Relaxin immunoactivity in human plasma during a 24-hr period

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Summary. Relaxin was secreted episodically in all 6 human subjects studied. A 24-hr rhythm was detected in the pooled data, with maximum secretion in the early-mid-morning hours and a nadir in the early evening.

Many anterior pituitary hormones in man are secreted episodically during a 24-hr period and their release is partly controlled by the central nervous system in relation to the sleep-waking cycle (Sassin *et al.*, 1969, 1972; Rubin, Kales, Adler, Fagan & Odell, 1970; Weitzman *et al.*, 1971; Kapen, Boyer, Hellmann & Weitzman, 1973). Steroid hormones have been shown to be subject to a diurnal release pattern in cyclic rhesus monkeys (Spies, Mahoney, Norman, Clifton & Resko, 1974), in pregnant women (Munson, Yannone & Mueller, 1972), and in normal men (Lacerda, Kowarski, Johanson, Athanasion & Migeon, 1973). It was of interest therefore to determine whether relaxin immunoactivity exhibits fluctuations over a 24-hr period.

Methods

Two normal cyclic women aged 21 and 28 years, a pregnant woman (first trimester) aged 22, a post-menopausal woman aged 50, and a normal 23-year-old male were acclimatized to the sleep laboratory for at least 12 hr of adaptation before sampling. All were healthy, none had ever received hormone therapy or was taking drugs. The phase of the menstrual cycle and the occurrence of ovulation was determined from the basal body temperature curves. In one woman in the early follicular phase of the cycle oestradiol benzoate was infused (total amount 500 µg/1000 ml saline) over the 24-hr period of study. A catheter was inserted into an antecubital vein and during the next 24 hr blood was sampled every 20 min from an adjoining room, using the technique described previously (Kirk & Sassin, 1969). Plasma was immediately separated and frozen until hormonal assays were performed. Samples were transported on solid CO₂ and kept frozen until assayed.

Subjects were monitored polygraphically by standard techniques for recording electroencephalogram, electro-oculogram and electromyogram (Rechtschaffen & Kales, 1968). Sleep recordings were scored in 1-min epochs as Stages W (wakefulness), 1, 2, 3, 4 or REM (rapid eye movement) sleep.

Relaxin radioimmunoassay was performed as described by Bryant (1972) but modified to a double-antibody separation procedure (Bryant & Stelmasiak, 1974). Since human relaxin immunoactivity in plasma is parallel to the porcine relaxin standard (NIH-R-P1) concentrations are expressed in terms of this standard. The sensitivity of the assay in routine use was such that 6.3 ng relaxin/ml plasma could be distinguished from zero and was measured with a maximum percentage error ($P = 0.05$) of $\pm 50\%$ when estimates at 3 concentrations were performed. Further work on the specificity of the radioimmunoassay for plasma relaxin has been reported (Kwok, McMurtry & Bryant, 1976; Bryant, 1976).

Results and discussion

The mean relaxin concentration for each subject studied is shown in Table 1; the concentrations were lowest in the post-menopausal and male subjects and were within the same range in the other

women. When the data were pooled for all 6 subjects (Text-fig. 1), relaxin immunoactivity was high in the morning hours, reaching its peak in the early afternoon, and thereafter decreasing rapidly until the onset of sleep. A rise in concentration occurred during the sleep period which continued through the early morning hours until afternoon. It is not known whether the increase which commences at the time of sleep is due to sleep, or time of day.

Table 1. Mean $\pm$ S.E.M. relaxin concentrations for each male or female human subject

Subject	No. of samples	Relaxin conc. (ng/ml)
Perioviulatory	107	142.9 $\pm$ 17.4
Follicular phase + oestradiol benzoate	61	130.9 $\pm$ 21.4
Follicular phase	65	112.8 $\pm$ 24.9
Pregnant	76	128.7 $\pm$ 19.1
Post-menopausal	74	46.5 $\pm$ 7.5
Male	65	91.5 $\pm$ 13.8

However, relaxin was secreted episodically in all the subjects studied. Text-figure 2(a) shows a typical example of one subject studied for 36 hr over two sleep periods at approximately the time of ovulation. Less episodic activity was shown by the relaxin concentrations in the post-menopausal woman (Text-fig. 2b), as might be expected for a peptide arising from the ovaries and uterus, although two short bursts of secretion were evident during the sleep period and a few during the morning hours.

From the current sampling time it was not possible to show any ultradian rhythm for the rapid peaks of relaxin immunoactivity but this possibility cannot be excluded. Relaxin secretion in women probably represents the integration of ovarian and uterine discharge with a frequency and magnitude varying according to the particular physiological state of the individual. In pregnancy the possible contribution of the placenta to the total relaxin immunoactivity must be considered. The source in men is at present unknown but may be the testes.

It is of interest to speculate whether immunoactivity arising from the reproductive organs has its own programmed rhythmicity or whether secretion is under direct control of the nervous system or of an hormonal substance arising elsewhere, e.g. the pineal. Little is known about the role of nerves and catecholamines in ovulation and normal ovarian function (Bahr, Kao & Nalbandov, 1974).

Text-fig. 1. The combined plasma relaxin values for 6 human subjects during a 24-hr period. The samples taken every 20 min were combined in threes to give an hourly average which was expressed as a % of that subject's 24-hr mean. The values for all 6 subjects were then pooled.

Text-fig. 2. The plasma relaxin immunoactivity of samples collected every 20 min from (a) a woman in the periovulatory phase of the menstrual cycle, and (b) a post-menopausal woman.

There appears to be no correlation between the relaxin immunoactivity and the diurnal variations of the pituitary hormones which have been studied (Sassin *et al.*, 1969, 1972; Rubin *et al.*, 1970; Weitzman *et al.*, 1971; Kapen *et al.*, 1973). Information will be gained regarding the controlling mechanisms if the overall 24-hr pattern of relaxin release can be altered with shifts of the sleep-wake or light-dark cycles. In addition, with the development of assay methods specific for the various sources of relaxin immunoactivity, it may be possible to understand more clearly the reproductive functions of this hormone(s).

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