Growth hormone, prolactin and cortisol nyctohemeral variations during naloxone-induced opiate receptor blockade in man

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Abstract. To evaluate the role of endogenous opioid peptides in prolactin (Prl), growth hormone (GH) and cortisol neuroregulation, 50 mg of the opiate antagonist naloxone was infused over 24 h to 6 normal male volunteers. An additional naloxone dose (5 mg) was given iv as a bolus injection at 20.00 h. Blood specimens were collected hourly by means of a portable constant withdrawal pump.

Naloxone failed to alter 24 h secretion of GH and Prl. The sleep-related GH and Prl rise was also unaffected by the opiate blocker. Moreover, naloxone failed to alter the circadian rhythm of cortisol and its 24 h concentration.

The results do not suggest a major role of opiate receptors in spontaneous GH, Prl and cortisol secretion in man.

Extensive investigation in recent years has established the presence within the central nervous system of opiate receptors and of several peptides with opiate-like activity (Hughes et al. 1975; Pert & Snyder 1973). Since then, progress has been made in the investigation of the role of opioid endogenous substances in the regulation of the endocrine system and many studies have provided evidence that morphine, endorphins and their receptors plays a role in regulating pituitary hormone secretion in experimental animals (Meites et al. 1979). The relation of opiate receptors to human neuroendocrine function is less clear. Studies reporting the effects of the opiate receptor blocker naloxone on basal and stimulated pituitary hormone release led to inconclusive results (Rubin et al. 1979; Morley et al. 1980; Grossman et al. 1981; Delitala et al. 1981; Spiler & Molitch 1980; Serri et al. 1981). However, morphine and β-endorphin have been shown to elevate prolactin (Prl) but not growth hormone (GH) or cortisol (Tolis et al. 1975, 1978; Catlin et al. 1980). Further, a long acting analogue of met-enkephalin stimulated GH (and Prl) while decreasing cortisol and gonadotrophin secretion through naloxone sensitive mechanisms in man (Von Graffenried et al. 1978; Stubbs et al. 1978).

Although the data suggest a role for opiate receptors in modulating secretion of some pituitary hormones, the question of whether endogenous substances with opiate like activity have a physiological role in the control of the hypothalamus-pituitary-axis in man remains. To investigate this further, we have studied the effects of naloxone, a pure opiate antagonist, on circadian variations of GH, Prl and cortisol in normal man.
Materials and Methods

Six normal males, aged 24–31 years, volunteered for this study. They were non-obese and none of them was taking any medication that could interfere with the assay of the hormones under study. The experiments were performed after 3 days' acclimatization to hospital life; during the test the subjects were free to carry out normal activity. Regular hospital meals were consumed at 08.00, 12.30 and 19.30 h. The collection of specimens were carried out by means of a portable constant withdrawal pump in accord to Kowarski et al. (1971); a 2.5 ml volume of blood was collected hourly. The constant withdrawal sampling permits the measurements of the mean concentration for a single fraction of time (integrated concentration, IC) and then the average of the concentration is related to a single period of the day (Giordano & Giusti 1979). An iv cannula was placed in the opposite arm for the infusion of naloxone or 0.9% saline: 50 mg naloxone (2.08 mg/h) or 0.9% saline (each in a volume of 40 ml) were infused over 24 h via a constant infusion pump. An additional naloxone dose (5 mg) or 0.5 ml normal saline were given over 1 min, at 20.00 h. Blood samples were immediately centrifuged and stored at −20°C until assayed. Every subject gave informed consent and all studies were performed single (subject) blind. Lights were turned off between 22.00–07.00 h and during this period sleep was continuously monitored by standard techniques and recording eye movements.

Serum GH and Prl were measured by a double-antibody radioimmunoassay using MRC standard 71/222 for Prl and 66/127 for GH. 125I-labelled antigens and antisera were provided by Biodata, Milan. The intra- and inter-assay coefficients of variations, estimated on reference pools, were 4.0 and 8.7% for Prl and 3.6 and 7.8% for GH. Serum cortisol was measured by competitive protein binding (Cortipac, Amersham). The intra- and inter-assay coefficients of variations were 5.0 and 9.0%, respectively.

Results are reported as mean ± SEM. Two-tailed paired t-test was used for data evaluation.

Results

Growth hormone (Fig. 1, Table 1).

IC-GH showed major fluctuations during saline infusion and in each subject there was the expected rise in GH 1 to 2 h after sleep onset (12.2 ± 4.5 ng/ml). Elevated IC-GH lasted 2 h and then fell suddenly to low levels. During naloxone infusion each subject showed the expected nocturnal GH rise 1 to 2 h after sleep onset (12.3 ± 2.8 ng/ml). In both experiments, each subject showed the nocturnal GH rise during slow wave sleep. No statistically significant difference could be found in IC-GH during waking, sleep or the entire 24 h period, between saline treated and naloxone treated subjects (P > 0.05).

Prolactin (Fig. 1, Table 1)

During saline infusion the nyctohemeral rhythm of IC-Prl was clearly evident in 4 subjects while IC-Prl was elevated at 19.00 h (10.7 ng/ml) and 08.00 h (10.1 ng/ml) in the other two volunteers. However, subsequent IC-Prl levels were normal. Since both subjects were re-cannulated at that time, these increased values were likely to be related to the venepuncture. In each of the 6 subjects serum IC-Prl began to rise 60–120 min after sleep onset, achieved maximal levels in the early morning and declined soon after morning awakening. When naloxone was infused there was the same release
Table 1.
Integrated concentration (mean ± SEM) of prolactin (ng/ml), growth hormone (ng/ml) and cortisol (μg/100 ml) during saline (S) and naloxone (N) infusion.

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pattern and IC-Prl again rose during sleep. The average IC-Prl related to a single period of the day, waking, during sleep or the entire 24 h was comparable in both experiments (P > 0.05).

Cortisol (Fig. 2, Table 1)
A circadian rhythm of cortisol was detected in each subject both during saline and naloxone infusion. In both experiments mean IC-cortisol reached its nadir at the 23.00 h period of sampling; the highest IC-cortisol was observed at 07.00 h both in naloxone treated and saline treated subjects. No statistically significant differences could be found: 24 h IC-cortisol was comparable during saline and naloxone infusions in each subject (P > 0.05).

Sleep
As described in detail elsewhere (Rodriguez et al., subm. for publ.) naloxone reduced the proportion of total sleep time spent in rapid eye movement (REM) sleep (20.7 vs 8.1%) and slightly increased slow sleep duration (76.3 vs 87.4%) without significantly altering sleep latency or total sleep time (saline 422 ± 29 min; naloxone 408 ± 47 min, P > 0.05). No side-effects were observed during naloxone infusion.

Discussion
The values of 24 h IC of GH, Prl and cortisol found in the present study are in agreement with previously published experiments performed using a constant withdrawal pump (Kowarski et al. 1971; Thompson et al. 1972; De Lacerda et al. 1973; Copinschi et al. 1978; Giordano & Giusti 1979). The well-known elevation of GH and Prl associated with sleep was clearly apparent in all subjects although the sleep-related peak of these hormones was lower than that obtained with different sampling techniques (Takahashi et al. 1968; Sassin et al. 1972, 1973). This difference is likely to be due to the continuous sampling procedure which integrates high frequency variations and tends to smooth any pulsatile hormonal secretion. The results show that the opiate antagonist naloxone had no effect on the 24 h pattern of GH and Prl secretion or on the circadian rhythm of cortisol. Moreover, naloxone failed to influence the nocturnal rise in serum GH and Prl in all subjects.

Exogenous and endogenous opiates have been shown to exert a variety of effects on pituitary hormone secretion in experimental animals. The ability of morphine, β-endorphins and met-enko-
and has basal dose-dependent action. Doses of GH significantly rose from 2.08 mg/h for 24 h. The failure of naloxone to produce any effect on cortisol secretion is not easily explained, since the drug has recently been shown to stimulate cortisol secretion significantly in man (Morley et al. 1980; Delitala et al. 1981). This discrepancy may be related to the different protocol of this study or, possibly, to the different naloxone dose infused in the present experiment. Since the effect of met-enkephalin is blunted by only 0.4 mg of naloxone, the opiate antagonist, a dose of 2.08 mg/h for 24 h is likely to be effective in blocking central opiate receptors. Moreover, we found this dose to be highly effective in stimulating gonadotrophin and testosterone secretion in normal males (unpublished results). The reported ability of naloxone, but only when given in large amounts, to stimulate cortisol release might suggest, by analogy with experimental evidences in rodents (Eisenberg 1980) a different sensitivity of the ACTH-cortisol axis to opiate receptor blockade. However, the possibility that cortisol secretion induced by naloxone was smoothed over by the continuous sampling procedure used in our experiment cannot be discarded.

In conclusion, our observations indicate a failure of naloxone to alter 24 h secretion of GH, PRL and cortisol in normal subjects and do not suggest a major role of opiate receptors in the physiological regulation of these hormones in man.

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References


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