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VARIABILITY OF PLASMA CATECHOLAMINE LEVELS: AGE, DURATION OF POSTURE AND TIME OF DAY

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1 In order to define factors which influence plasma catecholamine levels, and which might be controlled in the interests of reproducability and comparability, plasma noradrenaline plus adrenaline (NA + A) was measured repeatedly in normal subjects by a double isotope technique.

2 Age and posture were important determinants for plasma NA + A levels, whereas time of day was less important.

3 Levels were higher in old men aged 70–92 years than in young men aged 19–28 years, measured after 30 min or 9 h (overnight) recumbency, or after 5 min standing.

4 Duration of recumbency and of upright posture were both important. Recumbent levels were lower after 9 h (overnight) than after 30 min (mid-morning). Upright posture caused an increase in levels within 2 min. Levels peaked between 5 and 20 min, were lower after 12 h than after 20 min, but upright levels were always higher than levels after 9 h recumbency.

5 During continuous recumbency, levels were lowest at 24.00 h, and increased thereafter. During a second hospitalisation, levels at 09.00 h were again higher than levels at 06.00 h after overnight recumbency.

6 Variability of levels obtained by repeated sampling was lower while sitting, during a normal upright day, than after 30 min recumbency. Upright levels may be a better index of cardiovascular sympathetic activity.

Introduction

The assumption that circulating catecholamines reflect sympathetic nervous system activity is based on observations that procedures known to alter sympathetic activity are associated with changes in plasma catecholamine levels. These include the change from recumbency to upright posture (Cryer, Santiago & Shah, 1974), exposure to cold (Winer & Carter, 1977), and dynamic (Haggendal, Hartley & Saltin, 1970) or isometric (Lake, Ziegler & Kopin, 1976) exercise. Levels are reduced in patients with autonomic dysfunction (Cryer & Weiss, 1976), in patients quadriplegic due to cervical cord transection (Mathias, Christensen, Corbett, Frankel, Goodwin & Peart, 1975) and after ganglion blockade (Louis, Doyle & Anavekar, 1973). Involvement of the sympathetic nervous system in the pathophysiology of diabetes mellitus (Cryer, Silverberg, Santiago & Shah, 1978), thyroid disease (Coulombe, Dussault & Walker, 1976) and hypertension (Axelrod, 1976) is receiving considerable attention.

The effect of sampling conditions on levels has received relatively little attention, but is of particular importance in light of the lability of plasma catecholamines (Carruthers, Conway, Taggart, Bates & Somerville, 1970). The present study was designed to establish the effects of time of day, posture, and duration of posture on the variability of plasma catecholamines.

Methods

Subjects

Subjects were normotensive (BP consistently less than 140/90 mm Hg), non-smokers, on no medication and in good health. Biochemical screening showed normal serum levels of electrolytes, creatinine, urea and urate. Subjects in the circadian study were admitted to the metabolic ward the night before the study and were given a liquid diet every 6 h consisting of 500 ml Sustagen (a high protein formula made up in milk yielding 2040 Kcal/day). Free access to water was permitted. Subjects in other studies were on unrestricted diet but avoided alcohol for 24 h and coffee for 6 h before blood sampling.

Procedures

Blood pressure and heart rate were measured by the same trained observer before blood sampling using a standard mercury sphygmomanometer and by

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measurement of radial pulse. Blood (20 ml) was collected from an antecubital vein into chilled (0–2°C) vials containing 12.5 units/ml heparin and 1.5 mg/ml ascorbic acid. Plasma was separated at 0–4°C and stored at -20°C until assayed.

In the study comparing 9 h (overnight) and 30 min recumbency in young (aged 19–28 years) and old (aged 70–92 years) men, venepuncture was used.

In the studies of the effect of time of day and upright posture on levels in young men aged 21–23 years, an indwelling intravenous cannula (16 g Jelco) was used. Patency of the cannula was maintained with a solution of 5% dextrose containing 1000 units heparin per litre running at 600 ml/24 h. After insertion of the cannula at 21.00 h day 1, subjects were recumbent (including during drinking and voiding) and blood was collected from 06.00 h on day 2 three hourly until 09.00 h on day 3. In a subsequent study of the effects of posture, the same subjects were readmitted to hospital at least 10 days later, and remained recumbent from 21.00 h on day 1 to 09.00 h on day 2, when they stood up. After standing, subjects were allowed to sit, stand or walk but not to lie down until sampling finished at 21.00 h. In this study, in addition to 3 hourly sampling, blood samples were collected 2, 5, 10 and 20 min after standing, and blood pressure and heart rate measured hourly.

In the study of variability in samples obtained from four males and two females aged 24 to 43 years, venepuncture was employed on separate mornings at intervals of 3–7 days between 10.00–11.00 h.

Laboratory

Plasma levels of noradrenaline plus adrenaline (NA+A) were measured using the double isotope radioenzymatic assay as described by Engelman & Portnoy (1970) which does not measure dopamine. Plasma samples from each subject were assayed in the same assay run and were determined twice in separate 3 ml aliquots. The lower limit of sensitivity for this volume of plasma was 0.6 pmol/ml NA+A, and would be inadequate for measurement of A alone. Collection of larger volumes would have made separate measurement of NA and A possible, but was considered undesirable because of possible effects of repeated sampling. Duplicate determinations of levels in the range 0.6 to 2.5 pmol/ml differed from their mean by an average of 3.5 ± 1.9 (s.d.) %. This method gave reproducible results with intraassay and interassay coefficients of variation of 3.3% and 6.0% respectively.

Statistics

Preliminary tests based on normal order statistics showed no evidence of serious departures from normality (Pearson & Hartley, 1972). Results are expressed as the mean value $\pm$ s.e. mean or as the percent of their mean value. Blood pressure is expressed as 'mean' (diastolic + 1/3 (systolic-diastolic)). Statistical probability was calculated using Student's *t*-test for paired or unpaired data and a two way analysis of variance.

Results

Age

Levels were higher in old than in young men (Table 1), whether standing for 5 min ($P < 0.0001$) or lying for 30 min ($P < 0.01$). In a larger sample of ten old men aged 70–92 years (mean 1.867 ± 0.257 pmol/ml) and 13 young men aged 19–28 years (mean 1.28 ± 0.142 pmol/ml), levels were higher ($P < 0.05$) after 9 h (overnight) recumbency as well.

Table 1 Plasma levels in young and old men after recumbency (9 h or 30 min) and upright posture (5–10 min).

	Plasma noradrenaline + adrenaline (pmol/ml)					
	Young men			*Old men*		
	Recumbent		*Upright*	*Recumbent*		*Upright*
Age (years)	*9 h*	*30 min*	*5–10 min*	*Age (years)*	*9 h*	*30 min*	*5–10 min*
22	0.898	0.987	2.636	92	0.875	4.776	6.667
22	1.218	1.330	3.263	75	1.200	1.856	—
22	1.395	1.507	2.896	77	1.389	3.144	7.430
27	1.643	1.897	3.251	70	1.992	5,414	10.905
22	1.649	2.086	4.072	77	2.228	2.748	—
28	2.010	2.175	4.285	78	3.109	4.049	6.608
Mean	1.469	1.664	3.401		1.799	3.664	7,903
s.e. mean	0.158	0.190	0.265		0.333	0.542	1.018

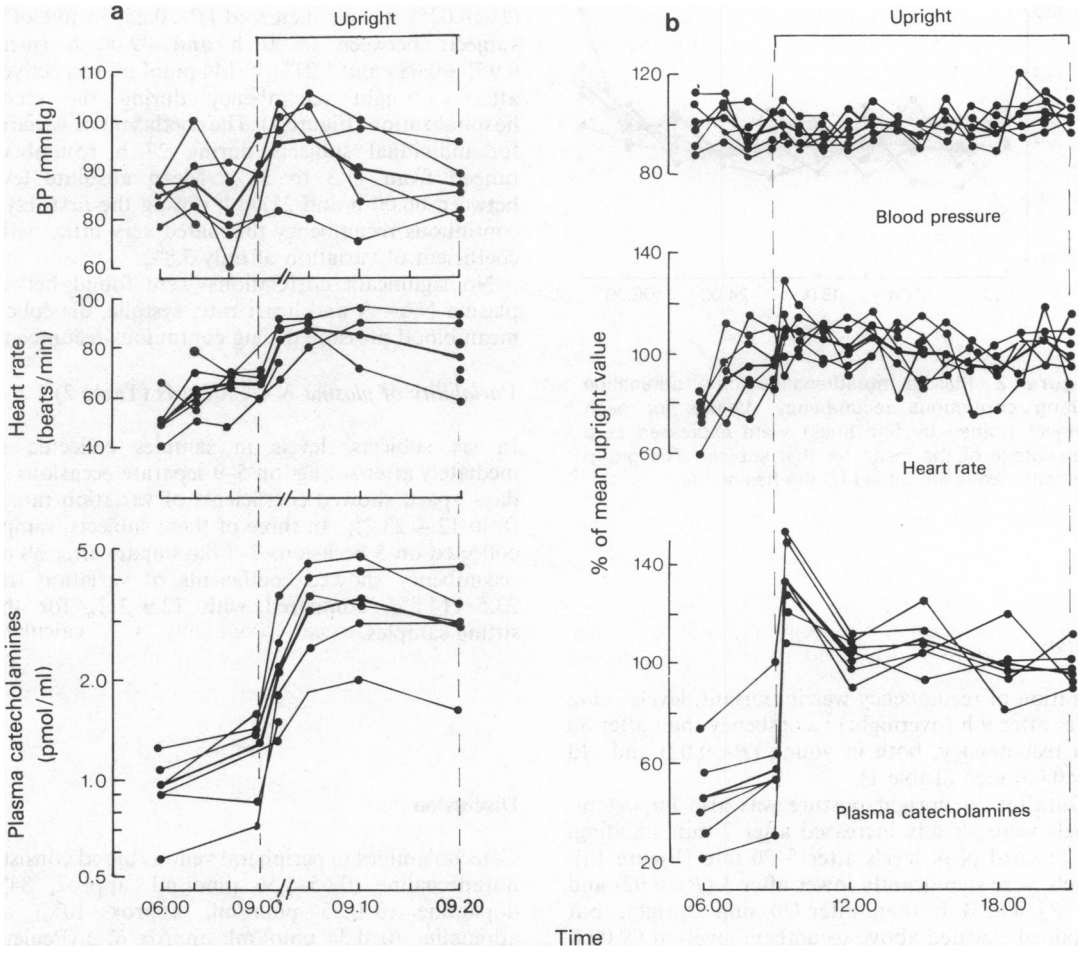

Figure 1 Plasma noradrenaline plus adrenaline, heart rate and blood pressure during the last 3 h of 12 h recumbency and during 12 h upright posture. Values for indivudal subjects are joined by fine lines and group means by heavy lines.
Figure 1a shows catecholamine levels 2, 5, 10 and 20 min after standing and Figure 1b after 20 min, 3, 6, 9 and 12 h standing. In Figure 1b, levels for each subject are expressed as a percentage of the mean upright value for that subject.

Table 2 Variability of plasma levels obtained at 3–7 day intervals after either 30 min recumbency (rec) or immediately after sitting (sit). Sex and age in years are shown in brackets.

	Plasma Noradrenaline+adrenaline (pmol/ml)									
Subjects	BB	(M, 24)	TE	(M, 30)	NS	(F, 34)	RD	(M, 43)	CS (M, 29)	FD (F, 38)
	rec	*sit*	*rec*	*sit*	*rec*	*sit*	*sit*	*sit*	*sit*	*sit*
	3.52	1.64	2.69	2.95	7.23	1.51	3.01	2.54	1.56	3.28
	3.51	2.14	3.82	2.89	6.77	1.72	1.98	2.97	1.46	2.62
	16.07	2.20	2.73	2.64	7.01	2.55	2.48	2.12	1.62	2.61
	2.31	2.69	2.08	2.20	2.63	1.84	3.99	2.19	1.59	2.53
	1.14	2.16	2.46	2.32	1.24	2.22	2.59		1.09	2.44
Coefficient of variation (%)	114.8	17.1	23.5	12.9	56.7	21.0	23.2		14.9	12.4

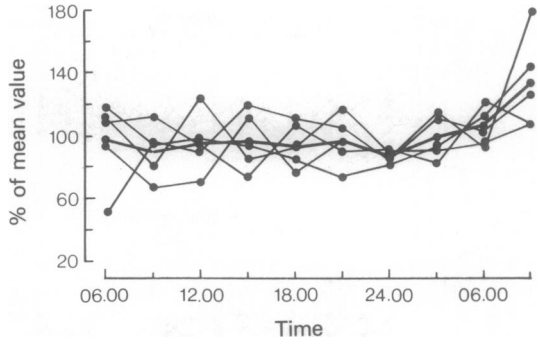

Figure 2 Plasma noradrenaline plus adrenaline during continuous recumbency. Values for each subject (joined by fine lines) were expressed as a percentage of the mean for that subject. The group percent means are joined by the heavy line.

Posture

Duration of recumbency was important, levels being lower after 9 h (overnight) recombency than after 30 min recumbency, both in young ($P<0.02$) and old ($P<0.05$) men (Table 1).

Duration of upright posture was also important. Levels were already increased after 2 min standing, but reached peak levels after 5–20 min (Figure 1a). Levels were significantly lower after 3 ($P<0.02$) and 12 ($P<0.025$) h than after 20 min upright, but remained elevated above recumbent levels at 09.00 h ($P<0.02$) (Figure 1b). Levels in individual subjects during 3–12 h of upright posture had coefficients of variation ranging from 5.7–19.7%, compared with 11.3–32% during continuous recumbency.

Blood pressure was not altered by the change from recumbency to upright posture, and no significant relationship between plasma NA+A and blood pressure was found. There was however, a significant positive correlation ($r=0.728$, $P<0.001$), between plasma NA+A levels and heart rate measured before and after assumption of upright posture.

Time of day

Levels during continuous recumbency are shown in Figure 2, expressed as percent of the mean for individual subjects to explore any rhythmical changes. Mean level was lowest (1.09 ± 0.15 pmol/ml) at 24.00 h and rose thereafter to 1.19 ± 0.09 at 03.00 h 1.34 ± 0.23 at 06.00 h and 1.60 ± 0.15 pmol/ml at 09.00 h. The mean level at 09.00 h was significantly higher than the level at 24.00 h ($P<0.02$) and 03.00 h ($P<0.025$). Levels increased ($P<0.25$) in five of six subjects between 06.00 h and 09.00 h (means 0.951 ± 0.089 and 1.217 ± 0.144 pmol/ml respectively) after overnight recumbency during the second hospitalization (Figure 1). The coefficient of variation for individual subjects during 27 h recumbency ranged from 11.3 to 32%. Mean absolute levels between 06.00 h and 21.00 h during the first day of continuous recumbency fluctuated very little, with a coefficient of variation of only 3.8%.

No significant correlations were found between plasma NA+A and heart rate, systolic, diastolic or mean blood pressure during continuous recumbency.

Variability of plasma NA+A levels (Table 2)

In six subjects, levels in samples collected immediately after sitting, on 5–9 separate occasions 3–7 days apart, showed coefficients of variation ranging from 12.4–23.2%. In three of these subjects, samples collected on 5 occasions 3–7 days apart after 30 min recumbency showed coefficients of variation from 23.5–114.8%, compared with 12.9–21% for their sitting samples.

Discussion

Catecholamines in peripheral venous blood consist of noradrenaline (0.65–3.56 pmol/ml, approx, 84%), dopamine (0–0.55 pmol/ml, approx 10%) and adrenaline (0–0.34 pmol/ml, approx 6%) (Peuler & Johnson, 1977). The method employed in this study (Engelman & Portnoy, 1970) does not measure dopamine, but noradrenaline plus adrenaline (NA+A). Since adrenaline change less than noradrenaline in response to upright posture (Cryer *et al.*, 1974) or exercise (Christensen & Brandsborg, 1973), the changes observed in NA+A in these studies reflect predominantly changes in noradrenaline.

The higher level of plasma NA+A in this study in older subjects is in accord with the finding of Lake, Zeigler, Coleman & Kopin (1977), of increasing plasma noradrenaline levels with age. In contrast, DeChamplain & Cosineau (1977) found no correlation between plasma NA+A and age, and Cryer & Weiss (1976) found no correlation between plasma noradrenaline or adrenaline and age. Ziegler, Lake & Kopin (1976), in attempting to explain higher plasma noradrenaline levels with age, postulated decreased vascular sensitivity. This would be critical during the haemodynamic adjustments to upright posture, and least after prolonged overnight recumbency. In the present study, the difference between levels in old and

young men was greatest during upright posture, and least after overnight recumbency.

Duration of recumbency is clearly very important. Higher NA+A levels were seen after 30 min recumbency at 10.00–11.00 than after overnight recumbency at 06.00 h–08.00 h, in both young and old men. Plasma NA+A levels may be higher after only 30 min recumbency than after overnight recumbency because the state of the sympathetic nervous system is closer to 'basal' after 9 h of recumbency, plus sleep. Lake *et al.* (1976) did not find significant differences between noradrenaline levels obtained after shorter periods of daytime recumbency (0.3 versus 3.0 h). Turton & Deegan (1974), on the other hand, found a progressive decline in plasma catecholamines during a recumbent day in subjects who had not been recumbent overnight before the study commenced. These discrepancies emphasize the need to standardize so-called 'basal' conditions for measuring plasma catecholamines, in order to control the various effects of age, wakefulness and duration of recumbency.

Plasma catecholamine levels increase sharply following assumption of upright posture (Cryer *et al.*, 1974), and this was evident in the present study measuring NA+A. As far as we are aware, the present study is the first to employ prolonged periods of upright posture (12 h standing, sitting or walking). Upright levels remained elevated when compared with 9 h recumbent levels for as long as 12 h, although peak levels occurred after only 5–20 min. If plasma catecholamine levels reflect sympathetic nervous system activity, such activity is greater immediately after standing than during prolonged periods of standing, consistent with greatest involvement of the sympathetic nervous system in acute haemodynamic adjustments. Significant activity persists, however, during many hours of upright posture.

Plasma levels of NA+A during upright posture both during a single day, and from day to day, were more consistent than levels obtained after a short period (30 min) of recumbency. Plasma catecholamine levels presumably reflect overall activity of the sympathetic nervous system in both cardiovascular and non-cardiovascular tissues. Since the activity of the sympathetic nervous system is probably more vital to blood pressure regulation during upright posture than in the recumbent position, it is reasonable to suggest that upright levels may be a better index than recumbent levels for sympathetic activity in the cardiovascular system. This may explain why patients with primary autonomic dysfunction had basal noradrenaline levels indistinguishable from normal, but stimulated (upright) levels were clearly subnormal (Cryer & Weiss, 1976).

Watson, Reid, Hamilton & Littler (1978) reported higher levels of plasma noradrenaline at 06.00–09.00 h (subjects awake but still recumbent) than between 20.00 and 02.00 h while sleeping, in untreated patients with hypertension. Aronow, Harding, DeQuattro & Isbell (1973), on the other hand, found higher daytime than night-time levels of plasma noradrenaline in only two of ten subjects. They did find an increase between 08.00 and 12.00 h, but did not give details of posture.

As far as we are aware, the present study is the first to measure plasma catecholamine levels during strictly controlled, prolonged recumbency. By removing the powerful effect of upright posture, and reducing the effect of duration of recumbency by commencing sampling only after 9 h recumbency, conditions should be suitable for recognition of any powerful effects of time of day. While no definite circadian rhythm appeared during 27 h of continuous recumbency during the first hospitalization, a significant increase was observed between 24.00 h and 09.00 h after more than 24 h recumbent. Circadian rhythms can be obsecured by the stress of hospitalization (Bartter, Delea & Halberg, 1962), and only emerge after several days. During the second hospitalization, an increase in plasma NA+A levels was observed between 06.00 and 09.00 h following overnight recumbency, and before rising. Littler, West, Honour & Sleight (1978) found lower levels of blood pressure during sleep in all except one of ten mildly to moderately hypertensive subjects; that subject did not sleep very well. In the present study, repeated measurements of blood pressure and pulse during the night might well have affected the level of arousal of our subjects, and hence sympathetic nervous system activity. Furthermore, measurement of NA+A may have obscured changes occurring in one or the other. Further studies, also employing strict postural control, are required to decide whether a true circcadian rhythm exists. If possible, a method of measuring blood pressure should be employed which does not disturb sleep.

In conclusion, the results of this study emphasize the need for standardization of sampling conditions for plasma catecholamines, when used to evaluate sympathetic nervous system activity. The value of a single sample is limited by fluctuations in levels which may be more marked in some individuals. Because of greater cardiovascular sympathetic activity during upright posture, and more consistent levels observed in this study during upright posture, it is suggested that samples collected soon after sitting may be more useful than those collected after relatively short periods of recumbency.

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