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THE LOSS OF CIRCADIAN RHYTHM FOR INTACT PARATHYROID HORMONE AND NEPHROGENOUS CYCLIC AMP IN PATIENTS WITH PRIMARY HYPERPARATHYROIDISM

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SUMMARY

The measurement of serum intact parathyroid hormone (PTH) (1–84) over a 24-h period has shown the existence of a circadian rhythm in normal males which is absent in patients with primary hyperparathyroidism. The physiological significance of this observation is reflected in the presence of parallel changes in nephrogenous cyclic adenosine monophosphate (N-cAMP) in normals which are also absent in primary hyperparathyroidism. Serum calcium, adjusted for variations in albumin concentrations, showed a transient fall in normal subjects prior to the nocturnal rise in PTH (1–84). A similar transient fall in serum adjusted calcium was observed in the hyperparathyroid patients. Serum phosphate showed a circadian rhythm in normal subjects, and an attenuated rhythm persisted in primary hyperparathyroidism. These data suggest that both ionic factors and higher centres play important roles in the fine control of PTH (1–84) secretion.

The recent development of immunoassays capable of measuring intact parathyroid hormone (PTH)(1–84) (Nussbaum *et al.*, 1987; Brown *et al.*, 1987; Logue *et al.*, 1989) has resulted in improved diagnostic discrimination of patients with hypercalcaemia and an ability to measure changes in the concentration of PTH (1–84) within the reference range in normal subjects. A study of the secretion of PTH (1–84) in normal volunteers has shown the existence of a circadian rhythm for PTH (1–84) with a broad peak of secretion occurring between 0200 and 0600 h (Logue *et al.*, 1989). Cyclic adenosine 3,5-monophosphate (cAMP) is a second messenger of PTH (1–84), and the estimation of nephrogenous cAMP (N-cAMP) is a measure of the biological activity of PTH (Broadus *et al.*, 1977). A significant elevation of N-cAMP is seen overnight in normal volunteers with N-cAMP rising in parallel with PTH (1–84) (Logue *et al.*, 1989).

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Patients with primary hyperparathyroidism have inappropriately detectable or high serum concentrations of PTH (1–84) in the presence of hypercalcaemia (Nussbaum *et al.*, 1987; Blind *et al.*, 1988; Chu & Chu, 1988) and an elevated N-cAMP in the majority of cases (Broadus *et al.*, 1977, 1978). Some of the available evidence indicates that in primary hyperparathyroidism the adenomatous parathyroid tissue functions autonomously, whilst other evidence suggests that the gland is not totally autonomous and an alteration in the set-point for PTH secretion exists. Thus we would expect that the circadian rhythm observed in normal subjects could be lost in primary hyperparathyroidism. Studies measuring N and C terminal PTH have shown either the existence (Sinha *et al.*, 1975; Lo Cascio *et al.*, 1982) or absence (Riggs *et al.*, 1971; Jubiz *et al.*, 1972) of a circadian rhythm for PTH in primary hyperparathyroidism.

In this study we attempt to resolve this inconsistency by measuring the biologically active intact PTH (1–84), a marker of its bioactivity, N-cAMP, serum calcium, and serum phosphate, in normal volunteers and in patients with primary hyperparathyroidism.

MATERIALS AND METHODS

Six normal male volunteers (age 29–40; mean 33 years) and six patients (three male, three female) with primary hyperparathyroidism (surgically proved with histological diagnosis, age 47–79; mean 58 years) were studied. Venous blood samples were obtained via indwelling cannulae at 30-min intervals for 24 h, and urine collections were made every 4 h during the day, one sample prior to retiring to bed and one sample on awakening in the morning. During the study period, all normal subjects ate meals at identical times, were ambulant but avoided exercise, and all lay down to sleep at an identical time. All patients were hospitalized during the study, they ate hospital diet at identical times, were ambulant but avoided exercise, and all lay down to sleep at an identical time. Whole blood was allowed to clot and the serum was separated and snap frozen within 30 min and stored at $-50°C$ prior to analysis. Plasma (K-EDTA) was separated immediately after collection and stored at $-50°C$ prior to analysis.

PTH (1–84) was measured on serum using an immunoradiometric assay described previously (Logue *et al.*, 1989). Calcium phosphate, albumin and creatinine were measured on a Hitachi 704 using standard methodologies, and calcium was adjusted for albumin concentration (Gardner *et al.*, 1981). Plasma c-AMP was measured using a commercially available assay system (Amersham Internationl plc, Aylesbury, Bucks, UK), urinary cAMP was measured by an in-house RIA (O'Reilly *et al.*, 1986) and N-cAMP calculated according to the method of Broadus *et al.* (1977).

PTH (1–84) and N-cAMP values showed a wide scatter in patients with primary hyperparathyroidism so, for clarity, the results were calculated for each subject as percentage difference from their 24 h mean value.

Statistical analysis was performed using a paired difference *t*-test to analyse significant changes within 24-h profiles. The Mann–Whitney *U*-test was used to analyse differences between groups. Statistical analysis of circadian rhythm parameters was carried out using the Cosinor technique (Nelson *et al.*, 1979). In this procedure a cosine curve with a period of 24 h was fitted to the data for each individual using the method of least squares. An *F*-statistic was then used to test the zero-amplitude hypothesis for a circadian rhythm. The following parameters were calculated: the mesor (rhythm-adjusted mean), the amplitude (half the total extent of the predictable change) and the acrophase (crest time of the

best-fit cosine function, in relation to local midnight). Ethical Committee approval was obtained for the study, and all patients gave informed consent before inclusion in the study.

RESULTS

Figures 1 and 2 show the 24-h profiles for PTH (1–84), N-cAMP (both expressed as percentage of the individual 24-h mean), adjusted serum calcium, and serum phosphate for normal subjects and for primary hyperparathyroid patients. The measured PTH (1–84) and N-cAMP concentrations throughout the 24-h periods in both groups are summarized in Table 1. The results of cosinor analysis of the data for the four measurements in each group studied are shown in Table 2.

A pronounced circadian rhythm was observed for PTH (1–84) in the normal subjects with a significant rise in PTH (1–84) overnight, the acrophase occurring at 0405 h. This circadian rhythm was lost completely in patients with primary hyperparathyroidism with no synchronized rise in PTH (1–84) seen at any time during the 24 h in these patients.

In normal subjects, the greatest variability in serum PTH (1–84) occurs between 0630 and 1000 h. In patients with primary hyperparathyroidism, concentrations of PTH (1–84) occasionally fell within the reference range during the 24-h period, and in one patient these results coincided with normal values for adjusted calcium.

A similar pattern of significant increase in N-cAMP was present in normals and was abolished in patients with primary hyperparathyroidism. In two patients, values for N-cAMP within the reference range were obtained intermittently throughout the 24 h.

The three male patients with primary hyperparathyroidism had the highest concentrations of PTH (1–84) and N-cAMP (Table 1). However, these subjects also had the highest concentrations of adjusted calcium, and so we interpret Table 1 as reflecting the severity of the condition rather than a fundamental difference in PTH (1–84) secretion between men and women.

Adjusted calcium varied very little during the 24 h in both groups. A transient, statistically significant decrease ($P<0{\cdot}05$) in mean adjusted calcium from 2·44 to 2·40 mmol/l at 0130 h in normals, and from 2·94 to 2·84 mmol/l at midnight in patients with primary hyperparathyroidism, was observed when compared with the group mean concentration between 1400 and 1800 h. The adjusted calcium mean concentration in the patient group was predictably higher than that in the normal group throughout the 24 h ($P<0{\cdot}001$).

Serum phosphate was significantly lower in the primary hyperparathyroid patients throughout the 24 h ($P<0{\cdot}001$). A marked synchronized rhythm in phosphate was observed in both groups with a transient decrease following the evening meal, which was the main meal of the day, a significant nocturnal rise and early morning fall being seen in both groups. The percentage change in phosphate during the 24 h was greater in normal subjects than in those with primary hyperparathyroidism.

DISCUSSION

Two-site immunometric assays for PTH (1–84) enable subtle changes of PTH (1–84), within the physiological range, to be measured, and have established the existence of a significant circadian rhythm in normal male subjects (Logue *et al.*, 1989). The present

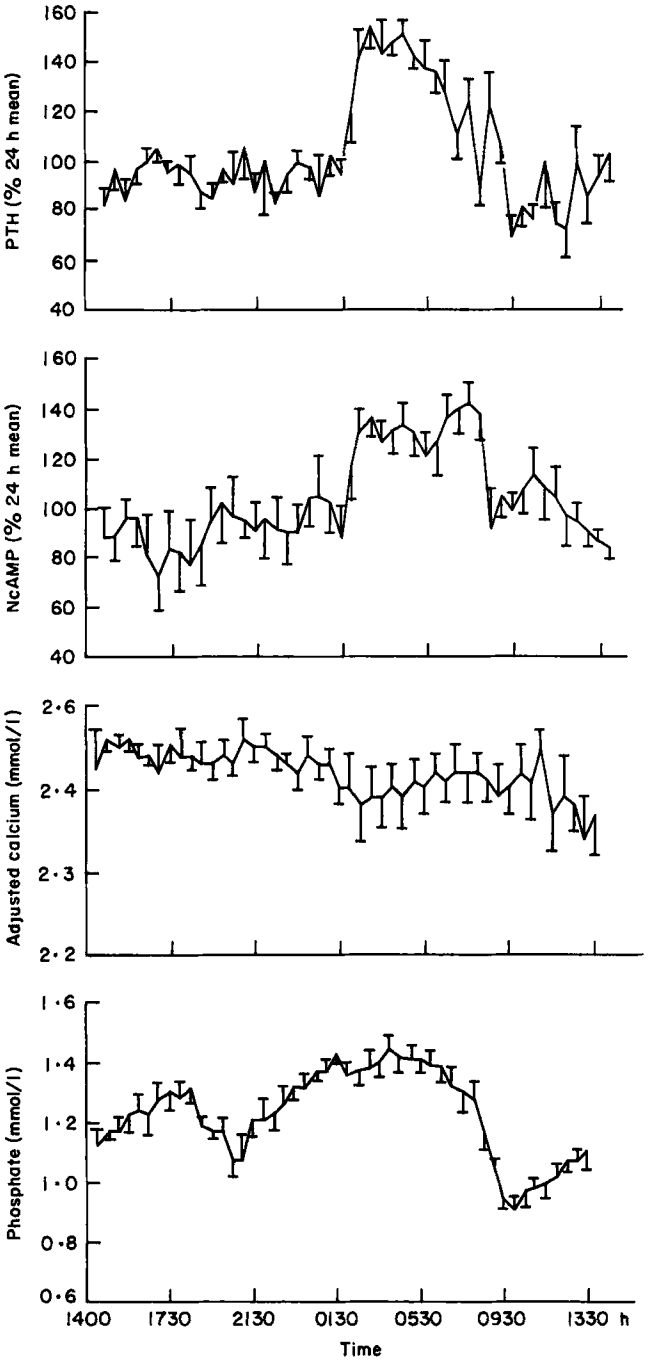

Fig. 1. 24-Hour profiles in normal subjects.

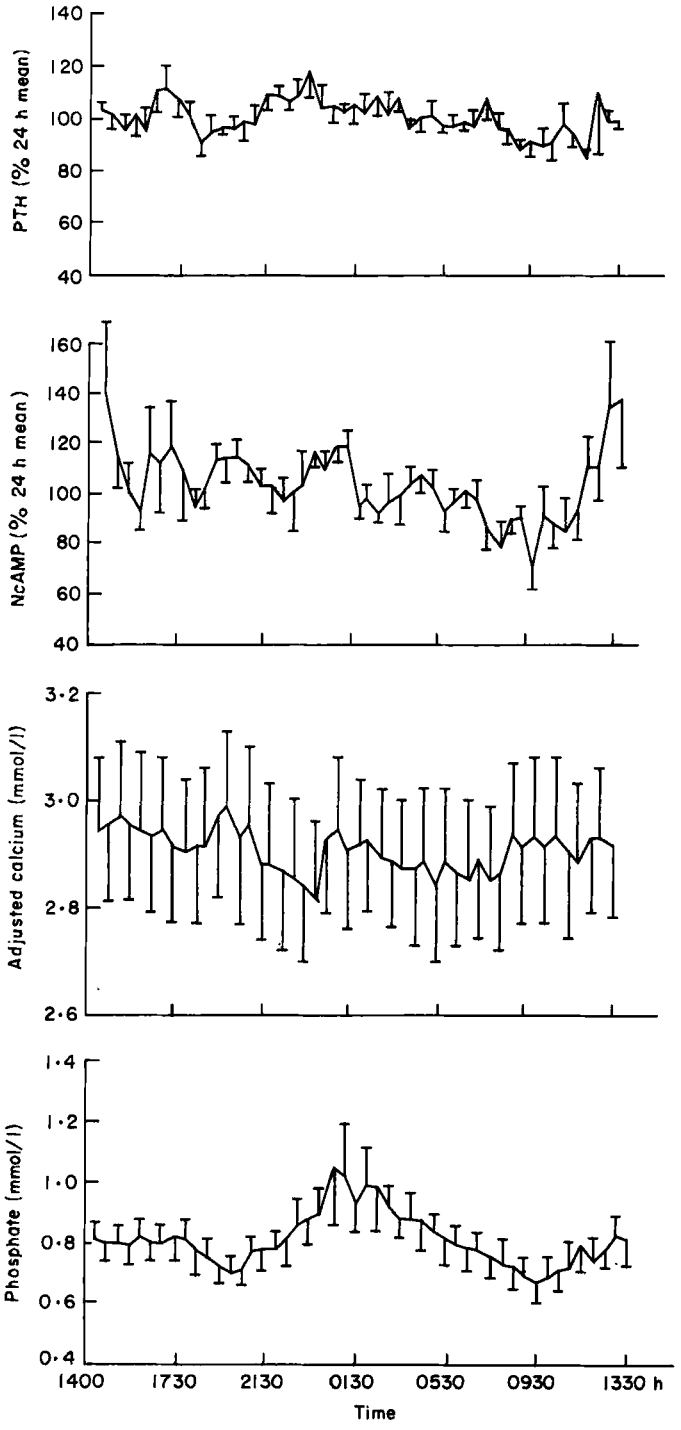

Fig. 2. 24-Hour profiles in hyperparathyroid patients.

Table 1. PTH (1–84) and N-cAMP concentrations over the 24-h period

		PTH (1–84) (pmol/l)			N-cAMP (nmol/l GF)		
	Sex	24-h Mean	Range	CV	24-h Mean	Range	CV
Normal subjects							
1	M	2·3	1·1–4·5	36·7	23·0	4·9–26·9	37·5
2	M	3·9	1·6–6·5	25·1	19·4	6·7–32·3	32·0
3	M	4·6	2·1–7·6	32·2	33·4	14·2–56·7	38·8
4	M	3·2	1·4–5·6	35·2	27·0	10·6–40·6	31·4
5	M	2·6	0·9–4·2	30·6	13·9	4·5–22·7	33·4
6	M	3·2	2·0–4·2	14·8	31·5	20·6–39·7	13·3
Hyperparathyroid subjects							
TC	M	13·9	11·2–17·2	11·0	46·6	20·7–72·9	25·3
PT	F	7·3	4·7–11·9	20·4	37·7	13·8–74·4	43·8
EC	M	20·8	12·6–25·8	10·9	104·0	81·9–126·0	10·3
SB	F	5·5	4·0–12·0	22·7	54·7	30·3–75·6	23·6
TD	M	24·6	16·0–33·0	14·4	73·9	42·6–101·4	17·6
EMcC	F	13·1	9·0–20·0	19·1	38·8	18·2–99·0	44·6

Table 2. Cosinor analysis of 24-h profiles

	Acrophase	Mesor	Amplitude
Normal subjects			
PTH (1–84)	0405	3·3	0·72 ($P<0·05$)
N-cAMP	0524	25·0	5·9 NS
ACa	2003	2·4	0·02 NS
PO_4	0155	1·2	0·14 ($P<0·001$)
Hyperparathyroid patients			
PTH (1–84)	2339	14·2	0·80 NS
N-cAMP	1804	58·0	5·6 NS
ACa	1613	2·9	0·03 NS
PO_4	0116	0·8	0·07 NS

study has shown that the rhythm is not present in patients with primary hyperparathyroidism. The loss of this rhythm is reflected by the absence of the circadian rhythm for N-cAMP in primary hyperparathyroidism.

These observations raise important questions for the control of PTH (1–84) secretion in normal and abnormal states. Previous studies have demonstrated a fall in ionized calcium during the night in both normal subjects and patients with primary hyperparathyroidism (Lo Cascio *et al.*, 1982). In this study we have also shown a transient but significant lowering of adjusted calcium in both groups between midnight and 0200 h. The aetiology of such a transient fall in calcium remains unclear.

A reduction in ionized calcium might be expected to trigger a compensatory surge in PTH (1–84) secretion and therefore provide the mechanism of the nocturnal rise in PTH

(1–84) in normal subjects. However, this explanation appears to be an oversimplification, for it is known that continuous infusion of calcium into normal subjects at a dose just sufficient to obliterate the nocturnal fall in serum calcium does not eliminate the nocturnal rise in PTH. Predictably, infusion of calcium at higher doses leads to decreased nocturnal PTH secretion (Jubiz *et al.*, 1972; Sinha *et al.*, 1975). Clearly, therefore, factors other than changes in ionized calcium concentration are important in the circadian rhythm of PTH (1–84) in normal subjects.

Patients with primary hyperparathyroidism have been shown to respond to the lowering of serum calcium with increased PTH secretion (Murray *et al.*, 1972; Lockefeer *et al.*, 1974). It has been further suggested that the basis of primary hyperparathyroidism is an alteration in the calcium–PTH set-point rather than autonomous secretion of PTH by the parathyroid gland (Brown, 1983; Parfitt, 1969; Gardin *et al.*, 1988). In this study we have shown the absence of a synchronized nocturnal increase in PTH (1–84) in the hyperparathyroid patients, despite the detection of a transient lowering in adjusted calcium consistent with the previous data on ionized calcium (Lo Cascio *et al.*, 1982). Although the precise incremental fall in serum calcium required to produce a response in hyperparathyroid patients has not been defined, this is further evidence against the fall in calcium being the main trigger to circadian changes in PTH (1–84).

If changes in calcium concentration do not fully explain the circadian rhythm of PTH (1–84), then the influence of phosphate must also be considered. This study demonstrates a pronounced circadian rhythm of phosphate in normal subjects. A small decrease in phosphate, which may be due to the movement of phosphate intracellularly to support carbohydrate metabolism, occurs following the evening meal. This small decrease is followed by a much larger increase in phosphate prior to the nocturnal surge of PTH (1–84). This increase in phosphate could be regarded as the stimulus for PTH release, which in turn would cause phosphaturia and the observed subsequent fall in serum phosphate. However, it is known that the phosphate circadian rhythm can be abolished by prolonged fasting, but that the nocturnal surge of PTH persists in these individuals (Jubiz *et al.*, 1972). Furthermore, suppression of PTH secretion by infusion of an appropriate dose of calcium results in an exaggerated phosphate circadian rhythm (Jubiz *et al.*, 1972; Sinha *et al.*, 1975). Thus, it would appear that phosphate has little or no influence on the secretion of PTH during the night, but that the PTH status of the individual may well influence the extent of the phosphate circadian rhythm. Further support for this latter conclusion is obtained from the phosphate results in the hyperparathyroid patients in this study, for the phosphate rhythm is attenuated in the presence of consistently elevated PTH (1–84) concentrations.

An important incidental observation from this study is that the discrimination between normal subjects and patients with primary hyperparathyroidism is time dependent. Thus, using PTH (1–84) as the discriminant, the optimal time for sampling is 1000–1600 h, when the variability within and between normal subjects is at its minimum. Similarly, the optimal time for sampling using N-cAMP as discriminant is between 1100 and 1500 h and not first thing in the morning as recommended by previous authors (Broadus *et al.*, 1977; Holmegaard, 1982).

Combining all the available data leads to the conclusion that changes in calcium and phosphate, either alone or in combination, can not fully explain the circadian rhythm of PTH (1–84) secretion in normal subjects. It remains to be established whether the rhythm is modified by the sex or the age of the subject, but it is tempting to speculate that

neuroendocrine factors from the hypothalamus, or other higher centres, may exert influence on PTH (1–84) secretion as well as on the secretion of hormones from the anterior pituitary. Further work is also required to establish whether the loss of circadian rhythm of PTH (1–84) in primary hyperparathyroidism is an early event in the natural history of the disease or whether it is a secondary consequence of increases in the plasma ionized calcium beyond critical threshold concentrations.

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