Abstract. To further investigate the role of opioids in the regulation of the pituitary-adrenal axis we studied the effect of morphine and naloxone on human corticotropin-releasing hormone (hCRH)-induced ACTH, immunoreactive (ir) β-endorphin, and cortisol release in normal subjects. Protocols: 1. 30 mg of a slow-release preparation of morphine or placebo was given orally 3 h prior to administration of hCRH (0.1 mg iv) (N = 7). 2. Naloxone (4 mg as bolus iv) or placebo was given 5 min prior to hCRH (N = 7). 3. Naloxone (4 mg iv as bolus followed by a continuous infusion of 6 mg over 75 min) or placebo was started 15 min prior to hCRH (N = 6). hCRH was injected at 11.00 h (protocol 1, 2) or at 17.00 h (protocol 3). Oral morphine not only suppressed basal hormone levels (P < 0.02), but also the peak response to hCRH compared with placebo (cortisol: 270 ± 50 vs 559 ± 80 nmol/l; ACTH: 5.1 ± 1.5 vs 13.1 ± 2.7 pmol/l; ir β-endorphin: 48.5 ± 8.7 vs 88 ± 14 pmol/l; mean ± SEM, P < 0.02). Similarly, the maximum incremental changes and the area under the curve were significantly reduced for all three hormones compared with placebo (P < 0.05). After 4 mg of naloxone in the morning, no significant hormonal changes in response to hCRH were observed. However, 10 mg of naloxone in the afternoon led to higher maximum hormone concentrations in response to hCRH compared with placebo (cortisol: 636 ± 30 vs 437 ± 65 nmol/l; ACTH: 19.6 ± 4.4 vs 8.7 ± 1.1 pmol/l; ir β-endorphin: 180 ± 44 vs 94 ± 18 pmol/l, P < 0.05). The effect of high-dose naloxone on the hCRH-induced hormone release alone supports the concept of a physiologically significant inhibition of the ACTH release by endogenous opioids via receptors of relative naloxone resistance (δ- or κ-receptors) located at the pituitary level. The μ-agonist morphine may act at suprahypophyseal sites by inhibition of CRH potentiating factors.

In man, opiates have been shown to inhibit the pituitary-adrenal axis (Grossman 1983). Thus, β-endorphin suppresses basal ACTH and cortisol secretion (Taylor et al. 1983). Similarly, the μ-opiate receptor agonist morphine sulphate reduces baseline cortisol concentrations (McDonald et al. 1959) and blocks the cortisol response to surgical stress (George et al. 1974; Brandt et al. 1978). The met-enkephalin analogue FK 33-824, a synthetic derivative acting at μ- and δ-opiate receptor sites (Kream & Zukin 1979) completely blocks the ACTH response to lysine-vasopressin (del Pozo et al. 1980a) and human corticotropin-releasing hormone (hCRH) (Allopio et al. 1985). As high doses of the specific opiate antagonist naloxone are required to reverse the effect of FK 33-824 on ACTH secretion, it has been assumed that this action is mediated via δ-opiate receptors (Gaillard et al. 1981; Alloio et al. 1982). Recently it has been reported that the racemic benzomorphan κ-agonist MR 2033 also decreases ACTH and cortisol secretion (Pfeiffer et al. 1985). Thus, there is evidence that the inhibitory action of opiates on the pituitary-adrenal axis involves μ-, δ- and κ-binding sites.

However, the physiological role and the site of action of endogenous and exogeneous opioids remain to be elucidated. Moreover, data are lacking as to whether administration of opiates (e.g. morphine) may carry the risk of significant impairment of the adrenocortical responsiveness in critically ill patients.

The aim of our study was, therefore, to investig...
igate the effect on the hCRH-induced hormone release of a long-acting morphine preparation, which is frequently used for analgesia in cancer patients. In addition, we studied the effect of two different doses of naloxone on the CRH-induced hormone secretion.

Patients and Methods

A total of 17 healthy volunteers (11 males, 6 females, aged 22–48 years) participated in the study after having given informed consent. The study was approved by the local ethical committee (Universitätskliniken Köln).

On two occasions, separated by at least 3 days, all participants underwent a hCRH-test after a resting period of 60 min using synthetic human CRH (0.1 mg iv, Fa. Bachem, Bubendorf, Switzerland).

An indwelling needle was placed in a forearm vein 30 min before the first blood sampling and kept patent by a slow saline infusion. All protocols were performed in a randomized, cross-over single blind fashion.

1. Morphine sulphate

After an overnight fast, seven volunteers (6 males, 1 female) received 30 mg of a slow-release morphine sulphate preparation (1 tablet MST 30, Mundipharma, Limburg, FRG) or placebo at 08.00 h. hCRH was injected at 11.00 h.

11. Naloxone

a. After an overnight fast, seven volunteers (5 males, 2 females) received 4 mg of naloxone (Narcanti, Du Pont, Frankfurt, FRG) or placebo (0.9% saline) as an iv bolus injection at 10.55 h followed by hCRH at 11.00 h.

b. Six volunteers (3 males, 3 females) received 4 mg of naloxone as an iv bolus injection at 16.45 h followed by a continuous infusion of 6 mg of naloxone over 75 min or placebo (0.9% saline). hCRH was injected at 17.00 h. All subjects had been fasting for at least six hours at the time of the hCRH injection.

Blood samples for cortisol, ACTH, immunoreactive β-endorphin (ir β-endorphin), and prolactin were collected at −30, 0 (time of hCRH injection), 15, 30, 45, 60, 90 and 120 min.

Plasma ACTH and ir β-endorphin were measured by radioimmunoassay (Allolio et al. 1981; Jeffcoate et al. 1978) after extraction from plasma (Voigt et al. 1974; Jeffcoate et al. 1978).

Our antibody against β-endorphin showed 58% cross-reactivity with β-lipotropin. Serum cortisol and prolactin were determined by radioimmunoassay using commercially available reagents (NEN, Dreieich, FRG; Serono, Freiburg, FRG). All samples from each subject were assayed in the same assay.

To compare the secretory response to hCRH, the area under the curve were also calculated taking basal secretion into account.

The results are expressed as mean ± SEM. Statistical analysis of the data was performed using Wilcoxon's non-parametric test for paired data.

Results

I. Morphine sulphate (Fig. 1)

Compared with placebo, oral morphine resulted in a significant (P < 0.02) suppression of basal serum cortisol (124 ± 17 vs 275 ± 58 nmol/l), plasma ACTH (1.2 ± 0.1 vs 2.9 ± 0.6 pmol/l), and plasma ir β-endorphin (28 ± 5.4 vs 47 ± 8.2 pmol/l) at 180 min, whereas serum prolactin was increased throughout the study period (Table 1). In addition, peak hormone values in response to hCRH were significantly reduced (cortisol: 270 ± 50 vs 559 ± 80 nmol/l; ACTH: 5.1 ± 1.5 vs 13.1 ± 2.7 pmol/l; ir β-endorphin: 49 ± 8.7 vs 88 ± 14 pmol/l, P < 0.02), as were the maximum incremental changes (cortisol: 135 ± 61 vs 283 ± 30 nmol/l, P < 0.05; ACTH: 3.8 ± 1.5 vs 10.2 ± 2.4 pmol/l, P < 0.02; ir β-endorphin: 20 ± 4.9 vs 41 ± 7.7 pmol/l, P < 0.05). Similarly, the integrated secretory response to hCRH as calculated from the area under the curve was reduced compared with that to placebo (cortisol: 119 ± 84 vs 320 ± 39 h · nmol/l; ACTH: 3.5 ± 1.3 vs 7.2 ± 1.6 h · pmol/l; ir β-endorphin: 12.2 ± 5.0 vs 25 ± 8.3 h · pmol/l, P < 0.05).

<table>
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<tr>
<th>Time from hCRH injection (min)</th>
<th>Serum prolactin (µg/l)</th>
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<tr>
<td></td>
<td>After morphine</td>
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<tr>
<td>−30</td>
<td>6.4 ± 1.6*</td>
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<td>0</td>
<td>6.5 ± 1.6*</td>
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<td>30</td>
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*P < 0.02.
Plasma ACTH, serum cortisol and plasma immunoreactive β-endorphin concentrations after hCRH administration (0.1 mg iv at 0 min) in seven subjects after placebo (●-●) and after the same stimulus plus morphine sulphate (30 mg orally at −180 min) (○-○). Maximum hormone concentrations are depicted on the right.

Plasma ACTH, serum cortisol and plasma immunoreactive β-endorphin concentrations after hCRH administration (0.1 mg iv at 0 min) in seven subjects after placebo (0.9% NaCl) (●-●) and after the same stimulus plus naloxone (4 mg iv at −5 min) (○-○).
II. Naloxone (Figs. 2 and 3)
Administration of 4 mg of naloxone as a bolus dose at -5 min did not alter the hormone response to hCRH (Fig. 2). Although hormone concentrations tended to be higher after naloxone than after placebo, the differences were not significant.

In contrast, compared with placebo, high-dose naloxone (10 mg) significantly enhanced the hormone release after hCRH (Fig. 3).
Peak hormone concentrations were significantly higher (cortisol: 636 ± 30 vs 437 ± 62 nmol/l; ACTH: 19.6 ± 4.4 vs 8.7 ± 1.1 pmol/l; ir β-endorphin: 180 ± 44 vs 94 ± 18 pmol/l, P < 0.05) as were secretory areas (cortisol: 447 ± 61 vs 297 ± 53 h·nmol/l; ACTH: 12.6 ± 3.0 vs 6.8 ± 0.5 h·pmol/l; ir β-endorphin: 104 ± 29.7 vs 38 ± 15 h·pmol/l, P < 0.05).

No side effects of naloxone were observed.

The test volunteers were unable to differentiate between naloxone and placebo. Morphine induced sedation and euphoria, which in some cases went unnoticed by the participants.

Discussion
Our study demonstrates that the µ-agonist morphine suppresses not only the basal secretion of cortisol, ACTH and ir β-endorphin, but also the increase in these hormones in response to hCRH. These findings are in accordance with recent results of Rittmaster et al. (1985) who found inhibition of the pituitary-adrenal response to ovine CRH by iv morphine sulphate (0.14 mg/kg). As CRH acts at the pituitary corticotropes, inhibition of CRH-induced hormone release suggests a direct action of morphine at the pituitary level. However, the blockade by morphine is incomplete and may, therefore, operate also at supr ahypophyseal sites by inhibition of endogenous CRH or factors which potentiate the action of CRH (e.g. vasopressin, catecholamines) (Vale et al. 1983; Gillies et al. 1982; Lamberts et al. 1984). This assumption is supported by the observation that morphine lacks a direct effect on rat pituitary cells in vitro (Rittmaster et al. 1985). However, opioidergic control of the pituitary-adrenal axis may be different in rats, as high doses of morphine stimulate ACTH and β-endorphin secretion in this species (Kokka et al. 1973; Haracz et al. 1981), whereas in humans only inhibitory effects have
been described (Grossman 1983). Thus, studies with intact human pituitary cells may be necessary to elucidate whether there is a direct effect of morphine at the pituitary gland in man.

Owing to the high affinity of naloxone to μ-opiate receptors, the action of μ-receptor agonists is easily blocked by low doses of naloxone. Thus, 0.4 mg of naloxone completely abolishes the stimulatory effects of FK 33-824 on prolactin and growth hormone release (Stubbs et al. 1978). In our study, a ten times higher dose (4 mg) failed to alter the hormonal response to hCRH, indicating that endogenous μ-opiate receptor agonists have no physiological role in the control of pituitary ACTH and ir β-endorphin secretion during stimulation with CRH.

Whereas in patients with Addison's disease, a dose of 4 mg of naloxone provokes a significant increase in plasma ACTH (Allolio et al. 1982), this dose is ineffective in normal subjects (del Pozo et al. 1980b). Only doses of 10 and 20 mg of naloxone have been demonstrated to induce a clear-cut increase in plasma ACTH and cortisol in normals (Volavka et al. 1979). In our study 10 mg of naloxone increased the hormonal response to hCRH, demonstrating again that high doses of the specific opiate antagonist are required to influence the pituitary-adrenal axis. This is in agreement with the findings of Conaglen et al. (1985) who reported an enhanced ACTH response to CRH after pre-treatment with 20 mg of naloxone. Thus, the opioidergic control of ACTH and ir β-endorphin release seems to be mediated via relatively naloxone-insensitive receptors (δ- or κ-receptors), which may be located in the pituitary gland. Previous studies with the μ- and δ-agonist FK 33-824 strongly suggest a role of δ-opiate receptors, as only high doses of naloxone reverse the effect of this substance on the pituitary-adrenal axis. Moreover, the vasopressin- and hCRH-induced ACTH release is completely abolished by FK 33-824 (del Pozo et al. 1980a; Allolio et al. 1985). In contrast, the effect of the κ-agonist MR 2033 is more variable and vasopressin-triggered increases of plasma ACTH are not blocked (Pfeiffer et al. 1985).

In conclusion, under physiological conditions, the plasma ACTH and ir β-endorphin secretion are under inhibitory opioidergic control via relatively naloxone-insensitive opiate receptors, probably δ-receptors located at the pituitary level. Additional supraphyseal sites cannot be excluded. Endogenous μ-opiate receptor agonists are without physiological significance in the regulation of ACTH release at the pituitary level, whereas the exogenous μ-agonist morphine leads to pronounced suppression of ACTH and ir β-endorphin, probably acting at suprathyseal sites.

The suppression by morphine of the adrenocortical response to surgery has been interpreted as the result of reduced stress (Brandt et al. 1978). Our study now shows that the decrease in serum cortisol and plasma ACTH concentrations is not only a consequence of reduced stress owing to sedation, euphoria, and analgesia, but that also the physiological regulation of ACTH release by CRH is impaired. This raises the question whether prolonged treatment with morphine may lead to significant adrenocortical insufficiency. Slow-release oral morphine is successfully used for long-term control of pain in cancer patients. After ingestion of 30 mg of morphine in a slow-release preparation, high levels of plasma morphine are reached after 3 hours and are maintained for up to 12 hours (Welsh et al. 1983). The increasing prolactin concentrations in our volunteers indicate that the maximum response to this morphine preparation has not yet occurred at 180 min (Tolis et al. 1975). No data are available for assessing the duration of adrenocortical suppression after 30 mg of slow-release morphine. Moreover, as many patients take up to 4 tablets per day, it becomes important to know whether adrenocortical suppression persist during long-term treatment or whether an escape phenomenon exists. The clinical signs of impaired adrenocortical function (anorexia, weakness, easy fatigue) may be falsely attributed to the underlying illness.

Acknowledgments

We are indebted to Miss D. Vollmar, Mrs K. Wehner, Mrs H. Hofmann, Mrs G. Roßbach, and Mrs G. Hermeiling-Mayer for skilful technical assistance. This study was supported by Landesamt für Forschung, NRW.

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Received May 5th, 1986.
Accepted November 20th, 1986.