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Evidence for ACTH-unrelated Mechanisms in the Regulation of Cortisol Secretion in Man*

H.L. Fehm, R. Holl, K. Steiner, E. Klein, and K.H. Voigt
Abteilung Innere Medizin I and Abteilung Physiologie I, Universität Ulm

Summary. In an attempt to elucidate the significance of ACTH independent mechanisms in the regulation of cortisol secretion in man, the dynamics of plasma ACTH and cortisol levels were studied in response to different stimuli. The cortisol response to small amounts of exogenous ACTH and to insulin induced hypoglycemia was preceded by an increase in ACTH levels appropriate to explain the increase in cortisol. In contrast, after administration of methamphetamine, there was an increase in cortisol levels in the absence of any changes in ACTH concentrations. Apparently, the methamphetamine induced cortisol secretion was not mediated by radioimmunoassayable ACTH. A diurnal rhythm was observed for the responses to hypoglycemia and to methamphetamine with larger cortisol responses in the evening as compared to the forenoon. These changes were not accompanied by parallel changes in the ACTH responses. From these differences, additional evidence is provided for the importance of ACTH independent mechanisms in the regulation of cortisol secretion.

Key words: ACTH – Cortisol – Methamphetamine – Pituitary gland

Introduction

It is generally accepted that synthesis and release of adrenal corticosteroids are mediated by pituitary ACTH. However, there is a large number of animal studies suggesting important extrapituitary influences on adrenal morphology and function. Already in 1959 Halasz and Szentagothai (see also [15]) provided evidence for the existence of a neural pathway from the adrenal gland to the hypothalamus. Later on, it has been demonstrated that hypothalamic stimulation or neurogenic stress is capable of increasing adrenocortical secretion in the absence of the pituitary gland [23, 36]. More recently, an increasing number of reports deals with extrapituitary influences on certain aspects of adrenocortical function in the rat: Engeland and Dallman (1976) demonstrated that adrenal growth after unilateral adrenalectomy is mediated by both afferent and efferent neural elements. Several authors reported about the existence of a nycthemeral rhythm in adrenal responsiveness to ACTH that is dissociable from the rhythm in ACTH [1, 8, 20, 21]. After stress-induced activation of adrenocortical secretion, a period of decreased response to subsequent stress has been observed and this altered response appeared to be mediated by a non-adrenocorticotropin mechanism [9]. Similarly, it has been shown that the rapid decreases in adrenal and plasma corticosterone concentrations after drinking are not mediated by changes in plasma ACTH concentrations [39]. According to Ottenweller and Meier (1982) adrenocortical rhythmicity can be maintained in hypophysectomized rats by extrapituitary mechanisms and can be suppressed by disruption of adrenal innervation (see also [25, 29]). However, the significance of adrenal innervation for rhythmicity has been doubted by several other authors [16, 27, 38].

To our knowledge, in humans the exclusive role of ACTH in the regulation of cortisol secretion has never been questioned. We now provide evidence that the well known cortisol response to methamphetamine is not mediated by radioimmuno-assayable ACTH by measuring plasma cortisol and ACTH levels at short intervals (5 and

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Offprint requests to: Prof. Dr. H.L. Fehm (address see page 24)

10 min respectively). The results are compared with those obtained with other established stimuli of cortisol secretion, i.e. insulin induced hypoglycemia and administration of exogenous ACTH. Because of the known circadian periodicity in the responsiveness of the pituitary-adrenal system to these stimuli, all examinations were performed in the forenoon and in the evening.

Experimental Subjects

32 healthy young men, aged 22–30 years, participated in the study as paid volunteers after giving informed written consent. The study has been approved by the human research committee of the University of Ulm.

Material and Methods

All studies were performed in the forenoon, starting at 9 or 10 a.m., and in the evening, starting at 6 p.m. All subjects were at bedrest in a quiet environment for the duration of the study.

Insulin Tolerance Test (ITT)

Regular insulin (0.15 U/kg body weight) was administered intravenously as a bolus. In each case blood glucose fell below 40 mg/dl after 15 min with the usual symptoms of hypoglycemia.

ACTH Administration

In an attempt to produce a rise in plasma cortisol levels comparable to ITT, small amounts of ACTH (0.01 I.U./kg body weight) were infused at constant rate during 15 min. As human ACTH was not available, highly purified natural porcine ACTH (Acortan simplex®, Ferring Co., 62 U/mg) was used instead. To ensure comparable starting levels for plasma cortisol, the forenoon studies included pretreatment with dexamethasone (1 mg orally 10 h before the test).

Methamphetamine Administration

15 mg methamphetamine-HCl (Pervitin®, Temmlerwerke Marburg) were given intravenously. Blood pressure and heart rate were monitored during the procedure; untoward reactions were not observed.

Blood was drawn through an indwelling cannula from a cubital vein at 5 or 10 min intervals. Plasma ACTH was estimated by radioimmunoassay after extraction of ACTH from the plasma as previously described [13, 37]. The antiserum employed yielded identical displacement curves with human and porcine ACTH. Parallel displacement curves were obtained with synthetic ACTH 1–28 and ACTH 1–24. There was no cross-reactivity with ACTH 1–13 or ACTH 18–39, and a weak and incomplete cross-reactivity with ACTH 11–24. In order to prove whether ACTH precursor molecules were also recognized by the antiserum, rat pituitary extracts were subjected to SDS disc electrophoresis and ACTH-like immunoreactivity of the protein fractions was estimated. By this procedure several peaks with ACTH-like immunoreactivity were obtained, the largest having a molecular weight of about 31 K, thus corresponding presumably to pro-opiomelanocortin. From these characteristics of the antiserum it appears that all ACTH-related peptides with known steroidogenic activity will be recognized by our radioimmunoassay system, whereas inactive ACTH fragments will not interfere. Plasma cortisol was measured by competitive protein binding analysis [28].

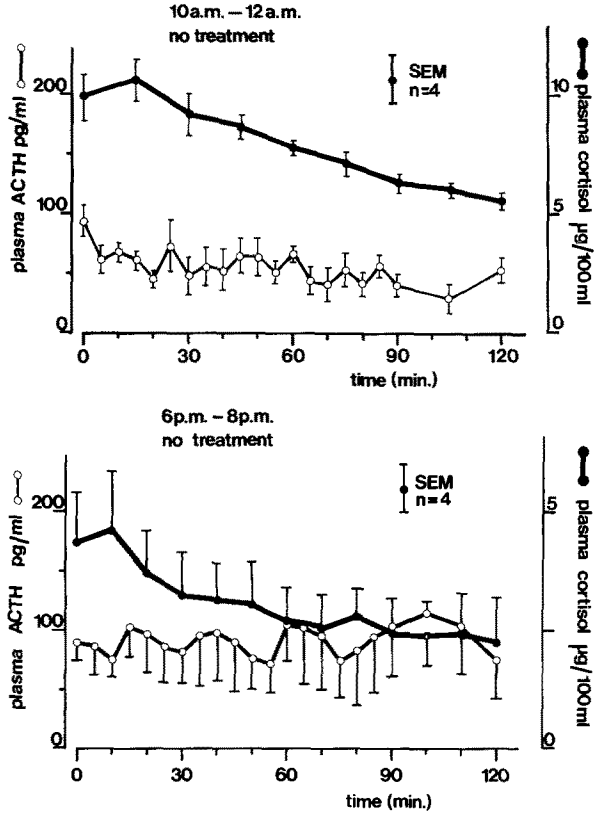

Fig. 1. Temporal pattern of plasma ACTH and cortisol levels in healthy subjects under basal conditions in the forenoon and in the evening (control groups)

Results

In 8 subjects without treatment (control group) plasma cortisol levels decreased continuously throughout the observation period (Fig. 1). As one would expect, there was a difference in starting values between forenoon and evening (10.2 ± 1.5 µg/dl; $\bar{x} \pm$ SEM and 4.4 ± 1.0 µg/dl, respectively). Individual plasma ACTH levels exhibited marked oscillations, in the mean they were uniform without any tendency to decrease in parallel to cortisol.

Hypoglycemia induced an increase in plasma cortisol concentrations with an identical maximum of about 13.5 µg/dl in the forenoon and the evening group (Fig. 2). However, there was a pronounced difference in starting values (8.0 ± 1.5 µg/dl vs. 4.8 ± 0.5 µg/dl). The rise in cortisol was preceded in each case by a rise in plasma ACTH with similar slopes for increase and decrease in the forenoon and evening groups. However, in the forenoon a maximal value of 367 ± 50 pg/ml was

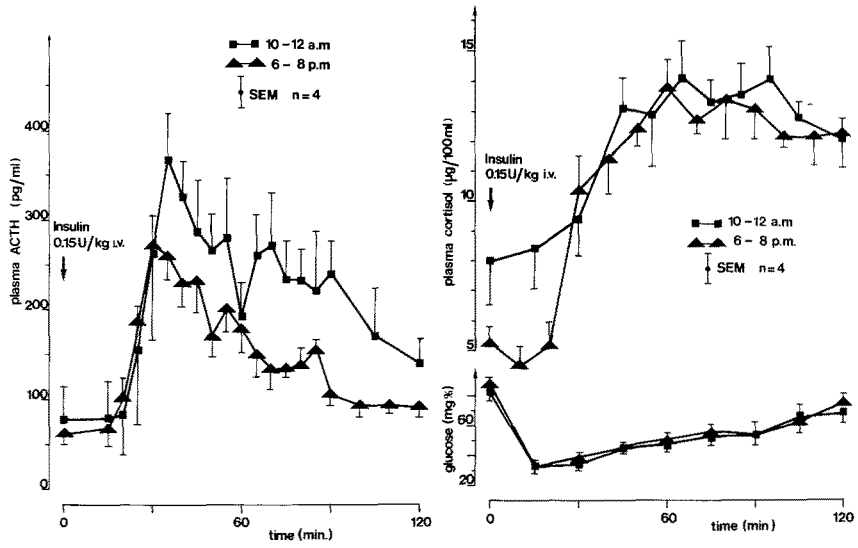

Fig. 2. Responses of plasma ACTH and plasma cortisol to insulin induced hypoglycemia. Insulin was administered either at 10 a.m. (forenoon) or at 6 p.m. (evening)

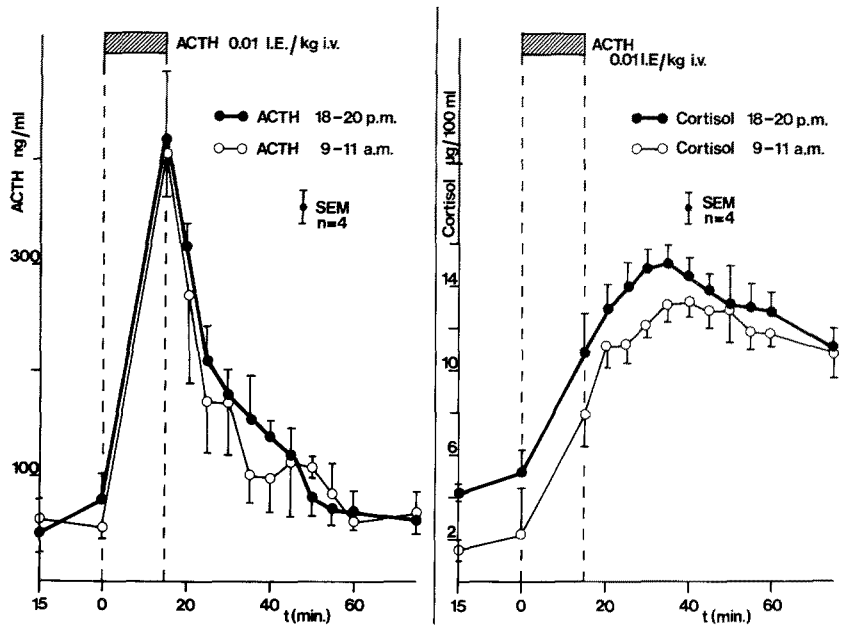

Fig. 3. Responses of plasma ACTH and plasma cortisol to administration of small amounts of exogenous ACTH. The forenoon group was pretreated with 1 mg dexamethasone per os to ensure comparable starting values of plasma cortisol

achieved after 35 min, corresponding to a value of 271 ± 35 pg/ml at 30 min in the evening group. Analysis of variance with repeated measures revealed a significant interaction between the factors daytime and cortisol concentrations ($p < 0.05$, $F_{3,9} = 2.26$). The corresponding ACTH values did not differ significantly; however, there was a trend towards lower ACTH responses in the evening ($p = 0.22$; $F_{3,18} = 1.31$). Thus in the forenoon the cortisol response to hypoglycemia was smaller in the presence of a larger ACTH response as compared to the evening.

Administration of small amounts of porcine ACTH at 6 p.m. induced an increase in plasma cortisol levels from 5.3 ± 1.0 µg/dl to 15.2 ± 0.8 µg/dl (Fig. 3). In the forenoon – after pretreatment with dexamethasone – starting levels were slightly lower (2.3 ± 2.3 µg/dl) and the maximal value to a similar extent (13.3 ± 0.8 µg/dl). When the differences in starting values were taken into account it appeared that the cortisol responses to exogenous ACTH were almost identical in both groups. The same was true for the maximal ACTH values at the end of the infusion period (about 400 pg/ml). After cessation of the ACTH infusion, plasma ACTH levels fell rapidly reaching starting levels within about 40 min.

After MA administration in the evening cortisol levels rose immediately to a plateau of 9.2 ± 0.4 µg/dl (starting value 3.7 ± 0.5 µg/dl) after 20 min

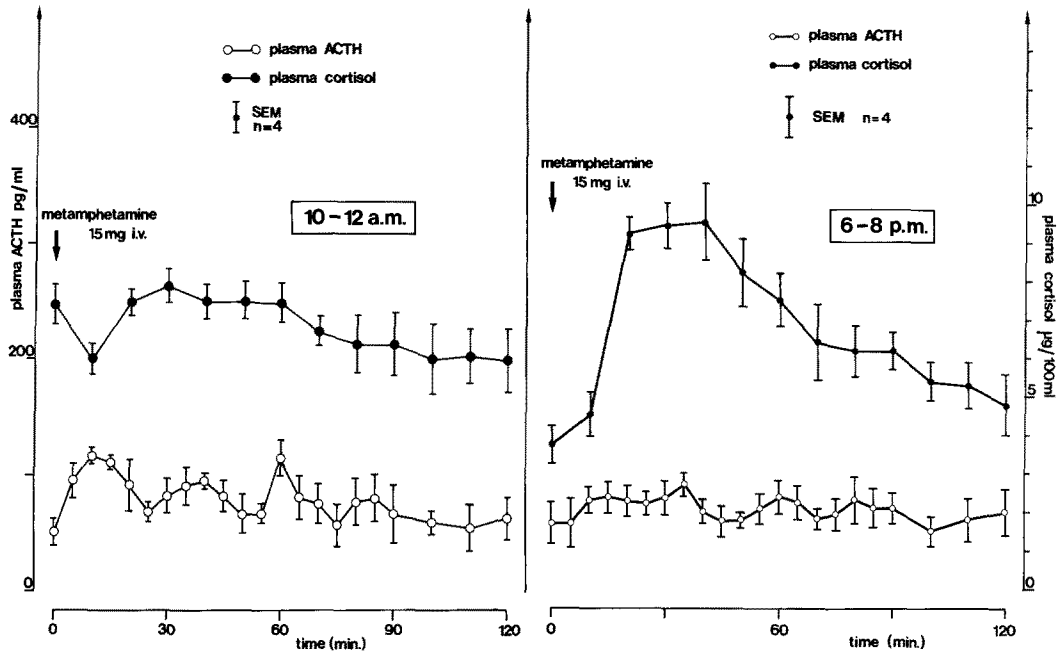

Fig. 4. Plasma cortisol responses to methamphetamine in the forenoon and in the evening. The increase in cortisol values occurred in the absence of an appropriate increase in radioimmunoassayable ACTH

(Fig. 4). However, this clearcut cortisol response was not preceded by an adequate increase in plasma ACTH levels; in fact, the values never exceeded 90 pg/ml. MA administration in the morning induced only a slight increment in cortisol levels; again, plasma ACTH concentration exhibited no significant changes.

Discussion

Our results pertaining to the behavior of cortisol in healthy recumbent subjects revealed a continuous decrease in plasma levels. Under these resting conditions no secretory bursts could be observed. This confirms recent reports by Quigley and Yen (1979) and Brandenberger and Follenius [4, 14]. This uniform decrease in cortisol levels was not paralleled by a corresponding decrease in ACTH levels.

The responses of plasma ACTH and cortisol to insulin induced hypoglycemia are in concordance with many other authors [10, 24, 34]. Ichikawa et al. (1972) reported similar diurnal variations in the cortisol responses to hypoglycemia which were not reflected by appropriate changes in the ACTH responses. When the maximal ACTH and cortisol levels after ACTH administration and ITT are compared, it appears that the ACTH levels obtained by insulin induced hypoglycemia were sufficient to explain the cortisol increments. However, ACTH unrelated mechanisms must be postulated to explain the inverse relationship of ACTH and cortisol responses to ITT in the forenoon and the evening.

By administering small amounts of exogenous ACTH it was possible to produce increments in plasma cortisol which can be called "physiological", when magnitude and duration of the increases are considered. There were no marked differences in the cortisol responses between forenoon and evening. The plasma ACTH levels necessary to induce such a "physiological" rise in cortisol levels were unexpected high (about 400 pg/ml).

The acute plasma cortisol response to MA in humans has been studied by Besser and coworkers [2, 3, 32] and Checkley [6]. These authors reported consistently that in normal subjects, intravenous doses of 15 mg MA stimulate cortisol secretion. The response was most pronounced when the drug was administered in the evening. Comparable results were obtained with dextroamphetamine [5, 7, 18, 33]. The interest in the neuroendocrine effects of these drugs was renewed by the observation that the cortisol response to MA or dextroamphetamine was absent in a group of endogenously depressed patients [6, 33]. The amphetamines are believed to affect cortisol secretion by their catecholaminergic influences on neuroendocrine pathway [12, 26]. This implicates that the cortisol response to amphetamines is mediated by pituitary ACTH. However, adequate data concerning the plasma ACTH response to these drugs are lacking.

As far as the cortisol response to MA is concerned, our data are in good agreement with the earlier work cited above. However, we were unable to find a corresponding increase in plasma ACTH levels. In comparison to the ACTH response to ITT and to the administration of small amounts of ACTH, the complete absence of an ACTH peak after MA must be taken to indicate that the MA induced cortisol secretion was not mediated by radioimmunoassayable ACTH.

The mechanism of the action of MA upon the adrenal cortex remains obscure. Several possibilities have to be considered:

1. MA may stimulate certain brain centers which influence the adrenal cortex via its autonomic innervation. Innervation of the adrenal cortex by postganglionic sympathetic fibers has been described by Kiss (1951) and more recently on the ultrastructural level by Unsicker (1971). The physiological significance of the innervation of the adrenal cortex is not known.

2. MA may stimulate the release of a pituitary factor or cofactor with steroidogenic properties unrelated to ACTH, which are not recognized by our radioimmunoassay system. The specificity of the ACTH antiserum employed in our studies is such that it would recognize all ACTH fragments with known adrenocorticotropic activity, and precursor molecules of ACTH including pro-opiomelanocortin. The existence of additional pituitary peptides with steroidogenic activity is very unlikely. Nevertheless, to exclude this possibility it would be necessary to measure the adrenocorticotropic activity in the plasma by bioassay.

3. MA may increase adrenocortical responsiveness to ACTH in such a way, that "normal" ACTH levels will stimulate cortisol secretion. The existence of an ACTH independent rhythm in adrenal responsiveness has been reported by several authors [8, 20, 21]. The mechanisms which bring about these changes in adrenal responsiveness are unknown.

4. MA may influence adrenocortical function by a peripheral site of action. It may either act on the presumed peripheral autonomic nerves supplying the adrenal cortex, or it may act directly upon adrenocortical cells. Another possibility would be that the increase in cortisol secretion is the consequence of an augmented adrenal blood flow or of changes in cortisol metabolism and distribution induced by MA.

The MA induced cortisol secretion stimulated speculations about the role of the catecholaminergic system in the control of ACTH secretion [2], and the disturbances of this system in depression [33]. In the light of our findings these interpretations have to be reconsidered. It is the main conclusion from our results that it is not allowed to infer from changes in cortisol secretion to corresponding changes in ACTH secretion. In studies of the system controlling the activity of the adrenal cortex the existence of ACTH unrelated mechanisms must be taken into account. The mechanism of the action of MA on the adrenal cortex is enigmatic and remains a matter of research.

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Prof. Dr. H.L. Fehm
Abt. Innere Medizin I
Zentrum f. Innere Medizin
d. Universität Ulm
Steinhövelstr. 9
D-7900 Ulm
Federal Republic of Germany