SHORT TIME KINETICS OF DEOXYCORTICOSTERONE, DEOXYCORTISOL, CORTICOSTERONE AND CORTISOL DURING SINGLE DOSE METYRAPONE TEST

By

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ABSTRACT

In 4 young healthy males, serum levels of 11-deoxycortisol (S), cortisol (F), 11-deoxycorticosterone (DOC) and corticosterone (B) were determined at short intervals after oral administration of 30 mg/kg of metyrapone (M) at midnight. 11-hydroxylase blockade was calculated from the formula $\frac{S \times 100}{S + F}$ or $\frac{DOC \times 100}{DOC + B}$. Significant blockade was demonstrable 15 to 30 min after drug administration. Maximum blockade ($> 90\%$) was found between 2 and 4 a.m., and fell to 60–70\% at 8 a.m. The profile of blockade was very similar in all the subjects, although the absolute early rise in steroid levels showed large inter-individual differences.

The F level at 8 a.m. (only slightly suppressed under these conditions) is not a safe indicator of adequate 11-hydroxylase blockade.

The single-dose version of the metyrapone test, first introduced by Jubiz et al. (1970b) is at present widely applied for testing pituitary ACTH release. In contrast to the time-consuming classical test (Liddle et al. 1959) using six doses of 750 mg metyrapone every 4 h, only a single dose of 30 mg metyrapone/kg body weight is administered orally at midnight. Even more than in the classical version it is important to be sure that a sufficient 11-hydroxylase blockade by metyrapone is achieved (Kaplan 1963; Schneider 1964; Sprunt et al. 1968;
Spark 1971). To avoid false-negative results, the simultaneous measurement of cortisol (F) and deoxycortisol (S) after metyrapone administration was recommended (Spark 1971). The literature provides some information on cortisol concentration at 8 a.m. after the single-dose administration of metyrapone at midnight (Jubiz et al. 1970b; Spark 1971; Meikle et al. 1975), which, however, exhibits a great variability.

In a prolonged kinetic study, Jubiz et al. (1970a) demonstrated that during the night further lowering of physiologically low cortisol by metyrapone does not induce pituitary ACTH release. Therefore, it should be possible to study at this time the changes of steroid concentrations which are due solely to 11-hydroxylase blockade.

Studies on serum concentrations of deoxycorticosterone (DOC) and corticosterone (B) during single-dose metyrapone test have not been reported so far. Due to an inhibited 11-hydroxylation of DOC, similar dynamics would be expected as those for the pathway from S to F.

From the following study on short time kinetics of the steroids mainly concerned with 11-hydroxylase inhibition, more information about the time-dependent degree of 11-hydroxylase inhibition during single-dose metyrapone test can be expected. Since serum concentrations of the steroid hormones controlled by AGTH are physiologically very low during the night, sensitive and specific methods of steroid estimations had to be applied.

METHODS

Sixteen normal subjects (4 females and 12 males, ranging in age from 23 to 42 years) without any evidence of pituitary-adrenal disease were studied by the single-dose metyrapone test (group I). The short-time kinetics were studied in 4 normal males, ranging in age from 30 to 42 years (group II). All the subjects received approximately 30 mg of metyrapone/kg body weight at midnight. In group I, blood samples for the estimation of DOC, S and F were taken at 8 a.m. on 2 consecutive days before and after metyrapone administration. During the kinetic study, blood for estimating serum DOC, S, B and F was taken at midnight immediately before the administration of metyrapone and subsequently at the following intervals: up to 1.30 a.m. every 15 min, at 2, 4, 5, 7, 8 a.m. and 4 p.m. Blood was drawn from a cubital vein. After cloting and retraction, the serum was separated by centrifugation and stored at -20°C until assayed.

Steroid determinations

Cortisol. – F was determined by a modification of the method described by Vecsei et al. (1972). 0.5 ml of serum, to which tracer amounts of [3H]F had been added, was extracted with 6 ml of carbon tetrachloride. F was then extracted from the plasma phase with 5 ml of methylene chloride. The organic phase was evaporated and the residue redissolved in a y-globulin borate buffer. In this buffer solution F was quantitated by a radioimmunological method.
Fig. 1.
Serum levels of deoxycortisol and cortisol during single dose metyrapone test. The ordinate is in logarithmic scale. Subjects and symbols: M. L. (●), W. O. (○), K. A. (□) and M. E. (x).

**Deoxycortisol and corticosterone.** – In group I, S was determined radioimmunologically without chromatography. 0.5 ml of serum, to which tracer amounts of \[^{3}H\]S had been added, was extracted with 10 ml of carbon tetrachloride. The serum phase was rejected and the organic phase was evaporated to dryness under a stream of nitrogen. The residue was redissolved in 1 ml of \(\gamma\)-globulin-borate buffer. Final quantitation of S was achieved by recovery measurement in a 500 \(\mu\)l aliquot and radioimmunological determination of unlabelled S in 100, 50 and 25 \(\mu\)l aliquots each filled up to 100 \(\mu\)l with buffer solution. In the case of serum samples obtained after metyrapone administration, the stock solution was diluted 1:10 before radioimmunological estimation. Standard curves were set up in duplicate. Doses of unlabelled S ranged from 3.12 to 800 pg each solved in 100 \(\mu\)l of buffer solution. Radioimmunoassay data were evaluated by a computer programme. The “spline-approximation” technique was used as standard curve model. Sensitivity of standard curve (2 standard deviations from zero point) was found to be 7.0 ± 9.2 (sd) pg. The amount of unlabelled S necessary for 50% displacement of activity at zero point was 101 ± 21 (sd) pg. Coefficients of intra-assay variability ranged from 21.6% for a steroid level of 0.2 \(\mu\)g/100 ml to 8.5% for 2 \(\mu\)g/100 ml, those of inter-assay variability ranged from 28.1% for a level of 0.5 \(\mu\)g/100 ml to 9.2% for 8 \(\mu\)g/100 ml. Accuracy determined as percentage recovery of known amounts of unlabelled S added to serum
samples ranged from $95 \pm 20$ (sd) /% for a level of 0.2 \( \mu g/100 \) ml to $109 \pm 4.9$ (sd) /% for 10 \( \mu g/100 \) ml.

In group II, S was determined simultaneously with B by radioimmunoassay after extraction with methylene chloride and purification by paper chromatography (Schöneshöfer et al. 1977).

**Deoxycorticosterone.** – DOC was extracted with carbon tetrachloride from a 3 ml serum sample, purified by paper chromatography and quantitated by radioimmunoassay (Schöneshöfer et al. 1975).

**RESULTS**

**Group I**

Mean serum concentrations of F and S at 8 a.m. before metyrapone administration were $14.76 \pm 6.6$ (sd) \( \mu g/100 \) ml and $0.24 \pm 0.21$ (sd) \( \mu g/100 \) ml, respectively. The corresponding values at 8 a.m. after metyrapone were found to be $8.93 \pm 3.6$ (sd) \( \mu g/100 \) ml and $9.12 \pm 2.4$ (sd) \( \mu g/100 \) ml. The mean concentration of F after metyrapone was significantly lower than that before metyrapone \( (P < 0.002) \).

The coefficients of the following correlations were calculated:

1. S after metyrapone (M) as against F after M,
2. S after M as against F before M,
3. \( S/F \) after M as against F after M.

The coefficients of these correlations were insignificant. Mean serum concentration of DOC at 8 a.m. after metyrapone was $3.0 \pm 1.32$ (sd) \( \mu g/100 \) ml, while normal 8 a.m. values range from 0.002 to 0.014 \( \mu g/100 \) ml (Schöneshöfer et al. 1975).

**Kinetic studies in group II**

Figs. 1 and 2 show the changes in serum S, F, DOC and B after metyrapone administration at midnight in 4 subjects. Corresponding to the reduced pituitary activity at this time (Weitzmann et al. 1971) the mean steroid concentrations were very low (S: 0.013 \( \mu g/100 \) ml; F: 2.5 \( \mu g/100 \) ml; DOC: 0.0013 \( \mu g/100 \) ml; B: 0.056 \( \mu g/100 \) ml). After metyrapone, serum S and DOC as well as serum F and B exhibit similar kinetic profiles. Fig. 3 demonstrates the significant correlations of F as against B (Fig. 3 a) and of S as against DOC (Fig. 3 b), respectively.

**Profiles of S and DOC.** – An increase in serum S and DOC is observed as early as 15–30 min after metyrapone administration. A first peak in steroid concentrations, especially accentuated in subjects M.L. and W.O., appears between 1 and 2 a.m. At 2 a.m., values of S and DOC are much higher in subjects M.L. and W.O. than in the other 2 subjects, as is evident from the
Serum levels of deoxycorticosterone and corticosterone during single dose metyrapone test. The ordinate is in logarithmic scale. Symbols for subjects as in Fig. 1.

logarithmic plotting of steroid concentrations in Figs. 1 and 2. It is noteworthy that M. L. and W. O. had suffered abdominal pain and/or nausea soon after taking metyrapone, while M. S. and K. A. felt comfortable throughout the test. After a transient decrease, serum S and DOC rise again between 4 and 8 a.m. The mean values at 8 a.m. were as follows: S: 8.42 µg/100 ml; DOC: 2.11 µg/100 ml. At 4 p.m., both steroid concentrations are still much higher than the normal concentrations at 8 a.m. (Schöneshöfer et al. 1977).

Profiles of F and B. – The initial fall of serum F and B is followed by a slight increase with peaks between 1 and 2 a.m. The higher concentrations of B in subjects M. L. and W. O. and the lower ones in subjects K. A. and M. E. correlate well with the corresponding levels of S and DOC. After a transient decrease, serum F and B rise again between 4 and 8 a.m. Mean values at 8 a.m. were as follows: F: 5.2 µg/100 ml; B: 0.71 µg/100 ml.

Changes of enzyme blockade. – As a measure of 11-hydroxylase inhibition, we used the serum concentrations of S and DOC expressed as the percentage
Correlations of steroid levels mainly concerned in 11-hydroxylase inhibition. Broken lines indicate standard deviations of correlation. a) Cortisol as against corticosterone: Equation of correlation: \( y = -0.052 + 0.16 \cdot x \); coefficient of correlation: 0.95. b) Deoxycortisol as against deoxycorticosterone: Equation of correlation \( y = 0.06 + 0.28 \cdot x \); coefficient of correlation: 0.941.

Relative serum concentrations of deoxycortisol \( S \times 100 \) (S + F) and of deoxycorticosterone \( \text{DOC} \times 100 \) (DOC + B) as functions of time during single dose metyrapone test. Mean values with SEM are shown.
of total serum concentrations of S plus F or of DOC plus B. Fig. 4 shows the ratios $S \times 100/(S+F)$ and DOC $\times 100/(DOC+B)$ after metyrapone administration at midnight. A significant increase in the ratio DOC $\times 100/(DOC+B)$ is observed 15 min after the oral administration of metyrapone, and a significant increase in the ratio $S \times 100/(S+F)$ after 30 min. The maximum, both of the ratio $S \times 100/(S+F)$ (92.1 ± 3.8 (sd) %) and the ratio DOC $\times 100/(DOC+B)$ (93.9 ± 3.6 (sd) %) is reached at 2 a.m. A significant decrease in the ratio DOC $\times 100/(DOC+B)$ is observed at 5 p.m., while a reduction in the ratio $S \times 100/(S+F)$ seems to occur earlier. This corresponds to a smaller ratio $S \times 100/(S+F)$ at 8 a.m. (63.9 ± 7.4 (sd) %) as compared with the ratio DOC $\times 100/(DOC+B)$ (72.4 ± 7.8 (sd) %). At 4 p.m. mean ratio DOC $\times 100/(DOC+B)$ still amounts to 46.4 ± 6.8 (sd) %.

DISCUSSION

11-Hydroxylase blockade and ACTH release

Berson & Yalow (1968) observed an increase in ACTH due to metyrapone induced 11-hydroxylase inhibition, but not observed until 3 h after drug administration. In 2 of our experimental subjects, S and DOC levels 1 h after taking the drug, were 10 to 20-fold higher than the pre-treatment F and B levels, which themselves also had increased slightly after 60 min. This indicates 1) that 11-hydroxylase blockade was effective at this time and 2) that the adrenals must have been stimulated, probably by the pituitary. Since these 2 subjects, in contrast to the others, suffered from nausea soon after taking metyrapone, this discomfort may have stimulated ACTH release. However, a direct stimulation of ACTH by metyrapone itself, as observed by Savano et al. (1972), or other stimulating mechanisms, cannot be excluded.

The second rise in all four steroids with onset around 4 a.m. exhibited a similar profile in all the subjects. Its course and magnitude is similar to that observed by Jubiz et al. (1970a) in a study of steroid kinetics during a classical long-term metyrapone test.

Despite the large fluctuations and inter-individual differences in steroid levels, (Figs. 1 and 2), it was interesting to find an almost uniform time-course of the calculated 11-hydroxylase blockade for the corresponding steroid pairs DOC-B and S-F (Fig. 4). As an authentic measure of 11-hydroxylase blockade, Cope et al. (1966) used the secretion rate of S as the percentage of the sum of the secretion rates of F and S. Since Plager et al. (1965) found no close correlation between the secretion rates of S and the plasma levels of S, once secretion is markedly stimulated, our method of calculating 11-hydroxylase blockade by the relative ratios of DOC or S plasma levels is only an approx}-
mation of the true degree of blockade. Blockade of S-hydroxylation seems to occur slightly later and persists for a shorter period than that of DOC hydroxylation. This difference may be due to the longer half life of F (Nattrass et al. 1972) compared with that of S (Peterson 1959). Hence, the ratio \( \frac{S \times 100}{(S + F)} \) probably underestimates the blockade of S-hydroxylation. We believe that the degree of 11-hydroxylase blockade calculated from the steroid concentrations of DOC and B is a better approximation to true blockade.

**Practical implications**

At 8 a.m. after metyrapone the ratio of \( \frac{DOC \times 100}{(DOC + B)} \) still amounts to 72\%, and in all healthy subjects, markedly elevated plasma levels of S and DOC are measurable. The single-dose metyrapone test seems therefore to be suitable for detecting patients with insufficient ACTH release. One practical disadvantage, however, has to be pointed out: plasma F levels at 8 a.m. after midnight metyrapone administration are only slightly, although significantly, suppressed compared with the control day, and no correlation between F and S serum concentrations was found at this time. In a single individual it may, therefore, be difficult to determine whether an unequivocal cortisol suppression has occurred. This is of importance in patients whose reliability in taking drugs is doubtful or in whom intestinal absorption of metyrapone may be impaired. To solve the problem of having a reliable reference measurement, three alternative approaches seem to be logical: 1. to leave the test unchanged and to measure plasma levels of reduced metyrapone and of S at 8 a.m.; 2. to administer metyrapone at 6 a.m. instead of 12 p.m. In this case, unequivocally suppressed cortisol levels should be demonstrable at 8 a.m.; 3. to inject metyrapone at 8 a.m., to measure plasma F and S at 8 a.m. and then after 2 or 4 h. Approaches 1 and 3 have been suggested previously by Meikle et al. (1975) and Kley & Krüskemper (1972). Because of the danger of enhancing cortisol deficiency in patients with suspected pituitary disease, we regularly admitted patients for the single dose metyrapone test for, at least, one night. Modifications 2 and 3 could be carried out in ambulatory patients if the prolonged blockade of 11-hydroxylase (about 48\% 16 h after drug administration) is taken into account and a glucocorticoid is given for 2 days following the test.

Our steroid measurements at 8 a.m. after metyrapone in group I revealed that mean DOC levels rose to 3 \( \mu g/100 \) ml and the S levels to 9.1 \( \mu g/100 \) ml. Thus, DOC represents a significant fraction (about 26\%) of total 11-deoxy-corticosteroids. This should be taken into account when competitive protein binding methods (Strott et al. 1969; Spark 1971; Kley & Krüskemper 1972; Nattrass et al. 1972) are used.
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