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Dose-Response Relationships Between Plasma Adrenocorticotropin (ACTH), Cortisol, Aldosterone, and 18-Hydroxycorticosterone After Injection of ACTH-(1–39) or Human Corticotropin-Releasing Hormone in Man*

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ABSTRACT. The effects of sc injections (at 1500 h) of increasing amounts of synthetic human ACTH-(1–39) (1.25–30 μg) on plasma ACTH, cortisol, aldosterone, and 18-hydroxycorticosterone were compared with those of iv injections of 30 and 100 μg synthetic human CRH in nine normal men. Five micrograms of ACTH, sc, was the lowest dose that significantly increased plasma levels of the three steroids. CRH (30 μg, iv) increased plasma cortisol and 18-hydroxycorticosterone, but not aldosterone, while 100 μg CRH also raised aldosterone secretion. The dose-response curve (peak plasma ACTH level *vs.* maximum increment of plasma cortisol within the first hour) was initially very steep. Plasma ACTH levels between 50 and 60 ng/L (11–13 pmol/L) stimulated cortisol to almost 80% of the maximal increment obtained with plasma ACTH levels above 300 ng/L (>66 pmol/L). This dose-response relationship is similar to that found in clinical tests of the pituitary-adrenal axis (insulin test, metyrapone test). The effects of plasma ACTH released by CRH on cortisol secretion were not significantly different from those of injected ACTH. Our results argue against the hypothesis that the effect of CRH on steroid secretion is mediated or modulated by POMC-derived peptides other than ACTH. (*J Clin Endocrinol Metab* **66:** 181, 1988)

NEY *et al.* (1) were the first to investigate the relationship between plasma ACTH and plasma 17-hydroxycorticosteroid concentration during the infusion of ACTH-(1–39) (porcine) for 24 h. In their study, maximum 17-OHCS levels were reached with plasma ACTH concentrations between 2 and 5 mU/mL (~200–500 ng/L or 44–110 pmol/L). Fehm *et al.* (2) concluded recently that plasma ACTH levels much higher than those in the early morning hours in man are necessary for maximal acute stimulation of cortisol secretion, as occurs during insulin-induced hypoglycemia. The relationship between peak plasma ACTH and cortisol levels after the iv injection of incremental doses of ovine (3) or human (4) CRH suggests that cortisol secretion is nearly maximally stimulated at ACTH levels around 40 or 50 ng/L (8.8–11 pmol/L). Since plasma ACTH cannot be increased above 80 ng/L (17.6 pmol/L) by injecting even 30 μg/kg ovine (o) CRH (3), we investigated ACTH-cortisol d response relationships by injecting incremental sc d of human (h) ACTH-(1–39). The results are compa with those obtained after iv injection of two stanc doses of hCRH. Since the effectiveness of CRH a stimulator of aldosterone is also debated (5–7), we m ured, in addition, plasma aldosterone and one of potential precursors, 18-hydroxycorticosterone(18-(B), after ACTH and CRH injections.

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Subjects and Methods

Nine normal men were studied. Their age varied betwee and 32 yr, and their body weight between 65 and 78 kg. were nonsmokers and were eating an unrestricted diet. protocol of the study was approved by the Ethical Commi of the Klinikum Steglitz, and each man gave written con to participate in the study.

ACTH or hCRH injections were always given at 1500 the afternoon after the men had eaten a light fat-free mea 1200 h and had been quietly seated in an armchair for 1 h 1400 h a plastic cannula for blood sampling was placed large forearm vein. Blood was collected in tubes containing EDTA for measurements of plasma ACTH, cortisol, aldos

ıe, and 18-OH-B at 1445, 1455, 1515 (after CRH only), 1530, 45, 1600, 1630, and 1700 h. The tubes were put into crushed ice and centrifuged immediately at 3 C, and the plasma was ored for up to 6 weeks at −70 C before RIA.

Synthetic hACTH-(1–39) was purchased from Peninsula boratories, Inc. (Belmont, CA; lot 009309). Before the study s begun, 500 μg ACTH were dissolved in 50 mL sterile 150 nol/L saline and passed through Millipore filters (0.22 μm; llex-GS, Millipore Corp., Bedford, MA) into 20 sterile plastic oes which were immediately frozen at −70 C. Each aliquot s thawed 1 h before injection. Dilutions of the thawed ACTH utions (theoretic concentration, 10 μg/mL) were compared eatedly with the standard ACTH used as constituent of the TH RIA kit (see below). In six assays, the ACTH injection ution was found to contain 10.8 ± 1.1 (±SD) μg/mL ACTH. e ACTH solution was drawn into sterile plastic syringes of ropriate size and injected sc, using very fine needles, into upper arm contralateral to that which had been cannulated. nore than 1 mL ACTH solution had to be given, two or three s were injected. The injections caused almost no pain. Each n received 1.25, 2.5, 5, 10, 20, and 30 μg ACTH in a random- l order at intervals of at least 3 days. On other occasions, men received 30 and 100 μg hCRH (CRF Bissendorf- tide GmbH, Wedemark, West Germany), iv, instead of TH. No adverse effects of ACTH injection were noted. ee men reported a mild warm flush after CRH injection, ch persisted for less than 5 min.

CTH was measured in unextracted plasma using commer- RIA kits obtained from Compagnie Oris Industrie S.A. (Gif Yvette, France). These kits contained a rabbit- anti-ACTH body directed against the 1–24 sequence of ACTH. hACTH- 9), calibrated against the ACTH MRC standard 74/555, used as standard. The antigen-antibody complex was pre- ated by adding a reagent containing an insoluble complex ieep antirabbit γ-globulin. The minimum ACTH concen- on detectable varied between 5 and 7 ng/mL (1.1–1.5 pmol/ The intraassay variability at a mean ACTH concentration).1 ng/L (4.4 pmol/L) was 11% (n = 9). The interassay ibility of a plasma pool with a mean ACTH concentration ng/L (11.2 pmol/L; n = 18) was 8.6%. In 40 normal men 40 yr of age), plasma ACTH levels (0800–0900 h) ranged less than 5 (n = 4) to 46.3 ng/L (<1.1–10.2 pmol/L), with an of 19.7 ± 10.3 (±SD) ng/L (4.3 ± 2.3 pmol/L). The na ACTH levels at 1500 h in the study subjects were 9.5 ± g/L (2.1 ± 0.35 pmol/L).

asma cortisol was measured using CEA Sorin kits with ody-coated tubes. The sensitivity of the assay was between nd 20 nmol/L. The intraassay variabilities at different ol levels (100, 350, and 1000 nmol/L; n = 15 each) were 3.9%, and 4.5%, respectively. The interassay variabilities e same concentration levels were 9.6%, 5.7%, and 5.5%, ctively. The areas under the curve for ACTH and cortisol ion were calculated by multiplying the mean of the hor- concentrations at the adjacent times of measurement up min by the time (in minutes), followed by summing the s of the individual time segments. Basal secretion (prein- n hormone level × 60) was then subtracted.

sma aldosterone and 18-OH-B were measured by RIA in the blood samples drawn before and at 1530, 1545, and 1600 h after ACTH injection. After hCRH injection, these steroids were measured at 1515, 1530, and 1545 h. The RIAs were preceded by paper chromatographic purification of the steroids (8, 9).

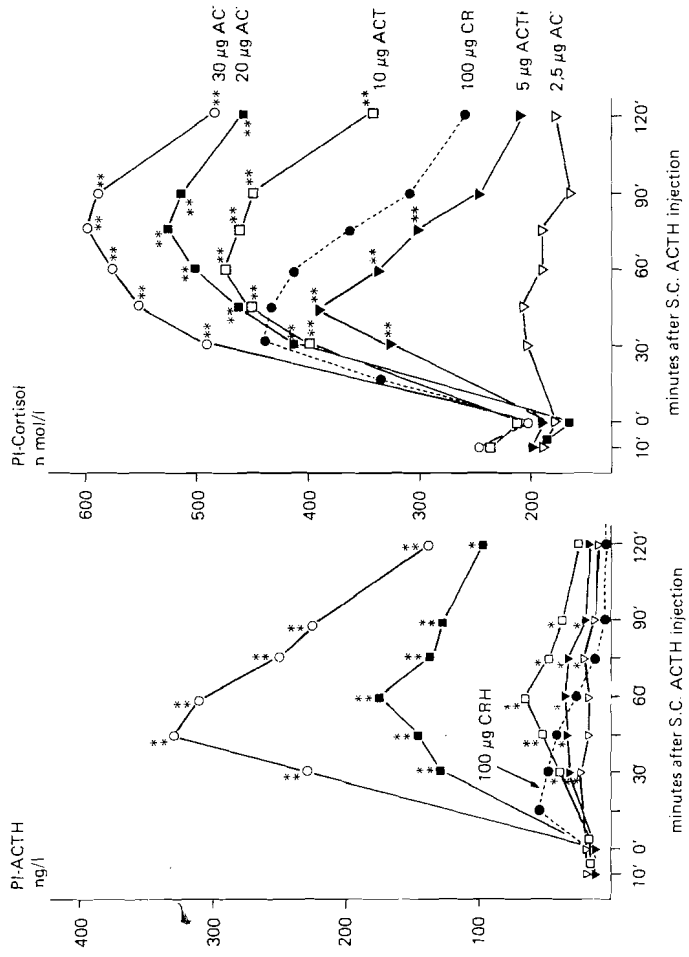
All ACTH and cortisol samples were analyzed in duplicate, and all samples from an individual man were analyzed at the same time. Statistical analyses of the results were performed using a STATS 2 program of Statsoft, Inc. (Tulsa, OK) and an IBM computer. Since all studies were performed in the same subjects, the Wilcoxon test for correlated samples was used exclusively.

Results

Figure 1 shows the mean plasma ACTH and cortisol concentrations after the injection of 2.5–30 μg ACTH, sc, and 100 μg CRH, iv. After the administration of 1.25 μg ACTH (not shown), neither plasma ACTH nor cortisol increased significantly. Plasma ACTH did rise after the injection of 2.5 μg ACTH, sc, from 11.3 ± 2.3 (±SEM) to 18.4 ± 1.2 ng/L ($P < 0.05$), but plasma cortisol did not increase significantly. After 5 μg ACTH, plasma ACTH rose to 27.8 ± 1.4 ng/L (6.2 pmol/L), and cortisol rose significantly to 399 nmol/L (Fig. 1 and Table 1). In the first half hour after the administration of 100 μg ACTH, cortisol rose almost as high as after the larger ACTH doses, although the mean peak ACTH level after 10 μg ACTH, sc, was much lower than that after 20 or 30 μg ACTH. After the higher ACTH doses, the rise in plasma cortisol lasted longer than after the smaller ACTH doses. The plasma ACTH peak after iv injection of 30 or 100 μg hCRH was earlier than the plasma ACTH peak after sc ACTH injection. The peak plasma ACTH and cortisol levels after 100 μg CRH, iv, were similar to those after 10 μg ACTH, sc, while the responses to 30 μg CRH, iv, resembled those to 5 μg ACTH, sc (Figs. 1 and 3). The areas under the plasma ACTH and cortisol curves in the first 60 min after injection also were similar for 5 μg ACTH and 30 μg hCRH and for 10 μg ACTH and 100 μg hCRH, as shown in Table 1.

Figure 2 is a plot of the peak plasma ACTH levels after the different dosages of ACTH sc or CRH iv as a function of the highest plasma cortisol increment within the first 60 min after injection. The dose-response curve initially was steep, but above peak ACTH levels of about 60 ng/L (13 pmol/L) it became very flat. However, the cortisol response to 20 μg ACTH was significantly higher ($P < 0.05$) than that to 10 μg ACTH, but the cortisol responses to 20 and 30 μg ACTH were not significantly different. The dose-response relationships after injection of ACTH sc or CRH iv fell almost on the same line (Fig. 2), although the timing of the peaks was different (Figs. 1 and 3).

Figure 3 shows the effects of the incremental ACTH



(12). They were able to mimic spontaneously occurring single plasma ACTH and cortisol peaks by injecting small doses of hCRH iv.

The fact that an acute increase in plasma ACTH to about 60 ng/L (13.2 pmol/L) stimulates cortisol secretion almost maximally is important for clinical tests of the pituitary-adrenal axis. In most normal subjects, plasma ACTH rises to levels above 150 or 200 ng/L (33 or 44 pmol/L) after insulin-induced hypoglycemia or metyrapone testing (13, 14). In patients with moderately impaired ACTH secretion, these tests will not reveal the disturbance if only the adrenal (plasma cortisol or 11-deoxycortisol) response is measured, since an increase in plasma ACTH to 50 or 60 ng/L (11 or 13 pmol/L) will lead to almost normal stimulation of the adrenal cortex. After comparison of steroid responses to insulin and metyrapone tests in a large number of patients with pituitary disease (15), we performed the short metyrapone test according to the method of Jubiz *et al.* (16), with plasma ACTH and 11-deoxycortisol measurements at 0800 h in 7 normal subjects and 35 patients with pituitary disease. In the normal subjects, plasma ACTH increased to 170–360 ng/L (37–79 pmol/L) and 11-deoxycortisol to 210–500 nmol/L. In the patients with pituitary disease, in whom plasma ACTH increased to between 50 and 210 ng/L (11 and 46 pmol/L), 11-deoxycortisol increased into the normal range after metyrapone injection. Only in patients with ACTH increases to less than 55 ng/L (12 pmol/L) was the rise of 11-deoxycortisol subnormal. This finding indicates that the results presented in this paper are applicable to the interpretation of clinical tests.

Reports on the effect of CRH on plasma aldosterone are controversial. Müller *et al.* (5) found no significant increase in plasma aldosterone after injection of 100 μg oCRH into normal subjects. We also found no significant change in plasma aldosterone in nine normal recumbent men given 100 μg oCRH at 1700 h (17), while cortisol and 18-OH-B increased markedly. Hermus *et al.* (6) and Conaglen *et al.* (7), however, found plasma aldosterone to rise slightly but significantly after the injection of 200 μg oCRH, iv, in normal subjects.

In this study, both plasma aldosterone and 18-OH-B were significantly stimulated by the same threshold dose of hCRH-(1–39) (5 μg) that significantly stimulated cortisol. However, with all ACTH and hCRH doses studied, the variability of the aldosterone and 18-OH-B responses was greater than that of the cortisol responses. While 30 μg hCRH significantly stimulated plasma cortisol and 18-OH-B ($P < 0.01$), plasma aldosterone rose minimally. Only 100 μg hCRH (100 μg) significantly stimulated aldosterone 45 min after injection, but the response was smaller than those of cortisol and 18-OH-B. Since 30 μg hCRH elicited plasma ACTH responses similar to those occurring after the injection of 5 μg ACTH, it appears that the injected ACTH was more effective in stimulating aldosterone than was the ACTH released by hCRH. This suggests the existence of POMC peptides or other CRH-induced factors that inhibit the aldosterone response to ACTH released by CRH. Altogether, the smaller aldosterone responses to ACTH and CRH, compared with those of cortisol and 18-OH-B, may be due to the relatively high sodium intake of our subjects, which can blunt aldosterone responses nonspecifically (18, 19). Differences in dietary sodium also may explain the contradictory findings of different researchers with regard to aldosterone stimulation by CRH (5–7).

CRH injection stimulates not only the release of ACTH but also that of other POMC-derived peptides (20, 21). β-Endorphin, β-lipotropin, and pro-γ-melanotropin have been reported to stimulate aldosterone secretion or enhance the effects of ACTH on aldosterone secretion (22–24). We found that hCRH-released ACTH tended to stimulate aldosterone secretion less than injected ACTH. This finding argues against the physiological significance of potential aldosterone-stimulating factors, other than ACTH, that may be released after hCRH injection.

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