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SHORT COMMUNICATION

One microgram is the lowest ACTH dose to cause a maximal cortisol response. There is no diurnal variation of cortisol response to submaximal ACTH stimulation

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Abstract

There are many suggestions in the literature that the adrenal gland is more sensitive to ACTH in the evening than in the morning. However, all these studies in humans were conducted when the basal cortisol level was not suppressed, and were based on the observation that, after stimulation, the increases in cortisol differed, though the peak values were the same. To examine this, we established the lowest ACTH dose that caused a maximal cortisol stimulation even when the basal cortisol was suppressed, and used a smaller dose of ACTH for morning and evening stimulation. The lowest ACTH dose to achieve maximal stimulation was found to be 1.0 μg, with which dose cortisol concentration increased to 607.2 ± 182 nmol/l, compared with 612.7 ± 140.8 nmol/l with the 250 μg test ($P > 0.3$). The use of smaller doses of ACTH (0.8 and 0.6 μg) achieved significantly lower cortisol responses (312 ± 179.4 and 323 ± 157.3 nmol/l respectively; both $P < 0.01$ compared with the 1 μg test). When a submaximal ACTH dose (0.6 μg) was used to stimulate the adrenal at 0800 and 1600 h, after pretreatment with dexamethasone, no difference in response was noted at either 15 min (372.6 ± 116 compared with 394.7 ± 129.7 nmol/l) or 30 min (397.4 ± 176.6 compared with 403 ± 226.3 nmol/l; $P > 0.3$ for both times). These results show that 1.0 μg ACTH, used latterly as a low-dose test, is very potent in stimulating the adrenal, even when baseline cortisol is suppressed; smaller doses cause reduction of this potency. Our data show that there is probably no diurnal variation in the response of the adrenal to ACTH, if one eliminates the influence of the basal cortisol level and uses physiologic rather than superphysiologic stimuli.

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Introduction

It was previously suggested (1–4) that the adrenal gland is more sensitive to stimuli in the evening than in the morning. These studies showed that, in humans, the increase in cortisol in the evening was higher than that in the morning, in response to adrenocorticotropic hormone (ACTH) (1), insulin-induced hypoglycemia (2) or corticotropin-releasing hormone (CRH) (3). However, in all these studies the peak cortisol concentration was similar, and unrelated to the time of day. The studies therefore do not necessarily indicate a greater response, but may reflect only the maximal ability of the adrenal to respond to ACTH (4). In rats, however, it was shown that, not only was the increase in corticosterone greater in the evening, but the peak cortisol value was also higher, thus truly demonstrating a greater evening response (5). In a previous study (4) we tried to eliminate the effect of the basal cortisol level on the final result, by pretreatment with dexamethasone. We found that, under these conditions, while the 30-min response to ACTH was the same, there seemed to be an earlier increase in cortisol in the evening. We concluded that this might indeed indicate a greater sensitivity of the adrenal to ACTH in the evening.

Latterly, after we introduced the 1.0 μg ACTH test (4), we became interested in identifying the lowest ACTH dose that would still cause a maximal adrenal response, unrelated to the basal cortisol level. We hypothesized that, by using a smaller dose than that at different times of the day, we might disclose a different adrenal response, representing a difference in adrenal sensitivity to ACTH at different times of day.

Subjects and methods

Ten normal volunteers (five women, five men, aged 20–56 years) were tested with the low-dose (1.0 μg)

and with the conventional (250 μg) ACTH tests, 8 h after pretreatment with 1.0 mg dexamethasone. Seven of the ten volunteers participated in both studies; the remaining three differed in the two studies. All tests were conducted between 0800 and 0900 h.

All ten normal volunteers from the previous 1.0 μg test group (five women, five men, aged 20–26 years), were then given 0.8 and 0.6 μg ACTH i.v. between 0800 and 0900 h, after pretreatment with 1.0 mg dexamethasone at midnight. At least 48 h were allowed to pass between the different tests, and the order of the tests was changed between participants.

In the third part of the study, 0.6 μg ACTH was injected i.v. to ten volunteers (five women, five men, aged 18–28 years) at 0800 h and at 1600 h, 8 h after pretreatment with 1.0 mg dexamethasone. At least 48 h were allowed between the two tests, and their order was reversed in 50% of the subjects.

Cortisol was measured using a solid phase RIA as previously described (4). ACTH solutions for i.v. injection were kept diluted to 5 μg/ml and refrigerated for up to 4 months. Further dilution was made shortly before the test. This procedure was proven to be satisfactory in earlier studies (4).

Analysis of data

Results are presented as means ± S.D. For multiple comparisons, ANOVA was performed. This test was followed by the Mann–Whitney test for the 1.0 and 0.8 μg tests. Student's *t*-test was performed for paired comparison of the morning and evening tests.

Results

Response to low ACTH concentrations

Figure 1 compares the cortisol response to stimulation with 0.6, 0.8, 1.0 and 250 μg ACTH after suppression of basal cortisol. No difference was found at 30 min between the 1.0 and 250 μg doses (607.2 ± 182 compared with 612.7 ± 140.8 nmol/l respectively, $P = 0.86$).

At 30 min, there was a significantly lower response to 0.8 μg than to 1.0 μg (312 ± 179.4 compared with 607.2 ± 182 nmol/l, $P < 0.01$). No further reduction in response to 0.6 μg was noted (323 ± 157.3 nmol/l, $P = 0.95$).

Only three of the volunteers in the 0.6 μg test, and three in the 0.8 μg test had a 30-min cortisol level of 500 nmol/l or greater, which would be considered a normal stimulation (6). In contrast, all volunteers showed a 30-min cortisol level of more than 500 nmol/l in response to the 1.0 μg test.

Effect of time of day

When the cortisol responses to 0.6 μg ACTH at 0800 h and 1600 h were compared after pretreatment with dexamethasone (Fig. 2), no difference was noted between basal levels (41.4 ± 35.9 compared with 38 ± 28.5 nmol/l) or at 15 min (372.6 ± 116 compared with 394.7 ± 129.7 nmol/l) or 30 min (397.4 ± 176.6 compared with 403 ± 226.3 nmol/l; $P > 0.3$ for both times).

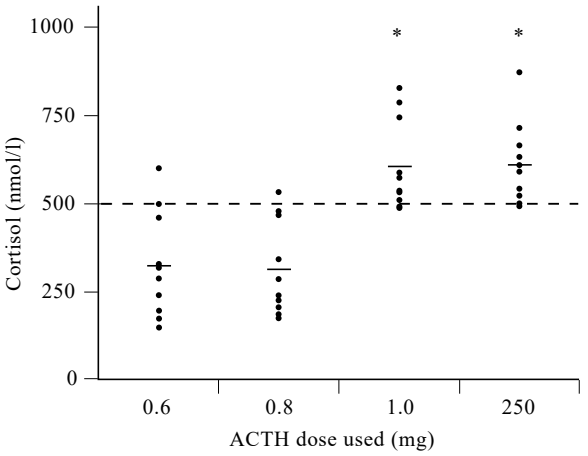

Figure 1 Plasma cortisol concentrations 30 min after i.v. injection of different doses of ACTH(1–24) to ten normal volunteers pretreated with 1 mg dexamethasone 8 h before the test. Vertical bars represent means. *$P < 0.01$ for responses to 1.0 and 250 μg ACTH compared with those to 0.8 and 0.6 μg ACTH.

Discussion

It has been claimed that there is circadian regulation of the sensitivity of the response of the adrenal cortex to ACTH (1–5, 7). The absolute increment in cortisol response to an ACTH stimulation test is greater when the test is performed at circadian nadir than at the peak

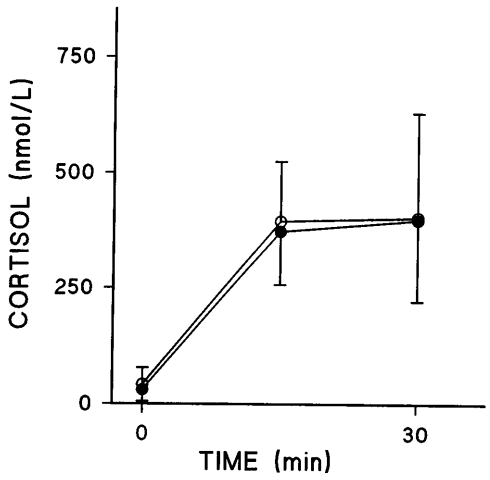

Figure 2 Plasma cortisol concentrations in response to 0.6 μg ACTH i.v. at 0800 and 1600 h, after pretreatment with 1.0 mg dexamethasone 8 h before the test.

(1, 8). This is true also for the cortisol response to insulin-induced hypoglycemia (2) and to CRH (3).

It is known that injection of very low doses of ACTH is enough to evoke some adrenal production of cortisol. It has been shown that 400 ng β-ACTH(1–24) caused an increment of about 190 nmol/l cortisol (9). DeBold (personal communication) found that i.v. injection of 2.0 ng β-ACTH(1–24) caused a mean increase in plasma ACTH of 5 pmol/l within 5 min, with a subsequent mean increase in plasma cortisol of 165 nmol/l 10 min later. However, these low doses of ACTH are effective only when introduced i.v.; s.c. injection with 2.5 μg (10) or even 4.0 μg (11) ACTH will cause no increase in plasma cortisol. Daidoh *et al.* (12) found that 0.5 μg ACTH i.v. was the smallest dose that caused maximal stimulation of cortisol under normal conditions, starting with normal basal cortisol levels of about 200 nmol/l.

We tried to identify the lowest dose of ACTH that will cause normal adrenal stimulation, even when basal cortisol level is suppressed. One microgram ACTH, the dose used for the low-dose ACTH test, was found to be that dose. This probably shows that the basal cortisol level is not important for interpretation of the low-dose ACTH test, and that this test can be performed at any time of the day.

A trial to reduce further the ACTH dose used produced a significant reduction in the adrenal response. Moreover, the response range widened considerably; some individuals showed a normal increase in cortisol, but most did not. This is further proof that the 1.0 μg dose used for the low-dose test is indeed the most appropriate dose. It should be emphasized again, however, that these studies were conducted after baseline suppression of cortisol. It might be, therefore, that many patients will continue to respond normally to these very low doses of ACTH (0.8 or 0.6 μg) if the baseline cortisol level is normal, as suggested by Daidoh *et al.* (12); others, however, will probably not, so that the specificity of the test will deteriorate.

We then used the sub-maximal dose of 0.6 μg ACTH to examine if there is truly a diurnal change in the adrenal response to ACTH (1–3, 6, 7). We did not consider an absolute increment in cortisol response to be good enough a criterion to prove such greater adrenal sensitivity, and believed that starting the test from the same basal cortisol levels is crucial for interpretation of the results. In our previous paper (4) we achieved that by pretreatment with dexamethasone, as did Kaneko *et al.* (5) in their study on rats, in which they indeed found a greater sensitivity of the adrenal in the evening. One must remember, though, that in the rat, a nocturnal animal, corticosterone levels are higher in the evening than in the morning, and a higher adrenal sensitivity in the evening should therefore not be surprising, unlike in humans. However, in our earlier study we used a pharmacologic rather than a physiologic dose of ACTH (250 μg), and under those conditions we found that the 30-min cortisol response was equal in the morning and in the evening, though at 15 min the evening response was significantly higher (4). We interpreted these results as proof of a greater adrenal sensitivity in the evening. However, from the present study, using a sub-maximal dose of ACTH, one can see that when the cortisol increase is less than maximal, no difference in the response can be noted between morning and afternoon results. In our opinion, this proves that there is no real diurnal difference in response, and that all the data previously presented that claimed such a difference in man merely represented achievement of the same maximal-possible cortisol level, starting from a different baseline and therefore representing a different increment.

We conclude that 1.0 μg ACTH is sensitive for measuring adrenal responses, even when the basal cortisol level is suppressed, and that this is probably the lowest ACTH dose that will produce a maximal response. Smaller doses of ACTH give variable responses when baseline cortisol is low. Use of such lower doses reveals no diurnal variation in adrenal cortisol response to a physiologic ACTH stimulus.

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