

# Effects of Loperamide on the Human Hypothalamo-Pituitary-Adrenal Axis *in Vivo* and *in Vitro*\*

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## ABSTRACT

Loperamide, an opiate agonist of high specificity for  $\mu$ -receptors, was recently reported to suppress ACTH and cortisol levels in normal subjects, but not in patients with proven ACTH-dependent Cushing's disease. However, there is little information on the site of action of loperamide in the hypothalamo-pituitary-adrenal axis of man. We investigated the effect of loperamide on pituitary hormone secretion *in vivo* and *in vitro*. In seven normal subjects, basal ACTH plasma levels were significantly suppressed 3 h after loperamide administration (16 mg, orally) from  $5 \pm 1$  to  $2 \pm 0$  pmol/L ( $P < 0.0001$ ). After the combined pituitary stimulation test (100  $\mu$ g human CRH, 100  $\mu$ g GnRH, 100  $\mu$ g GH-releasing hormone, and 200  $\mu$ g TRH), the ACTH peak (maximum increase at 30 min) was significantly blunted by loperamide from  $9 \pm 1$  to  $4 \pm 1$  pmol/L ( $P < 0.001$ ) and the area under the curve of ACTH from 0–120 min was reduced from  $35 \pm 5$  to  $23 \pm 4$  pmol/L $\cdot$ 2 h ( $P <$

0.05). In the insulin-hypoglycemia test (0.15 IU/kg BW), neither the ACTH peak nor the area under the curve of ACTH was affected by loperamide. In six patients with Cushing's disease and one patient with secondary adrenal insufficiency due to hypothalamic failure, neither basal ACTH and cortisol levels nor CRH-stimulated levels were influenced by loperamide. In four cultured human corticotrophic adenomas, loperamide was not able to reduce basal and CRH-induced ACTH secretion. In summary, loperamide is able to reduce basal and CRH-induced ACTH and cortisol levels in normal subjects, but not in patients with Cushing's disease or secondary adrenal failure of hypothalamic origin. Loperamide has no significant effect on insulin-hypoglycemia-induced ACTH and cortisol levels and, therefore, no effect on stress-induced elevation of cortisol levels. Loperamide might act at a suprapituitary site in man *in vivo*, but, nevertheless, a pituitary site cannot be excluded. (*J Clin Endocrinol Metab* 75: 552–557, 1992)

THE ANTIDIARRHEAL drug loperamide is an agent of the piperidine class known to act as an opiate agonist of high specificity for  $\mu$ -receptors (1, 2). Loperamide was recently reported to suppress ACTH and cortisol levels in normal subjects and patients with Addison's disease, but not in patients with proven ACTH-dependent Cushing's disease (3–5). The suppressive effect of loperamide on ACTH secretion was shown to be reversible by naloxone (4). Loperamide also modified the ACTH responses to CRH and lysine vasopressin in patients with Addison's disease (6). With regard to ACTH secretion, these findings are similar to those for a number of  $\mu$ -opiate agonists, where secretion was suppressed in normal subjects but not in patients with Cushing's disease (7–10). However, in spite of the well established suppressive effect of opiate alkaloids and peptides on ACTH secretion, their precise site of action has not been identified up to now. While some investigations suggest a direct pituitary site of action (9, 11), others postulate a suprapituitary modulation of some CRH-potentiating factors by the opioid system (10, 12).

One aim of our study was to elucidate the site of action of loperamide in the hypothalamo-pituitary-adrenal axis of man. While administration of releasing hormones stimulates

hormone secretion at the pituitary level (13), insulin-induced hypoglycemia activates the whole hypothalamo-pituitary-adrenal axis and stimulates suprapituitary centers (14, 15). Therefore, the combination of a specific hypothalamic and pituitary stimulation test should make it possible to localize the site of action of loperamide. Moreover, no data exist to indicate whether therapeutically or diagnostically used high dose administration of loperamide may carry the risk of a reduced adrenocortical responsiveness to stress in an ill patient. The insulin-hypoglycemia test was performed to address this problem. Additional information about the action of loperamide was sought by investigating the effect of this drug on CRH-induced ACTH secretion in patients with Cushing's disease *in vivo* and in human corticotrophic adenomas *in vitro*.

## Materials and Methods

### Materials

Loperamide was obtained from Janssen (Neuss, Germany). Human (h) CRH was purchased from Bissendorf Peptide GmbH (Wedemark, Germany). Regular human insulin was obtained from Novo Industrie GmbH (Mainz, Germany).

### Subjects

The combined pituitary stimulation test and insulin-hypoglycemia test was performed in seven healthy volunteers, aged 20–28 yr. The two females and five males had normal body weight according to the Broca index. All were free from any current life stress situations, physical

Received October 7, 1991.

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\* This work was supported in part by a grant from the Deutsche Forschungsgemeinschaft (Sta 285/1–4).

illness, and medication.

The CRH stimulation test was performed in six female patients, aged 20–45 yr, with proven Cushing's disease and in one male patient (47 yr) with secondary adrenal insufficiency.

The diagnosis of Cushing's disease was made by the following endocrine evaluation. Mean plasma cortisol concentrations, derived from five measurements at 4- to 5-h intervals within a 24-h period, were determined in the preoperative period and showed a cancelled diurnal rhythm of secretion. The range of mean plasma cortisol concentrations was 480–950 nmol/L (normal, 250–650 nmol/L). After the low dose dexamethasone suppression test (2 mg/day), there was a lack of suppression of cortisol secretion. In the insulin-hypoglycemia test (0.15 IU/kg BW), patients did not show an appropriate cortisol secretory response. The range of basal plasma ACTH concentrations was 7–24 pmol/L (normal, 4–17 pmol/L). All patients responded to a large dose of dexamethasone (8 mg/day) with a significant decrease in plasma cortisol levels. In addition, every patient was subjected to a sinus petrosus inferior catheterization in combination with a CRH stimulation test for determination of pituitary origin and localization of ACTH secretion. The radiological investigation consisted of a nuclear magnetic resonance scan. All patients were cured after transsphenoidal microsurgery.

The patient with secondary adrenal insufficiency due to a hypothalamic origin of his disease had no ACTH response to the insulin-hypoglycemia test (data not shown). However, a large rise in ACTH secretion was observed from a low basal level after the CRH stimulation test. Cortisol levels were not detectable. The hydrocortisone substitution (25 mg/day) was discontinued on the days of the tests.

All subjects were familiar with the nature of the study and had given written informed consent. Each person acted as his own control, and cross-over studies were performed at random at least a week apart.

### Protocols

Tests started at 0730 h (–210 min) after an overnight fast. The cubital vein was cannulated and kept open with iv 0.9% saline. At 0800 h (–180 min), 16 mg loperamide in liquid form (8 mL) or placebo was given orally. The combined pituitary stimulation test was performed with 100 µg hCRH, 100 µg GnRH, 100 µg GH-releasing hormone (GHRH), and 200 µg TRH. The CRH stimulation test was performed with 100 µg hCRH, and the insulin-hypoglycemia test with 0.15 IU/kg BW regular human insulin. Each agent was administered iv at 1100 h (0 min). Blood samples were collected between –180 and 120 min at 15- to 30-min intervals. Hypoglycemia was regarded as adequate if the blood glucose level was decreased to below 2.2 mmol/L and typical clinical signs were documented.

### Hormone measurements

ACTH was measured with a commercial immunoradiometric assay kit from Nichols Institute (San Juan Capistrano, CA). Intra- and inter-assay variabilities were 6.9% and 13.3%, respectively. The detection limit was 1 pmol/L. Normal values were 3–11 pmol/L. Cortisol was measured by a specific RIA (16). Intra- and interassay variabilities were 3.6% and 5.0%, respectively. The detection limit was 30 nmol/L. Normal values ranged from 140–550 nmol/L. PRL, LH, FSH, GH, and TSH were measured by double antibody RIAs (17).

### Human corticotropic adenoma cell culture

Cell culture was performed as described recently by Stalla *et al.* (18). In brief, after transsphenoidal surgery, adenoma tissue was transported to the laboratory in sterile culture medium. Routinely, the tumor transport medium was assayed for all anterior pituitary hormones. The four tumors were selected for these studies because they had no detectable levels of LH, FSH, TSH, GH, and PRL, in contrast to high ACTH levels (>2000 pmol/L) in the transport medium. The tissue was mechanically and enzymatically dispersed. Cells were diluted to a density of  $1.0 \times 10^5$  cells/mL and distributed in 24-well tissue culture plates. The plates were placed in a humidified CO<sub>2</sub> incubator for 4–6 days. Afterwards, the monolayers were washed with Dulbecco's Modified Eagle's Medium containing 0.5 g/L BSA. The hCRH standard (2 nM) and loperamide (1

and 10 µM) were added in small volumes (50 µL). Cells were preincubated for 30 min with loperamide and then incubated together with CRH or medium alone for a further 180 min. Then medium was removed for ACTH measurement by a RIA, as described previously (18).

### Statistics

Statistics were performed by analysis of variance in combination with the Scheffe test. All data are shown as the mean  $\pm$  SE. To eliminate intervariability between subjects, the data in Figs. 1, 2, 3, and 5 are shown as a percentage of the basal control value (100%). Three different parameters were used to estimate the basal and stimulated hormone levels in the *in vivo* studies: 1) 0', basal level at 0 min; 2)  $\Delta 30'$ , hormone level 30 min after stimulation minus basal level at 0 min; and 3) AUC, area under the curve from 0–2 h after stimulation minus basal level at 0 min.

## Results

### *In vivo* studies

**Normal subjects.** In the combined pituitary stimulation test, the basal plasma ACTH levels at –180 min were almost identical in the loperamide *vs.* placebo group. While the basal ACTH level did not change in the placebo group during the following 3 h, it significantly declined early (–120 min) during loperamide treatment and showed a nadir of  $43 \pm 6\%$  *vs.*  $100 \pm 0\%$  ( $5 \pm 1$  pmol/L) at 0 min ( $P < 0.001$ ). After combined pituitary stimulation, the peak ACTH levels in the loperamide *vs.* placebo group differed significantly up to 60 min ( $155 \pm 29\%$  *vs.*  $319 \pm 54\%$  at 45 min, respectively;  $P < 0.05$ ). At 120 min, ACTH levels in both groups had returned to basal values. Furthermore, the ACTH increase ( $\Delta 30'$ ) was significantly blunted by loperamide from  $9 \pm 1$  to  $4 \pm 1$  pmol/L ( $P < 0.001$ ), and the AUC of ACTH was significantly reduced from  $35 \pm 5$  to  $23 \pm 4$  pmol/L·2 h ( $P < 0.05$ ; Fig. 1).

Basal cortisol plasma levels at –180 min were similar in the loperamide *vs.* placebo group. Even at –120 min, a significant difference between the treatment groups could be observed with respect to cortisol secretion. At 0 min, cortisol secretion was suppressed by loperamide to  $39 \pm 5\%$  *vs.*  $100 \pm 0\%$  ( $300 \pm 60$  nmol/L;  $P < 0.01$ ). After combined pituitary stimulation, the cortisol levels during loperamide treatment showed significant differences from those in the placebo group at each time up to 120 min. In both groups, cortisol levels reached their maximum values at 60 min ( $172 \pm 27\%$  *vs.*  $252 \pm 23\%$ ;  $P < 0.01$ ) and returned to almost basal values at 120 min. In addition, the cortisol increase ( $\Delta 30'$ ) was significantly blunted by loperamide from  $330 \pm 30$  to  $170 \pm 30$  nmol/L ( $P < 0.05$ ), whereas the decline in the AUC was not significant (Fig. 1).

The basal level of PRL at –180 min was  $10 \pm 1$  µg/L and declined in the placebo group to  $5 \pm 0$  µg/L at 0 min; in contrast, in the loperamide group, PRL stayed at the elevated level of  $9 \pm 1$  µg/L ( $P < 0.0001$ ). Stimulated PRL levels as well as basal and stimulated hormone levels of LH, FSH, GH, and TSH did not show any significant differences between placebo and loperamide treatment groups (data not shown).

In the insulin-hypoglycemia test, the basal ACTH values at –180 min were almost identical in the loperamide *vs.*

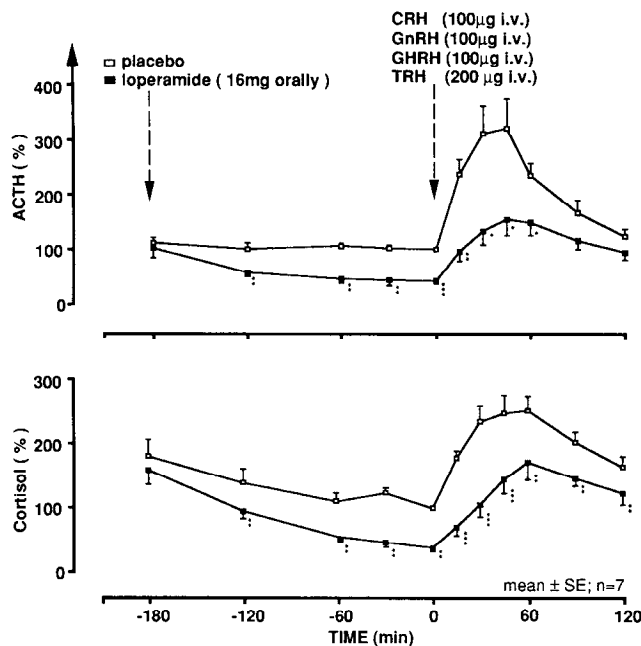


FIG. 1. Effects of loperamide and placebo on plasma ACTH and cortisol (mean  $\pm$  SE) before and after a combined bolus injection of 100  $\mu$ g hCRH, 100  $\mu$ g GnRH, 100  $\mu$ g GHRH, and 200  $\mu$ g TRH in seven normal subjects. Loperamide (16 mg) or placebo was given orally at  $-180$  min. hCRH, GnRH, GHRH, and TRH were given at 0 min. Data are shown as a percentage of the basal value at 0 min in the placebo group (100%). The corresponding ACTH value was  $5 \pm 1$  pmol/L, and the corresponding cortisol value was  $300 \pm 60$  nmol/L. Stars indicate times at which a significant difference occurred between loperamide and placebo treatments by the Scheffe test ( $\star$ ,  $P < 0.05$ ;  $\star\star$ ,  $P < 0.01$ ;  $\star\star\star$ ,  $P < 0.001$ ).

placebo group. The basal ACTH levels in the placebo group showed a slight decrease for the next 3 h, while in the loperamide group, ACTH levels were significantly diminished as early as  $-60$  min and reached  $51 \pm 3\%$  vs.  $100 \pm 0\%$  ( $4 \pm 0$  pmol/L) at 0 min ( $P < 0.0001$ ). After insulin injection, there was a delayed, but overwhelming, ACTH increase in the placebo group as well as in the loperamide group ( $977 \pm 128\%$  vs.  $730 \pm 103\%$  at 45 min), which could not be significantly suppressed by loperamide at any time. Peak ACTH was about 3–4 times higher than that in the combined pituitary stimulation test. In the second hour after insulin injection, ACTH levels in the loperamide and placebo group were nearly identical and reached basal levels at 120 min. Also, there was no significant decline of the ACTH increase ( $\Delta 30'$ ) and AUC after loperamide treatment compared to those after placebo administration (Fig. 2).

Basal cortisol levels at  $-180$  min were about 20% higher in the loperamide than in the placebo group, but were diminished significantly at 0 min  $62 \pm 7\%$  vs.  $100 \pm 0\%$  ( $250 \pm 30$  nmol/L;  $P < 0.01$ ). Corresponding to the ACTH values, there was a delayed cortisol increase during hypoglycemia. At 60 min, cortisol levels reached their maximum of  $312 \pm 28\%$  in the placebo group and  $292 \pm 23\%$  in the loperamide group and did not return to basal levels until 120 min. At no time during hypoglycemia were the cortisol levels blunted significantly by loperamide. Loperamide also failed to significantly decrease the cortisol increase ( $\Delta 30'$ ) or the AUC (Fig.

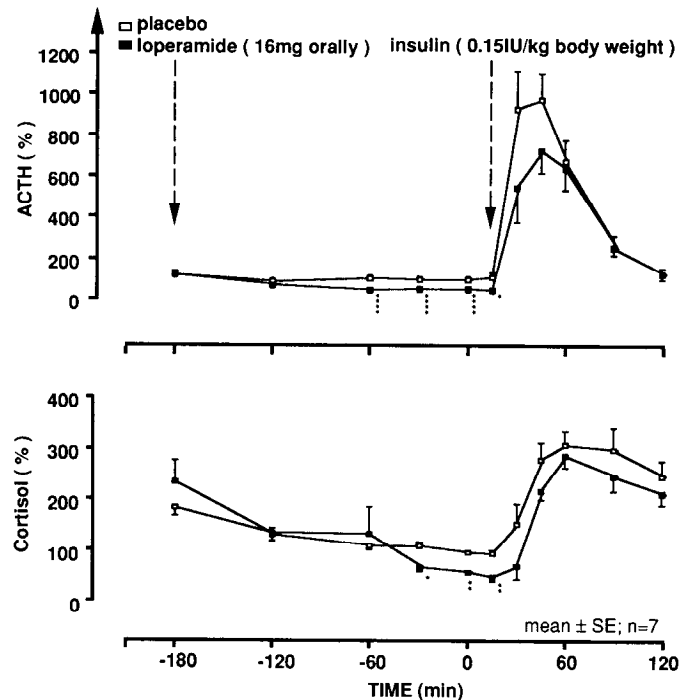


FIG. 2. Effects of loperamide and placebo on plasma ACTH and cortisol (mean  $\pm$  SE) before and after a bolus injection of 0.15 IU/kg BW regular human insulin in seven normal subjects. Loperamide (16 mg) or placebo was given orally at  $-180$  min. Insulin was given at 0 min. Data are shown as a percentage of the basal value at 0 min in the placebo group (100%). The corresponding ACTH value was  $4 \pm 0$  pmol/L, and the corresponding cortisol value was  $250 \pm 30$  nmol/L. Stars indicate times at which a significant difference occurred between loperamide and placebo treatments by the Scheffe test ( $\star$ ,  $P < 0.05$ ;  $\star\star$ ,  $P < 0.01$ ;  $\star\star\star$ ,  $P < 0.0001$ ).

2).

Blood glucose levels and symptoms of hypoglycemia were similar in the placebo and loperamide groups (data not shown).

**Patients with Cushing's disease.** The mean basal ACTH level (0 min) in the patients with Cushing's disease receiving placebo treatment was  $16 \pm 3$  pmol/L and similar to that in the loperamide group from  $-180$  min up to 0 min. Also, after the injection of hCRH, loperamide was not able to significantly suppress the ACTH increase, and ACTH levels in the placebo and loperamide groups reached peaks of  $298 \pm 58\%$  and  $234 \pm 54\%$ , respectively, at 30 min. At 120 min, ACTH levels in placebo and loperamide groups had returned to basal values. Furthermore, there was no significant suppressive effect of loperamide on the ACTH increase ( $\Delta 30'$ ) and AUC (Fig. 3).

Basal cortisol levels in the placebo ( $640 \pm 80$  nmol/L) and loperamide groups showed a slight decrease from  $-180$  min to 0 min, but loperamide had no significant effect at any time. Also, after hCRH stimulation, cortisol levels in the placebo vs. loperamide groups did not differ significantly and reached a maximum values of  $150 \pm 21\%$  and  $142 \pm 22\%$ , respectively, at 45 min. At 120 min, values had almost declined to basal levels in both groups. In addition, there was no significant suppressive effect of loperamide on the cortisol increase ( $\Delta 30'$ ) and AUC (Fig. 3).

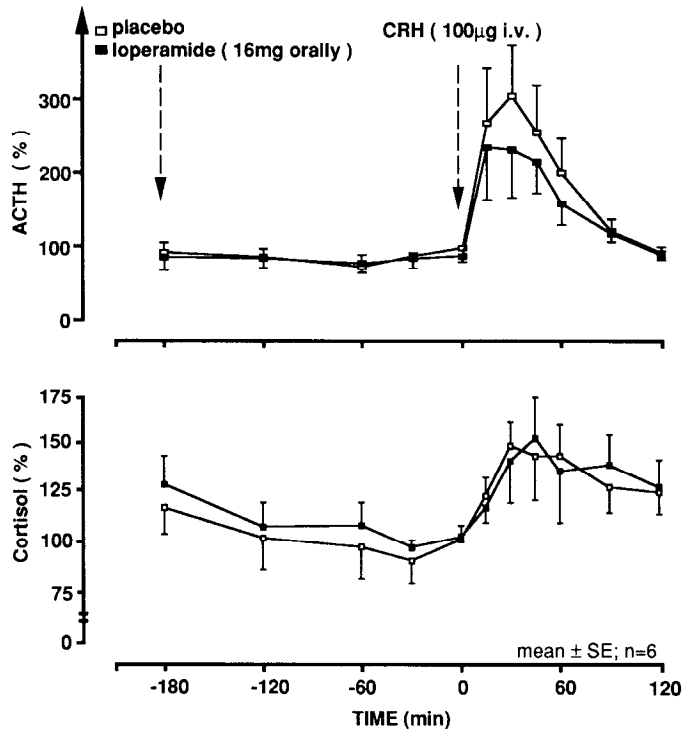


FIG. 3. Effects of loperamide and placebo on plasma ACTH and cortisol (mean  $\pm$  SE) before and after a bolus injection of 100  $\mu$ g hCRH in six patients with Cushing's disease. Loperamide (16 mg) or placebo was given orally at  $-180$  min. hCRH was given at 0 min. Data are shown as a percentage of the basal value at 0 min in the placebo group (100%). The corresponding ACTH value was  $16 \pm 3$  pmol/L, and the corresponding cortisol value was  $640 \pm 80$  nmol/L. At no time did a significant difference occur between loperamide and placebo groups, as determined by the Scheffe test.

*Patient with secondary adrenal insufficiency.* The patient's basal ACTH level (0 min) was approximately 2 pmol/L. The maximum values after hCRH injection were reached at 15 min, with a pronounced 12-fold increase in ACTH levels. At 120 min, the prolonged high values had reached a maximum of about 9 pmol/L. At no time were hormone measurements different between the loperamide and placebo groups (Fig. 4). Cortisol levels could not be detected, being lower than 30 nmol/L (Fig. 4).

No serious side-effects were observed after oral administration of 16 mg loperamide. The only minor complaints were constipation for 4 days (two cases) and xerostomia for some hours (five cases).

#### *In vitro studies*

The basal ACTH levels measured in the medium of four human corticotrophic adenomas differed as follows: 110 pmol/L (adenoma 1), 550 pmol/L (adenoma 2), 2202 pmol/L (adenoma 3), and 6606 pmol/L (adenoma 4). Stimulation with hCRH (2 nM) caused a 2-fold increase in ACTH secretion in adenomas 1 and 2, while CRH-induced ACTH levels in adenomas 3 and 4 were 3 times higher than basal. Loperamide in concentrations of 1 and 10  $\mu$ M had no significant effect on basal or CRH-induced ACTH secretion in any adenoma (Fig. 5).

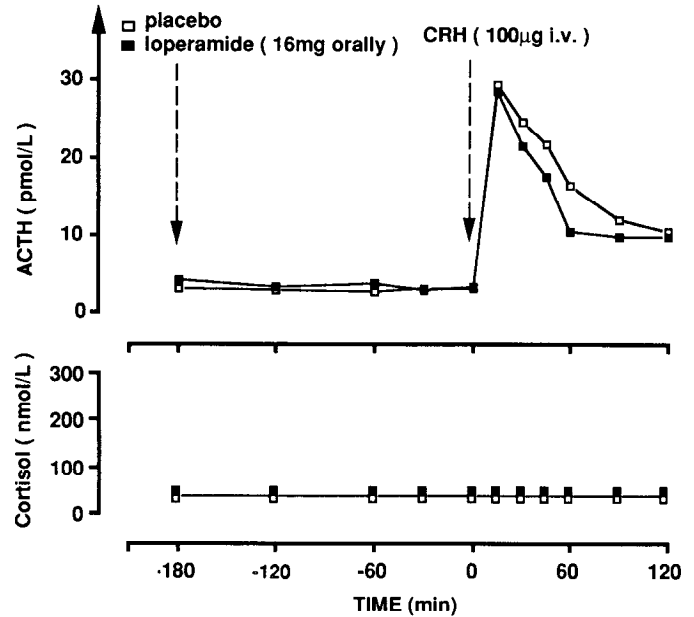


FIG. 4. Effects of loperamide and placebo on plasma ACTH and cortisol before and after a bolus injection of 100  $\mu$ g hCRH in a patient with secondary adrenal insufficiency due to primary hypothalamic failure. Loperamide (16 mg) or placebo was given orally at  $-180$  min. CRH was given at 0 min.

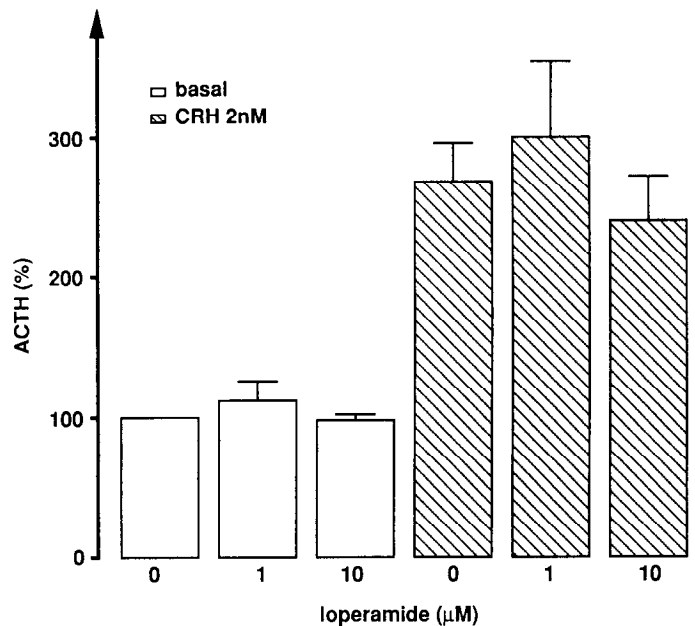


FIG. 5. Effects of loperamide on basal and CRH-induced ACTH levels in the cell culture of four human corticotrophic adenomas (mean  $\pm$  SE;  $n = 4$ ). Data are shown as a percentage of the basal value of the control group (100%). The corresponding ACTH value was  $2367 \pm 1483$  pmol/L. At no concentration did a significant difference occur between loperamide and control groups, as determined by the Scheffe test.

#### Discussion

We have been able to show that loperamide reduces basal and CRH-induced ACTH and cortisol levels in normal subjects. These findings are in agreement with those of Ambrosi

*et al.* (3), who reported a suppressive effect of loperamide on basal ACTH levels in normal subjects, and Bochicchio *et al.* (6), who described a suppressive effect of loperamide on CRH-induced ACTH secretion in patients with Addison's disease. In addition, we have shown for the first time that loperamide is unable to reduce the ACTH increase in the insulin-hypoglycemia test.

The administration of releasing hormones leads to hormone secretion by activating the anterior pituitary gland directly (13). Insulin-induced hypoglycemia activates the whole hypothalamo-pituitary-adrenal axis and stimulates suprapituitary centers (14, 15). Insulin-induced hypoglycemia provokes peak plasma ACTH levels that are greater than those after CRH, arginine vasopressin, or simultaneous CRH and arginine vasopressin administration (19). As insulin-induced hypoglycemia provokes the secretion of maximally stimulating endogenous CRH levels (20), our results might reflect a suppressive effect of loperamide on endogenous CRH secretion or some other cofactors of corticotropic stimulation. In the combined pituitary stimulation test, the potentiating effect of such cofactors on exogenous CRH is missing, and the ACTH increase is blunted after loperamide administration. In the insulin-hypoglycemia test, the strong stimulus of hypoglycemia has an overwhelming effect on suprapituitary structures, and it would appear that loperamide is too weak to diminish the secretion of CRH or some other cofactors of corticotropic stimulation. On the other hand, a pituitary site of action cannot be excluded. First, we do not know whether the decline in basal ACTH secretion is mediated by a suppressive effect of loperamide on the corticotrophs themselves or on a hypothetically existing suprapituitary trigger of basal ACTH secretion.

It was reported recently that in patients with secondary adrenal insufficiency who respond to exogenous CRH with a pronounced and prolonged ACTH response, the secondary adrenal insufficiency is not caused by a lesion of the pituitary gland itself, but by a lesion at a suprapituitary level (21). Our data for the patient with secondary adrenal insufficiency due to a hypothalamic lesion provided confirmation of these findings, since although he could not react to insulin-induced hypoglycemia, he showed a pronounced and prolonged ACTH increase after the CRH stimulation test. Loperamide was not able to influence his very low basal and large CRH-induced ACTH secretion, while his corticotrophs were still able to respond to exogenous stimuli. Although we only report a single case and, therefore, it is difficult to extrapolate to other cases, these results strongly suggest a suprapituitary site of action of loperamide.

In patients with Cushing's disease, we could show that basal and CRH-induced ACTH levels were not suppressed by loperamide; thus, our results are in agreement with those of Ambrosi *et al.* (4), who reported the failure of loperamide to suppress basal ACTH levels in these patients. Hypercortisolism in Cushing's syndrome is mostly generated by an autonomous ACTH-producing pituitary adenoma of monoclonal origin (22). It has been shown that the ACTH increase after CRH stimulation is considerably more pronounced in patients with Cushing's disease than in normal controls (13).

In addition, it has been reported that the main suppressive effect of corticosteroid excess on CRH biosynthesis and secretion takes place at a suprapituitary site (23, 24). Therefore, it may be possible that the hyperresponsiveness of CRH-induced ACTH increase in patients with Cushing's disease is due to an increased sensitivity of corticotrophs to CRH resulting from the prolonged absence of negative feedback by cortisol. In this context, a suppressive effect of loperamide on the already very low endogenous CRH levels in patients with Cushing's disease should not affect their autonomous ACTH secretion, while an effect of loperamide at the pituitary level should have diminished at least the CRH-induced ACTH increase. Thus, these data may support the idea of loperamide modifying the release of CRH or some other cofactors of corticotropic stimulation.

In addition, we found no significant effect of loperamide on basal and CRH-induced ACTH secretion of human corticotropic adenomas *in vitro*. While Lamberts *et al.* (11) reported about a suppressive effect of the  $\mu$ -opiate agonist FK 33-824 on ACTH secretion of rat anterior pituitary lobes *in vitro* that was not reversible by naloxone, Rittmaster *et al.* (10) found no effect of morphine on ACTH secretion of perfused rat anterior pituitary cells.

After loperamide administration, we found a significant elevation of basal PRL levels, which is in contrast to the findings of Ambrosi *et al.* (4). In agreement with our results, different  $\mu$ -opiate agonists have been reported to elevate PRL levels *in vivo* (25, 26) and *in vitro* (27).

Modulation of the pituitary-adrenal activity by a suprapituitary influence of loperamide would require this compound to cross the blood-brain barrier. While there are some reports to show that loperamide poorly penetrates the blood-brain barrier in rats (28) and that it lacks any central opiate-like effects in man (29), other researchers found small amounts of loperamide after oral application in brain tissue of rats (30) and reported an analgesic potency in the mouse hot plate test (1). Moreover, loperamide may act at the median eminence of the hypothalamus. The observation that loperamide has a high affinity for  $\mu$ -receptors (1, 2) and that there exists a high concentration of  $\mu$ -receptors in the hypothalamus (31) coupled with the finding that the  $\mu$ -opiate antagonist naloxone (4) antagonizes the loperamide effect on ACTH provide additional evidence to suggest that it probably works at a suprapituitary level.

In summary, loperamide is able to reduce basal and CRH-induced ACTH and cortisol levels in normal subjects, but not in patients with Cushing's disease and secondary adrenal insufficiency due to hypothalamic failure. Furthermore, loperamide has no effect on insulin-hypoglycemia-induced ACTH and cortisol levels in normal subjects. The fact that high dose loperamide administration fails to block adrenocortical responsiveness to stress in man is of clinical importance. One can speculate that there might exist a suprapituitary site of action of loperamide in man *in vivo*; nevertheless, a pituitary site cannot be completely excluded.

#### Acknowledgments

The authors would like to thank S. Bauer, K. Lechner, and B. Glockner (Department of Medicine, University of Munich) for excellent technical

assistance. We also gratefully acknowledge Drs. U. Renner and E. Arzt (Max Planck Institute of Psychiatry) for numerous helpful discussions, and Dr. C. Newton (Max Planck Institute of Psychiatry) for reviewing the manuscript for English usage.

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