# THE CIRCADIAN RHYTHM OF INTACT PARATHYROID HORMONE (1-84) AND NEPHROGENOUS CYCLIC ADENOSINE MONOPHOSPHATE IN NORMAL MEN

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## ABSTRACT

A pronounced circadian rhythm has been demonstrated for intact parathyroid hormone (1-84) in the serum of normal male adults. The broad nocturnal rise of parathyroid hormone (1-84) secretion appears to be of physiological significance, for it is accompanied by a significant rise in nephrogenous cyclic adenosine monophosphate. The rate of return of parathyroid hormone (1-84) to baseline concentrations varies between individuals, an observation which has implications for the optimal time of sampling for the investigation of possible mild hyperparathyroidism.

## INTRODUCTION

The measurement of parathyroid hormone (PTH) has been complicated by the presence of circulating fragments generated by peripheral and glandular metabolism of intact PTH (1-84). The recent development of two-site immunometric assays (Nussbaum, Zahradnik, Lavigne et al, 1987; Brown, Aston, Weeks et al, 1987; Logue, Perry, Chapman, et al, 1988) allows the direct measurement of intact PTH (1-84). Previous studies using assays mainly specific for the biologically inactive C-terminal end of PTH have reported nocturnal rises in immunoreactive PTH concentrations (Jubiz, Canterbury, Reiss et al, 1972; Sinha, Miller, Fleming et al, 1975) which may also be sleep related (Kripke, Lavie, Parker et al, 1978).

The biological action of PTH is mediated by cyclic adenosine monophosphate (cAMP). The measurement of the nephrogenous component of urinary cAMP (N-cAMP) has been shown to be a good index of PTH bioactivity in vivo (Broadus, Mahaffey, Bartter et al, 1977). Studies on cAMP metabolism have reported both the presence (Sagel, Colwell, Loadholt et al, 1973; Murad & Pak, 1972) and absence (Shaw, Oldham, Rosoff et al, 1977) of circadian variation in total urinary cAMP excretion. There have been no reports of circadian variation in N-cAMP.

We have investigated the circadian variation of intact PTH (1-84) and nephrogenous cAMP concentrations in six normal male subjects over a 24 h period.

## METHODS

Six healthy male volunteers (aged 29-40; mean

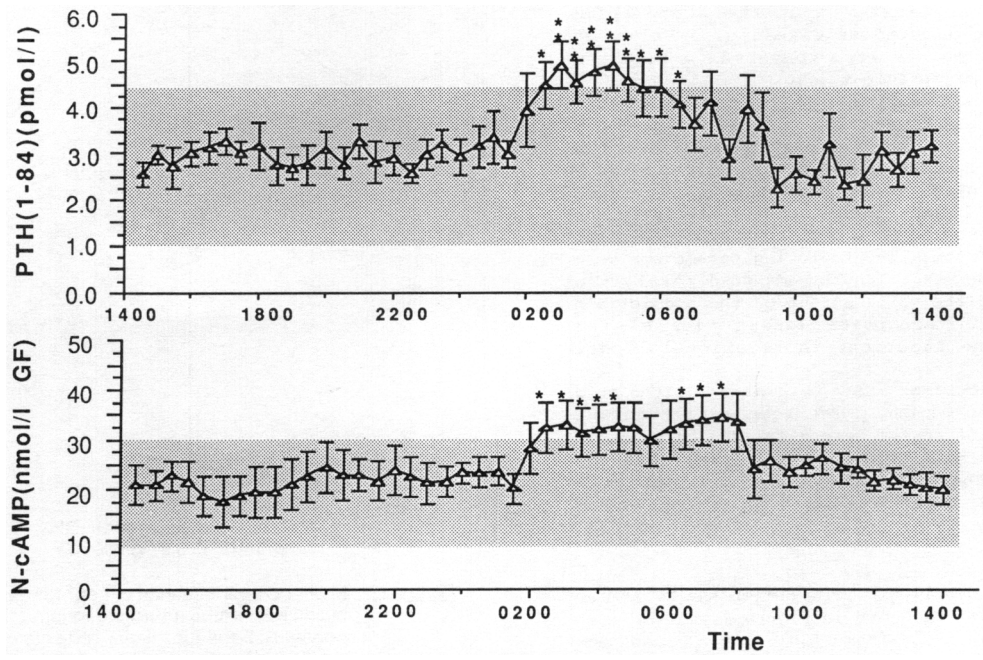


**Figure 1:** **Intact parathyroid hormone ( PTH(1-84) ) and nephrogenous cyclic adenosine monophosphate (N-cAMP) concentrations (mean +/- SEM) in six normal subjects over the 24 h period. Reference ranges are indicated by the shaded area. * p< 0.05 ** p<0.01 ( GF = glomerular filtrate )**

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33 years) were studied over the same 24 h period. Venous blood samples were obtained through an indwelling cannula at 30-min intervals, and timed urine collections were taken throughout the study period.

During the study all subjects ate an identical diet on the day of sampling, with meals being consumed at fixed times. The subjects were ambulant but avoided exercise during the study period. They lay down at 0100, were asleep by 0200 and were awakened at 0700 h.

Intact PTH (1-84) in serum was measured by an in-house IRMA (Logue, Perry, Chapman et al 1988) with a detection limit of 0.5 pmol/l and a range of 1.5 - 250 pmol/l, with an intra assay CV of less than 10%. The assay gives quantitative recovery of PTH (1-84) standard (mean recovery 107%) and is unaffected by a 200 molar excess of C-terminal fragments (53-84). The reference range for normal subjects (n = 40) at 1000 h is 1.0 - 4.5 pmol/l. Intact PTH (1-84) concentrations in 30 patients with primary hyperparathyroidism sampled at random were in the range 5.0 - 120 pmol/l. Subjects with hypoparathyroidism (n = 5) so far studied had PTH (1-84) concentrations of less than 0.5 pmol/l. Plasma cAMP was measured using kits purchased from Amersham International plc, Aylesbury, Bucks, UK. Urinary cAMP was measured using an in-house RIA method (O'Reilly, Fraser, Penney et al, 1986). Nephrogenous cAMP was calculated by the method of Broadus, Mahaffey, Bartter et al (1977). All samples from an individual subject were analysed in the same assay batch.

The 24 h hormone profiles were analysed statistically by two methods:

1) The individual mean concentrations for each subject over the first 4 h of the study (1400 - 1800 h) were adopted as baseline. Values at subsequent time points were compared to baseline using a paired difference t test.

2) Statistical analysis of circadian rhythm parameters was carried out using the Cosinor technique (Nelson, Tong, Lee 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).

The mean-cosinor data were then plotted on a polar co-ordinate graph. The mean amplitude and acrophase of the rhythm were displayed by means of the length and angle of the vector. The envelope representing the bivariate statistical confidence region for these parameters was also derived.

## RESULTS

The mean 24 h profiles of PTH (1-84) and N-cAMP in the six subjects are shown in Figure 1.

PTH (1-84) rises from 0130 h in a broad peak through the night. The concentrations from 0200 to 0600 h were significantly elevated compared to the baseline (1400 - 1800 h). The PTH (1-84) concentrations in all subjects returned to baseline values by 1000 h although the rate of fall varied markedly between individuals. The mean intra-

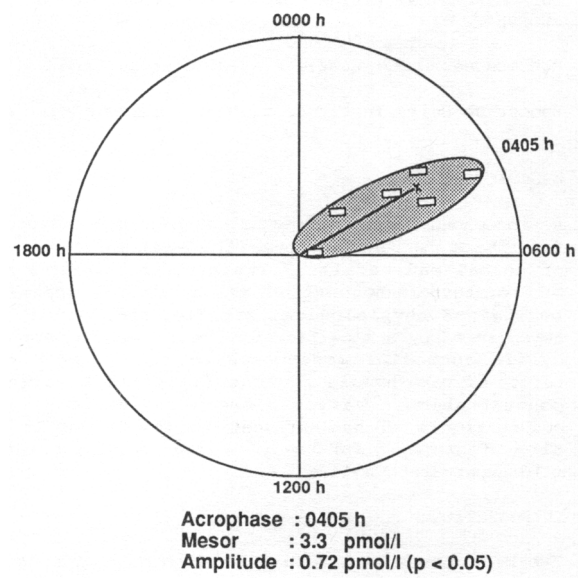


**Figure 2:** **Mean cosinor analysis of intact parathyroid hormone concentrations for the six subjects during the 24 h period. Polar co-ordinate plot. The shaded area represents the statistical confidence limits.**

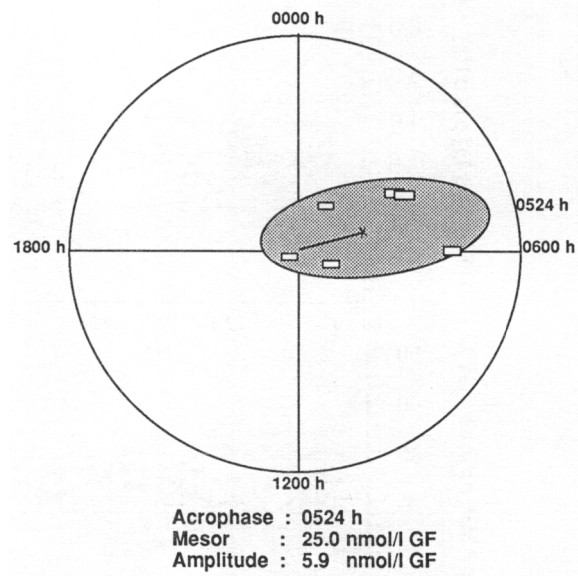


**Figure 3:** **Mean cosinor analysis of nephrogenous cyclic adenosine monophosphate concentrations for the six subjects over the 24 h period. Polar co-ordinate plot. The shaded area represents the statistical confidence limits. (GF = glomerular filtrate )**

individual CV between 0630 and 1000 h was 30% (range 12.9 - 42.5) compared to 17% (12.6 - 23.6) in the basal period.

The mean-cosinor analysis for PTH (1-84) is shown in Figure 2. The polar-coordinate plot indicates the tight phase and frequency synchronisation in the PTH (1-84) concentrations of the six subjects over the 24 h period. The results confirm a significant circadian rhythm in PTH (1-84) of mean amplitude 0.72 from a mesor concentration 3.3 pmol/l with the acrophase occurring at 0405 h.

The mean N-cAMP concentrations rose throughout the night in parallel to the PTH (1-84). The N-cAMP concentrations at eight of the eleven time points during the period 1200 to 0700 were significantly elevated compared to the baseline individual means.

The mean-cosinor data for N-cAMP is shown in Figure 3. Although there is phase synchrony in the N-camp concentrations of the six subjects occurring at 0524 h, the inter-individual variability in amplitude is such that the mean amplitude of 5.9 from the mesor concentration of 25 nmol/l glomerular filtrate is not statistically significant at the 5% level.

## DISCUSSION

Both serum PTH (1-84) and N-cAMP concentrations rise significantly during the night, with mean values above the day-time reference ranges for these analytes. The cosinor analysis confirms significant circadian rhythm in PTH (1-84), with an acrophase at 0405 h. This is consistent with the night-time rise in immunoreactive PTH previously reported using C-terminal specific assays (Jubiz, Canterbury, Reiss et al, 1972; Sinha, Miller, Fleming et al, 1975) and also with the hypothesis that the rise may be sleep related (Kripke, Lavie, Parker et al, 1978).

Our results show that the circadian variations in PTH (1-84) are accompanied by parallel variations in N-cAMP levels. This strongly supports the conclusion that the PTH (1-84) released through the night is physiologically active. The release of PTH (1-84) is probably secondary to the fall in serum ionised calcium levels observed during the night (Lo Cascio, Cominacini, Adami et al, 1982).

The elevated night-time PTH (1-84) concentrations had returned to basal values in all subjects by 1000 h. However, the fall to baseline concentrations varied between individuals, which gave rise to the increased intra-individual variation between 0630 and 1000 h. Notwithstanding the small number of subjects studied, these results have implications for the assessment of the basal PTH (1-84) concentrations in an individual. Early morning samples, such as those typically collected from hospital in-patients, may give spuriously elevated concentrations. We would suggest that maximum discrimination between normal subjects and patients with mild primary hyperparathyroidism is likely to be obtained after 1000 h each day.

Although the N-cAMP concentrations rose significantly, the cosinor analysis failed to detect a significant phase and frequency synchronised rhythm for the six subjects. This is probably related to the small number of subjects and the inherent variability in this parameter which is calculated from four separate measurements (cAMP and creatinine in plasma and urine).

It is of interest that the N-cAMP levels remained elevated (0600 - 0700 h) after the main peak of PTH (1-84). Further studies are required to define the rhythm parameters for N-cAMP.

The existence of a circadian rhythm in PTH (1-84) which is physiologically active raises a number of questions related to the controlling mechanisms of PTH (1-84) release. Studies are proceeding into the role of regulators of PTH secretion and of disease states likely to alter circadian rhythm.

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