 lead. Records were scored for sleep stages according to standard criteria (Rechtschaffen & Kales, 1968).

Specimen analysis

All urine specimens were collected in plastic bottles containing 5% acetic acid. The specimens were kept chilled during collection and then frozen until assay for epinephrine and norepinephrine. Blood for catecholamines was collected in chilled tubes containing reduced glutathione, an antioxidant, and EGTA, an anticoagulant; blood for cortisol was collected in chilled heparinized tubes. Plasma was separated within 10 min of collection and frozen until assay. Unconjugated epinephrine and norepinephrine in urine and plasma were assayed with a single-isotope radioenzymatic method (Peuler & Johnson, 1977; McCann & Huber-Smith, 1984). The assay sensitivity for both catecholamines is 20 pg/ml. Both the intra- and interassay coefficients of variation for norepinephrine are 11%, and for epinephrine 18%, based on sample contents of approximately 300 and 100 pg/ml, respectively. Cortisol was determined with a competitive protein binding assay (Wilens *et al.*, 1984). The assay sensitivity for cortisol is 0.4 μg/ml. The intra- and interassay coefficients of variation are both approx 7%. Creatinine in urine was determined by a colorimetric method with the "Rapid-Stat" kit.

Statistical analysis

Differences between subjects and between times, and the interaction of subjects by time, were determined. Because plasma levels of all three substances were demonstrated (by least squares regression analysis) to increase from the first to the last specimen drawn from each subject in Experiment I, analyses of covariance were performed in which these trends were removed statistically, and then differences of residual values were evaluated. A repeated-measures analysis of variance then was performed on these residuals. A multiple regression analysis also was performed, which additionally permitted tests of significance of sine and cosine components of the regression. A significant result indicated that a regular cyclical fluctuation in the data over 24 hr was present (Harris, 1975). Pearson product – moment correlations were calculated for each pair of hormones. Analyses of variance with two variables, subjects and time, were used in Experiment II.

Spectral analyses were performed within each subject and substance in Experiment I to determine if ultradian rhythms were present. Spectral analysis is a form of time series analysis by which data can be examined for consistent periodic fluctuations of different frequencies (Chatfield, 1980). Also, spectral analyses across substances, with and without temporal phase changes between substances, were studied to determine if plasma levels were associated (i.e., in phase) with each other. Finally, comparisons were performed (by analysis of variance) for each substance during different sleep stages in Experiment I.

RESULTS

Experiment I:

Three of four subjects showed statistically significant tendencies for plasma epinephrine levels to increase from the first to the last specimen (regression analysis, $p < 0.0005$, 0.002, and 0.05). Therefore, these trends were removed by analyses of

covariance. Substantial sample-to-sample variation was observed (Fig. 1). Although a decrease in mean level for the four subjects appears to have occurred at 0100 to 0200 hr, no statistically significant circadian fluctuation was demonstrated with either the repeated-measures or the regression analyses (Fig. 2). Sleep was normal in all four subjects. No ultradian rhythm or relation to sleep or sleep stage (Table I) was observed.

Two of four subjects showed highly significant increases from the first to the last norepinephrine specimen (regression analysis, $p < 0.0002$) and one other showed a trend ($p < 0.07$). Therefore, subsequent analyses again were performed on detrended data. By regression analysis, a significant difference over time for the mean level of the four subjects at each time point was observed ($p < 0.01$), with a significant cosine component ($p < 0.01$) but no significant sine component. The highest values were in the late morning and the lowest just after midnight (Fig. 2). However, the repeated-measures analysis was not statistically significant. Regression analyses for individual subjects indicated that one had a significant fluctuation ($p < 0.02$) and a second showed a trend ($p < 0.08$), but two others were not significant. Interestingly, the subject with the least fluctuation had the highest mean 24-hr norepinephrine levels. For the mean of the four subjects, the amplitude was small, with the peak being only 165% of the trough. No association with sleep stage was observed (Table I). All four subjects demonstrated ultradian rhythm peaks at 0.25, with spectral densities ranging from 2.0 to 4.9, suggesting an ultradian rhythm for norepinephrine of approximately 80 min. Two subjects also had peaks at 3 hr and two at 11 hr.
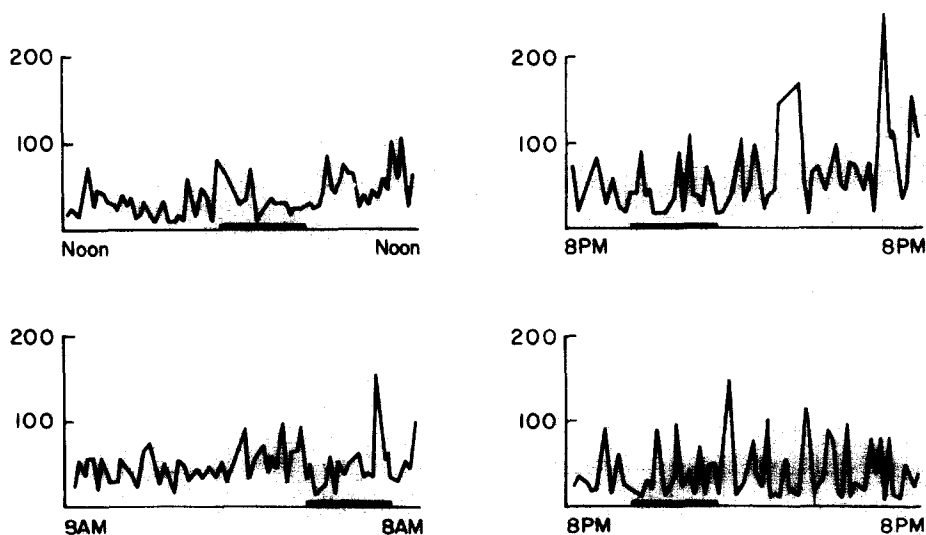

FIG. 1. Individual plasma epinephrine (E) values (pg/ml) every 20 min from each of four normal subjects, with time of start and end indicated for each subject. Thick black line on abscissa indicates time of lights out.
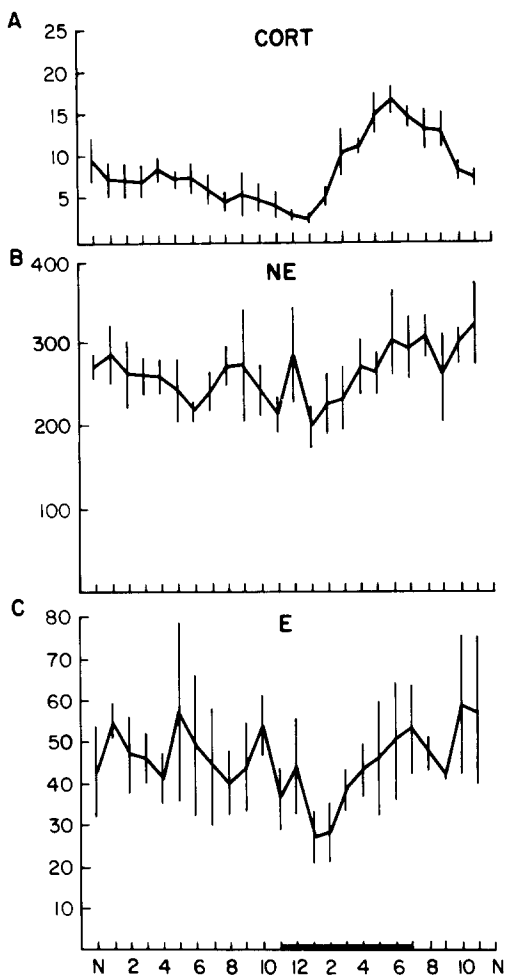
Fig. 2. Circadian pattern of plasma cortisol (Cort, μg/dl, panel A), norepinephrine (NE, pg/ml, panel B), and epinephrine (E, pg/ml, panel C) (mean ± S.E.M.) from 1200 hr (noon-N) to 1200 hr. Thick black line on abscissa is time of lights out. Each hourly point is the mean of 12 data points—three values at 20-min intervals for each of four subjects.

Cortisol also demonstrated a highly-significant variation over time (for both analyses, $p < 0.0001$), without significant rises from the first to the last specimen (Fig. 2). In agreement with prior research (Weitzman et al., 1971; Gallagher et al., 1973), the lowest level was at 0100 hr and the highest at 0600 hr. Thus, the levels observed for norepinephrine and cortisol document normal endocrine functioning in these subjects under the conditions of this study. As with the catecholamines, no significant association with sleep or sleep stage for cortisol (Table I) was observed, and, similar to epinephrine, cortisol showed no ultradian rhythm.

TABLE I.

Sleep stage	Awake	REM	NREM 1	NREM 2–4
N	214	13	17	48
Epinephrine (pg/ml)	+1.99	−7.02	+6.10	−9.11
Norepinephrine (pg/ml)	+5.34	+29.83	−27.71	−21.36
Cortisol (μg/dl)	−0.12	+3.27	+0.82	−0.66

Sleep stages include REM (rapid eye movement) and NREM (non-REM) stages 1 and 2 through 4 combined. N represents the total number of observations from all four subjects of Experiment I in each stage. The scores represent mean differences per determination above (+) or below (−) the overall grand means for each hormone. Although there appears to be a difference between REM and NREM for norepinephrine, and REM vs other times for cortisol, none of the differences are statistically significant. Mean total sleep time for the four subjects was 7.0 hr.

Correlations were performed between the three pairs of hormones studied over time, separately for each subject and also for the mean of all four subjects at each time point. In order to reduce within-subject variability between specimens, the means of three consecutive specimens for each subject (i.e., over 1 hr) were used. The correlations for individual subjects ranged from −0.09 to +0.70 for epinephrine × norepinephrine, −0.02 to +0.48 for cortisol × norepinephrine, and −0.17 to +0.15 for cortisol × epinephrine. The correlations for the combined data of all four subjects were +0.62 ($p < 0.002$), +0.46 ($p < 0.02$), and +0.30 ($p < 0.20$), for the same three comparisons, respectively. Thus, at least for the aggregate data, the levels of these substances are in phase with each other, although for cortisol × epinephrine the correlation was only weakly positive and not significant. Similarly-calculated correlations with phase shifts of up to 3 hr between all pairs of substances produced only weaker correlations.

Experiment II

Twenty-four hr urinary unconjugated epinephrine and norepinephrine, and creatinine, were collected as one 8-hr and four 4-hr aliquots. Creatinine levels indicated that urine collections were complete. For data analysis the catecholamine levels for the 8-hr aliquots were divided by two in order to produce five 4-hr estimates of urinary epinephrine and norepinephrine throughout 24 hr.

With normal posture and variations in rest/activity, there was a trend toward a circadian fluctuation of urinary epinephrine ($p < 0.10$), with the peak being approximately 250% of the trough (Fig. 3). The peak appeared in the late morning or early afternoon and the trough at night. Significant inter-subject differences were observed ($p < 0.001$). The peak level for urinary norepinephrine occurred in the afternoon, with the peak almost 250% of the trough (Fig. 3). This difference over time showed a weak trend toward significance ($p < 0.15$). For norepinephrine as well, there was a significant difference between subjects ($p < 0.005$).

In order to study the effects of posture and activity and to make comparisons with the results of Experiment I, the circadian variation in urinary catecholamines while subjects remained supine also was determined. Creatinine levels again indicated that urine collections were complete. Mean urinary epinephrine showed a trend toward significance

for daytime levels to be greater than night levels ($p < 0.10$), even though the magnitude was small (Fig. 3). Norepinephrine also was higher during the day than at night. However, the magnitude of the difference again was small and, due to variation across individuals, not significant. A significant difference between subjects was observed for epinephrine ($p < 0.05$), but not for norepinephrine. These data are in agreement with prior findings that day-time urinary catecholamine levels are higher than night-time levels, even when subjects maintain a supine posture.
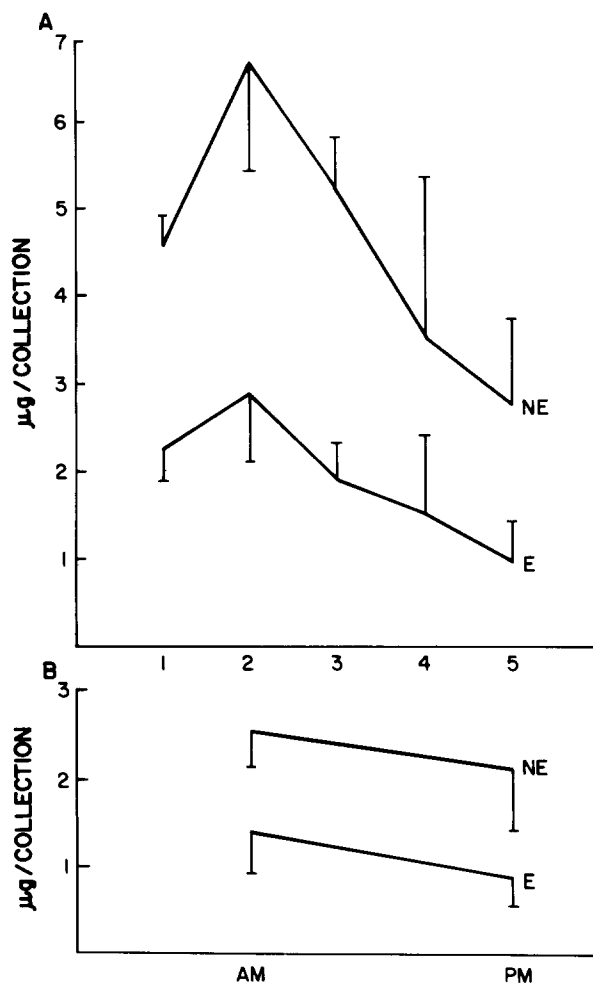

FIG. 3. Urinary unconjugated norepinephrine (NE) and epinephrine (E) in µg per collection (mean ± S.E.M.) for four 4-hr (1 – 4) and one 8-hr (5) collections, 0800 – 1200, 1200 – 1600, 1600 – 2000, 2000 – 2400, and 0000 – 0800 hr, respectively, appear in panel A (µg for collection 5 is one-half of complete 8-hr collection, in order to make all five collections comparable). Subjects were engaged in normal ambulant activities. Urinary unconjugated NE and E in two 12-hr collections, 0800 – 2000 hr (a.m.) and 2000 – 0800 hr (p.m.) from the same three subjects who remained supine, appear in panel B.

Large differences were observed between the urinary levels of epinephrine and norepinephrine when normal activity and posture was maintained compared to when supine posture was maintained. For the day-time 12-hr period 0800 hr − 2000 hr, the average epinephrine level across subjects was 1.38 μg supine vs 7.01 μg during normal activity (a 508% difference). For night-time epinephrine, the supine value was 0.86 μg vs 3.44 μg for normal activity (a 400% difference). For day-time norepinephrine, the values were 2.53 μg supine vs 16.46 μg for normal activity (651%), while during the night the results were 2.12 μg supine vs 8.98 μg for normal activity (424%). Thus, maintenance of upright posture and normal activity raised urinary levels of both catecholamines substantially, even in the night-time specimens when supine position was maintained part of the time.

DISCUSSION

In these experiments, circadian changes in epinephrine levels in healthy young adult male humans were studied. Normal levels of norepinephrine and cortisol indicate normal functioning of these subjects.

In contrast to a detailed previous study of plasma epinephrine (Linsell *et al.*, 1985), and also in contrast to most other hormones studied (Krieger, 1979), a significant circadian rhythm of epinephrine in plasma was not demonstrated in our study. A comparison of the two studies suggests a reason: in the prior study, subjects were not constantly supine. They ". . . kept to a fixed activity regimen. They sat quietly for 10 minutes before sampling, then walked around the ward for at least 10 of the following 20 minutes". Prior studies have demonstrated that acute postural change influences plasma epinephrine. Furthermore, both the present study and prior studies have demonstrated that upright posture and normal activity produce a very substantial (approx. five-fold) increase in urinary excretion of epinephrine and norepinephrine (Karki, 1956; Townshend & Smith, 1973). Thus, it is possible that postural and, especially, activity differences over several hours modified the levels of epinephrine and that this procedural difference accounted for the appearance of a significant epinephrine peak in the late afternoon and a trough at night in the prior study (Linsell *et al.*, 1985). The appearance of a brief drop in plasma epinephrine between 0100 hr and 0200 hr for the average of the four subjects in our study seems to suggest a slight tendency for a drop at night in supine subjects, which was also suggested by some of the prior studies in supine subjects. However, inspection of the data from individual subjects (Fig. 1) does not reveal a consistent drop at these times. Furthermore, in the study of Prinz *et al.* (1984), which also examined circadian fluctuations of plasma epinephrine, "a supine position was encouraged throughout and was required in the 15 minutes preceding blood sampling". This study of supine subjects also failed to demonstrate a significant circadian variation. Thus, it appears that, if there is any circadian rhythm of plasma epinephrine, it is very weak, and that posture and activity may either produce a circadian pattern or accentuate the very weak one already present. Also, metabolic and/or kidney functioning appears to produce a tendency towards a rhythm of catecholamine appearance in urine, even in supine subjects, and this rhythm also may be accentuated by activity and/or postural change (DeSchaepdryner & Leroy, 1961; Hollister & Moore, 1970; Froberg *et al.*, 1972; Townshend & Smith, 1973; Faucheux *et al.*, 1976).

Several other findings are of interest. No ultradian pattern or association with sleep or stage of sleep was observed for epinephrine; this agrees with the prior studies reviewed above. Furthermore, the data of Experiment I indicated that norepinephrine levels are significantly correlated with both cortisol and epinephrine across all four subjects. One could hypothesize that norepinephrine levels may be influencing the other substances, but prior research indicates that this is not correct. Infusion of norepinephrine lowers cortisol (Wilcox et al., 1975; Few et al., 1980), and negative correlations between cortisol and norepinephrine plasma levels have been reported (Sotsky et al., 1981). Further, systemic treatment with dexamethasone, a synthetic glucocorticoid, lowers norepinephrine (Stene et al., 1980). Thus, it seems more likely that cortisol and norepinephrine levels correlate because their fluctuations are in phase but not directly dependent on each other. Since a circadian fluctuation for epinephrine was not observed, this explanation does not seem adequate for the norepinephrine – epinephrine correlation. However, the combination of a possible slight fluctuation for epinephrine, with the lowest point at the same time as norepinephrine, plus the stress effects described below, which would be in phase for each individual subject, might account for the observed correlation. Further study will be necessary to determine if systemic norepinephrine levels directly influence epinephrine levels, or vice versa.

Another observation of interest relates to potential stress effects. Stress of various kinds increases systemic levels of epinephrine and norepinephrine. All subjects described the procedure of Experiment I as somewhat stressful (e.g., mild pain in the arm from which blood was drawn during the later part of the 24-hr period), even though cortisol levels were not obviously increased. Because start times for subjects were counterbalanced, this increase should not have influenced any circadian rhythm effect. But it might have increased the estimate of basal plasma levels of both catecholamines averaged over the 24-hr period. For plasma epinephrine the magnitude of this potential stress effect, calculated as the mean of the last specimen obtained from each subject minus the mean of the first, divided by two, was approx. 48 pg/ml. Subtraction of this value from the levels observed (Fig. 2) would reduce the true basal epinephrine level to 10 pg/ml or less. If epinephrine is a stress hormone, secreted from the adrenal medulla only under stressful conditions, a true basal level this low might not be implausible. However, it is below previously reported studies [indeed, below the limit of reliability of existing assays (Peuler & Johnson, 1977; Holly & Makin, 1983; McCann & Huber-Smith, 1984)]; therefore, this result should be interpreted cautiously.

For plasma norepinephrine, the magnitude of this stress effect was approximately 44 pg/ml. Subtraction of these values from the basal ranges observed (Fig. 2) suggests that norepinephrine ranges between approx. 150 pg/ml and 250 pg/ml over the 24-hr period in unstressed individuals; this is in the lower range of the basal plasma norepinephrine levels reported previously in normal subjects (Lake et al., 1976; Cryer, 1980; Stene et al., 1980; Ratge et al., 1982; Holly & Makin, 1983; McCann & Huber-Smith, 1984), suggesting that it is an appropriate estimate.

The lack of a significant circadian fluctuation for plasma epinephrine in the unstressed (or, possibly in the case of this study, in a mildly stressed) state should not necessarily be extrapolated to more stressful conditions. It has been observed in rats that, even though no circadian fluctuation in plasma epinephrine level was observed in an unstressed

situation, when a stressful circumstance (footshock) was introduced, the plasma levels of epinephrine did show circadian variation (McCarty *et al.*, 1981). The possibility of procedurally-related stress-induced elevations of catecholamines, as well as modifications of circadian patterns, should be considered in all studies of catecholamine levels.

The present study included only males of a specific, relatively narrow age range; extrapolations to females or to other age groups should be done cautiously. Some hormone levels are different between males and females, although catecholamines appear not to be (Karki, 1956; Stene *et al.*, 1980), and some show different levels in different age groups, including plasma norepinephrine (Lake *et al.*, 1976; Prinz *et al.*, 1979, 1984; Barnes *et al.*, 1982; Pfeifer *et al.*, 1983) but not epinephrine (Barnes *et al.*, 1982; Pfeifer *et al.*, 1983; Prinz *et al.*, 1984). Finally, even with this homogeneous experimental group, significant and substantial differences for plasma catecholamine levels between subjects were observed. Such large inter-subject differences are consistent with prior studies (Lake *et al.*, 1984).

The results of this and prior research indicate that norepinephrine probably has a small amplitude circadian fluctuation in supine subjects, and that epinephrine has either no rhythm or a very small amplitude rhythm; troughs were during the night. Neither sleep nor sleep stage had an effect, and no ultradian epinephrine rhythm was observed. Norepinephrine may have an ultradian rhythm between 1.3 and 3 hr. However, no attempt was made to remove "Zeitgebers" (temporal stimuli or signals) such as light − dark cycle, sleep, or standard meal times; the results might have been different if this had been done.

REFERENCES

Aronow W S, Harding P R, DeQuattro V, Isbell M (1978) Diurnal variation of plasma catecholamines and systolic time intervals. *Chest* **63**: 722 – 726.

Barnes P, Fitzgerald G, Brown M, Dollery C (1980) Nocturnal asthma and changes in circulatory epinephrine, histamine, and cortisol. *N Engl J Med* **303**: 263 – 267.

Barnes R F, Raskind M, Gumbrecht G, Halter J B (1982) The effects of age on the plasma catecholamine response to mental stress in man. *J Clin Endocrinol Metab* **54**: 64 – 69.

Brezinova V, Carruthers M (1974) Plasma-catecholamines in sleeping hypertensives. *Lancet* ii: 352.

Chatfield C (1980) *The Analysis of Time Series: An Introduction*. Second Edition, Chapman and Hall, London.

Cryer P E (1980) Physiology and pathophysiology of the human sympathoadrenal neuroendocrine system. *N Engl J Med* **303**: 436 – 444.

DeSchaepdryner A F, Leroy J G (1961) Urine volume and catecholamine excretion in man. *Acta Cardiol (Brux)* **16**: 631 – 638.

Faucheux B, Kuchel O, Cuche J L, Messerli F H, Buu N T, Barbeau A, Genest J (1976) Circadian variations of the urinary excretion of catecholamines and electrolytes. *Endocr Res Comm* **3**: 257 – 272.

Few J D, Gawel M J, Imms F J, Tiptoft E M (1980) The influence of the infusion of noradrenaline on plasma cortisol levels in man. *J Physiol* **309**: 375 – 389.

Frankenhauser M (1971) Behavior and circulating catecholamines. *Brain Res* **31**: 241 – 262.

Froberg J, Karlsson C-G, Levi L, Lidberg L (1972) Circadian variations in performance, psychological ratings, catecholamine excretion, and diuresis during prolonged sleep deprivation. *Internat J Psychobiol* **2**: 23 – 36.

Gallagher T F, Yoshido K, Roffwarg H D, Fukushima D K, Weitzman E D, Hellman L (1973) ACTH and cortisol secretory patterns in man. *J Clin Endocrinol Metab* **36**: 1058 – 1068.

Harris R J (1975) *A Primer of Multivariate Statistics*. Academic Press, New York.

Hollister L E, Moore F (1970) Factors affecting excretion of catecholamines in man: urine flow, urine pH, and creatinine clearance. *Res Comm Chem Pathol Pharmacol* 1: 193 – 202.

Holly J M P, Makin H L J (1983) The estimation of catecholamines in human plasma. *Anal Biochem* 128: 257 – 274.

Karki N T (1956) The urinary excretion of noradrenaline and adrenaline in different age groups, its diurnal variation and the effect of muscular work on it. *Acta Physiol Scand* 39 (Suppl 132): 1 – 96.

Krieger D T (1979) *Endocrine Rhythms.* Raven Press, New York.

Lake C R, Chernow B, Feuerstein G, Goldstein D S, Ziegler M G (1984) The sympathetic nervous system in man: its evaluation and the measurement of plasma NE. In: Ziegler M G, Lake C R (Eds) *Norepinephrine.* Williams and Wilkins, Baltimore, pp 1 – 26.

Lake C R, Ziegler M G (1985) Techniques for the assessment and interpretation of catecholamine measurements in neuropsychiatric patients. In: Lake C R, Ziegler M G (Eds) *The Catecholamines in Psychiatric and Neurologic Disorders.* Butterworth, Boston, pp 1 – 34.

Lake C R, Ziegler M G, Kopin I J (1976) Use of plasma norepinephrine for evaluation of sympathetic neuronal function in man. *Life Sci* 18: 1315 – 1326.

Levin B E, Rappaport M, Natelson B H (1979) Ultradian variations of plasma noradrenaline in humans. *Life Sci* 25: 621 – 628.

Linsell C R, Lightman S L, Mullen P E, Brown M J, Causon R C (1985) Circadian rhythms of epinephrine and norepinephrine in man. *J Clin Endocrinol Metab* 60: 1210 – 1215.

McCann D S, Huber-Smith M J (1984) Plasma catecholamines. *J Clin Immunoassay* 6: 308 – 312.

McCarty R, Kvetnansky R, Kopin I J (1981) Plasma catecholamines in rats: daily variations in basal levels and increment in response to stress. *Physiol Behav* 26: 27 – 31.

Mullen P E, Lightman S, Linsell C, McKern P, Sever P S, Todd K (1981) Rhythms of plasma noradrenaline in man. *Psychoneuroendocrinology* 6: 213 – 222.

Peuler J D, Johnson G A (1977) Simultaneous single isotope radio-enzymatic assay of plasma norepinephrine, epinephrine, and dopamine. *Life Sci* 21: 625 – 636.

Pfeifer M A, Weinberg C R, Cook D, Best J D, Reenan A, Halter J B (1983) Differential changes of autonomic nervous system function with age in man. *Am J Med* 75: 249 – 258.

Prinz P N, Halter J, Benedetti C, Raskind M (1979) Circadian variation of plasma catecholamines in young and old men: relation to rapid eye movement and slow wave sleep. *J Clin Endocrinol Metab* 49: 300 – 304.

Prinz P N, Vitiello M V, Smallwood R G, Schoene R B, Halter J B (1984) Plasma norepinephrine in normal young and aged men: relationship with sleep. *J Gerontol* 39: 561 – 567.

Ratge P, Kroll E, Diever U, Hadjidimas A, Wisser H (1982) Circadian rhythm of catecholamines, cortisol and prolactin is altered in patients with apallic syndrome in comparison with normal volunteers. *Acta Endocrinol* 101: 428 – 435.

Rechtschaffen A, Kales A (1968) *A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects.* Brain Information Service, Brain Research Institute, University of California at Los Angeles.

Robertson D, Johnson G A, Robertson R M, Nies A S, Shand D G, Oates J A (1979) Comparative assessment of stimuli that release neuronal and adrenomedullary catecholamines in man. *Circulation* 59: 637 – 643.

Rose R M (1980) Endocrine responses to stressful psychological events. *Psychiatr Clin N Amer* 3: 251 – 276.

Sotsky S M, Lake C R, Goodwin F K (1981) Adrenocortical function and plasma norepinephrine in normal human subjects. *Biol Psychiatry* 16: 643 – 651.

Stene M, Panagiotis N, Tuck M L, Sowers J R, Mayers D, Berg G (1980) Plasma norepinephrine levels are influenced by sodium intake, glucocorticoid administration, and circadian changes in normal man. *J Clin Endocrinol Metab* 51: 1340 – 1345.

Townshend M M, Smith A J (1973) Factors influencing the urinary excretion of free catecholamines in man. *Clin Sci* 44: 253 – 265.

Turton M B, Deegan T (1974) Circadian variations of plasma catecholamines, cortisol and immunoreactive insulin concentrations in supine subjects. *Clin Chim Acta* 55: 389 – 397.

Ward M M, Mefford I N, Parker S D, Chesney M A, Taylor C B, Keegan D L, Barchas J D (1983) Epinephrine and norepinephrine responses in continuously collected plasma to a series of stressors. *Psychosom Med* 45: 471 – 486.

Weitzman E D, Fukushima D K, Nogeire C, Roffwarg H, Gallagher T F, Hellman L (1971) Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *J Clin Endocrinol Metab* 33: 14 – 22.

Wilcox C S, Aminoff M J, Millar J G B, Keenan J, Kremer M (1975) Circulating levels of corticotrophin and cortisol after infusions of L-Dopa, dopamine, and noradrenaline in man. *Clin Endocrinol* 4: 191 – 198.

Wilens T E, Ritchie J C, Carroll B J (1984) Comparison of plasma cortisol and corticosterone in the dexamethasone suppression test for melancholia. *Psychoneuroendocrinology* 9: 45 – 55.

Winer N, Carter C H, Willoughby T L (1980) Circadian variation of norepinephrine, renin, blood pressure, and heart rate. Proc 62nd Ann Meet Endocrine Soc (Abst 195).