Multiple-sites of inhibition by intravenous metyrapone of human adrenal steroidogenesis

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Abstract. The in vivo influence of metyrapone on adrenal steroidogenesis has been studied by measuring plasma concentrations of pregnenolone, 17-OH-pregnenolone, progesterone, 17-OH-progesterone, 11-deoxycorticosterone, 11-deoxycorticisol, corticosterone, cortisol, 18-OH-11-deoxycorticosterone, 18-OH-corticosterone and aldosterone before, during and after a 5 h infusion of metyrapone ditartrate at doses of 0.2 g/h and 0.8 g/h respectively. Time courses of plasma steroids and corticotrophin indicate an inhibitory effect of metyrapone on total adrenal steroidogenesis in addition to the known inhibition of the 11- and 18-monoxygenase. The effect on total adrenal steroidogenesis is pronounced at high concentrations of metyrapone and may be compensated by corticotrophin. This effect and a concomitant suppressive effect of metyrapone on plasma corticotrophin itself may account for the frequently described falsely abnormal responses to the metyrapone test. From the present 'in vivo' data, no significant, metyrapone induced alterations of the 3β-hydroxysteroid dehydrogenase/Δ5-isomerase, 17-monoxygenase, 21-monoxygenase or the 18-hydroxysteroid dehydrogenase are apparent.

In clinical practice, metyrapone is predominantly used for the assessment of the pituitary capacity for corticotrophin release in response to hypocortisolaemia induced by adrenal 11-monoxygenase blockade. Secondary signals of pituitary activity, such as serum or urine levels of adrenal steroids preceding the 11-monoxygenase block are still widely used as response parameters (Liddle et al. 1959; Strott et al. 1969; Spark 1971; Mahajan et al. 1972; Spiger et al. 1975; Sakamoto et al. 1976; Leisti 1977; Best et al. 1980; Feek et al. 1981). Meanwhile, data have been published indicating additional inhibitory effects of metyrapone on adrenal enzymes other than 11-monoxygenase. Inhibition of 18-monoxygenase has been shown under in vitro (Erickson et al. 1966) and in vivo conditions (Schöneshöfer et al. 1980). Carballeira et al. (1976) and Cheng et al. (1974) demonstrated by in vitro experiments an inhibitory effect of metyrapone on the 'cholesterol-cleavage enzyme' which precedes the total corticosteroid biosynthesis. If significant under in vivo conditions, especially the latter effect might account for the false negative results of the metyrapone test sometimes observed when secondary steroid signals are measured (Schneider 1964).

In a previous experiment, the in vivo influence of metyrapone on the serum concentrations of eight adrenal steroids had been studied (Schöneshöfer et al. 1980). However, the oral way of drug application provoked strong, unspecific pituitary activity secondary to gastric irritations thus partially masking direct effects of metyrapone on in vivo steroidogenesis. In the present study, we reassessed potential in vivo effects of metyrapone the drug now being iv applied for 5 h at different dosages. As indirect parameters of adrenal steroidogenesis the peripheral plasma concentrations of pregnenolone (PL), 17-OH-pregnenolone (17-PL), progesterone (P), 17-OH-progesterone (17-OHP), 11-deoxycorticosterone (DOC), 11-deoxycorticisol (S), corticosterone (B), cortisol (F), 18-OH-11-deoxycorticosterone (18-OH-DOC), 18-OH-corticosterone (18-OH-B), aldosterone (Aldo) and of corticotrophin (ACTH) were measured.
Protocols

Experimental protocols
Five normal male medical students between 20 and 28 years of age volunteered for the study. They consented to participate after the protocol had been carefully explained to them. The same 5 students were studied in a control and in two iv metyrapone experiments. There was an interval of at least 3 weeks between the individual tests.

Control experiment
One half h prior to the first blood sampling, an indwelling cannula was inserted into the antecubital vein. Isotonic saline was infused throughout the test period. Blood samples for the estimation of plasma corticotrophin immunoreactivity and plasma concentrations of the eleven adrenal steroids were taken through the cannula from 8 a.m. at 30 min intervals, and then at 2 h intervals until 4 p.m.

Iv metyrapone experiments
In the low-dose experiment, 1 g metyrapone ditartrate (Ciba-Geigy, Basle, Switzerland) dissolved in 500 ml of 0.9% saline was infused via an antecubital vein from 8 a.m. to 1 p.m. A cannula was inserted into the opposite arm for blood sampling and kept patent with a saline infusion throughout the experiment. Basal blood samples were taken at 7.30 a.m. and at 8 a.m. followed by samples taken at 30 min intervals up to 11 a.m. and then at 1 h intervals up to 4 p.m. A final sample was taken by venepuncture at 6.30 p.m. In the high-dose experiment, 5 g metyrapone ditartrate were infused under comparable conditions over the 5 h experimental period.

During the high-dose experiment, 2 subjects complained of some burning along the course of the vein infused with metyrapone. No side-effects were experienced in the low-dose experiment.

Methods
Blood was withdrawn in chilled plastic tubes containing peptide stabilizing preservatives (EDTA, Trasylol®, mercaptoethanol). After centrifugation at 4°C, plasma was frozen and stored at −20°C until analysis. Plasma ACTH was measured radioimmunologically after silica extraction from 2 ml of plasma (Schöneshöfer et al. 1981a). Sensitivity of the method was 1.47 ± 0.29 pmol/l. After extraction from 1 ml of plasma and effective fractionation by high performance liquid chromatography, plasma steroids were estimated by radioimmunoassay. The protocol and analytical assay parameters have been detailed in an analytical paper (Schöneshöfer et al. 1981b). Statistic comparisons of mean values were performed by Student's t-test.

\[\text{Fig. 1.}\]
Changes of plasma ACTH during the control (a), the low-dose (b) and the high-dose experiment (c). Values are expressed as mean ± SEM. Horizontal bars indicate period of metyrapone infusion.

Results

Control experiments
The upper parts (a) of Figs. 1 to 4 show the time courses of plasma ACTH as well as of plasma adrenal steroids in the control experiment. The profiles of plasma steroids were determined by marked individual episodic fluctuations as indicated by considerable ranges of standard deviations. They were similar to that of plasma ACTH (Fig. 1a) characterized by episodic and circadian rhythmicity, the mean concentration slowly declining in the afternoon.

Responses of plasma corticotrophin
In the low-dose experiment, plasma ACTH consistently fell from 7.30 a.m. to 9.30 a.m. (Fig. 1 b). However, the levels at 8 a.m. and 9 a.m. did not differ significantly \((P > 0.05)\). Nor was there a significant difference between the 9 a.m.-levels in the control and the low-dose experiment (Fig. 1 a and 1 b). Likewise, the further time course of plasma ACTH did not significantly differ between both experiments.
Changes of plasma pregnenolone (A), 17-OH-pregnenolone (B), progesterone (C), and 17-OH-progesterone (D) during the control (a), the low-dose (b) and the high-dose experiment (c). Values are expressed as mean ± SEM. Horizontal bars indicate period of metyrapone infusion.

In the high-dose experiment, the initial fall of plasma ACTH was steeper and the levels at 8 a.m. (start of infusion) and 9 a.m. were significantly different ($P < 0.05$). When comparing the 9 a.m.-level with the corresponding value of the control experiment, the difference was significant at the $P < 0.01$-level. The delayed increase of plasma ACTH starting at about 10 a.m. was more pronounced and consistent than in the low-dose experiment. The peak level at 1 p.m. was about 350% higher than the minimum level at 9 a.m. Plasma levels remained elevated up to 6.30 p.m.
Responses of plasma pregnenolone, 17-OH-pregnenolone, progesterone and 17-OH-progesterone

Infusion of metyrapone caused similar time courses for plasma PL, 17-PL, P and 17-OHP (Fig. 2 A-D). There was a slight downward trend of the mean concentrations from 7.30 a.m. to 9 a.m. In the low-dose experiment, further changes differed considerably among the individual subjects thereby scattering around the basal levels (Fig. 2 Ab-2 Db). In the high-dose experiment, the initial fall was followed by consistent increases of plasma concentrations (Fig. 2 Ac-2 Dc). Plasma PL, 17-PL and P reached maximum values at about 4 p.m. and plasma 17-OH-P at about 1 p.m. For PL and 17-PL, the maximum values were distinctly higher, for P and 17-OHP, they were only slightly higher than basal levels at 8 a.m. In both metyrapone experiments, time courses of plasma P, 17-PL, P and 17-OH-P, were very similar to that of plasma ACTH.

Responses of plasma 11-deoxycorticosterone and 11-deoxycortisol

Infusion of low-dose metyrapone induced a significant increase of plasma S and DOC as early as 8.30 a.m. and 9 a.m. respectively, whereas increases of plasma S and DOC induced by high dose metyrapone were delayed by about half an hour if compared with those of the low-dose experiment. In the low-dose experiment, plasma DOC and S fell immediately after termination of the infusion (Fig. 3 Ab and 3 Bb). In the high-dose experiment, however, plasma S even further increased reaching a maximum at 3 p.m. (Fig. 3 Ac) and plasma DOC remained elevated up to 2 p.m. (Fig. 3 Bc).

Responses of plasma corticosterone and cortisol

The mean concentrations of plasma B and F fell consistently throughout the infusion period (Fig. 3 C and D). In the high-dose experiment, the fall in plasma B and F was steeper and more pronounced than in the low-dose experiment. Moderate reincreases of mean plasma concentrations started between 3 p.m. and 4 p.m. in both experiments.

Responses of 18-OH-deoxycorticosterone, 18-OH-corticosterone and aldosterone

There were no significant changes of plasma 18-OH-DOC apparent throughout the complete experiment (Fig. 4 A). The strong fluctuations of plasma concentrations were partially caused by analytical insufficiency since plasma concentrations were scattering around the detection limit of the analytical method. In both metyrapone experiment, changes of plasma 18-OH and Aldo were characterized by more or less consistent falls during the experiment (Fig. 4 B and 4 C). There were no reincreases discernible during the rest of the experiment.

Responses of the sum of all steroids measured

In the early phase of the metyrapone infusion period, the sum of all steroids measured consistently fell in both metyrapone experiments (Fig. 4 Db and c). In the low-dose experiment, the further time course of the cumulative steroid concentration (Fig. 4 Db) approximately followed the kinetics of plasma ACTH (Fig. 1 b). There were no significant differences if compared with the corresponding data of the control experiment (Fig. 4 Da). In the high-dose experiment, however, cumulative steroid concentrations remained suppressed up to the end of the metyrapone infusion and then consistently increased up to the end of the experiment.

Discussion

Plasma concentrations of adrenal steroids, used as an experimental signal in the present in vivo study, only reflect adrenal secretory and biosynthetic activity if metabolic clearance rates are not altered by the experimental conditions themselves. Accelerating effects of metyrapone on the metabolic clearance rate of adrenal steroids are known only for cortisol so far (Levin et al. 1978). To our knowledge, detailed data on the influence of metyrapone on the metabolic behavior of other steroids are lacking. When discussing the in vivo influence of metyrapone on enzymatic activities of adrenal steroidogenesis by interpreting time courses of absolute steroid concentrations, these potential effects of metyrapone on steroid catabolism have to be taken into account, in any case.

In most of the previous experiments on effects of metyrapone on steroidogenesis, high doses of metyrapone had been administered orally (Sawano et al. 1972; Schönshöfer et al. 1980; Matsuki et al. 1982). Due to gastric irritation, unspecific stimuli provoked ACTH surges with onset immediately after drug absorption thus masking direct metyrapone effects on the pituitary-adrenal axis (Sawano
Changes of plasma 11-deoxycorticosterone (A), 11-deoxycortisol (B), corticosterone (C), and cortisol (D) during the control (a), the low-dose (b) and the high-dose experiment (c). Values are expressed as mean ± SEM. Horizontal bars indicate period of metyrapone infusion.

et al. 1972; Schöneshöfer et al. 1980). In the present iv experiments, such unspecific 'stimuli' like nausea were not experienced by the subjects. Furthermore, the experimental data on plasma ACTH (Fig. 1) document not only the absence of unspecific ACTH surges but, moreover, a fall of plasma ACTH in the initial phase of metyrapone infusion. The molecular mechanism of this effect of metyrapone has not been elucidated so far (Schöneshöfer et al. 1983).
Fig. 4.
Changes of plasma 18-OH-11-deoxycorticosterone (A), 18-OH-corticosterone (B), aldosterone (C), and of the sum of all steroids measured (D) during the control (a), the low-dose (b) and the high-dose experiment (c). Values are expressed as mean ± SEM. Horizontal bars indicate period of metyrapone infusion.

Initial step of adrenal steroidogenesis
It was the primary intention of the present dose-response experiments to monitor alterations of the 'cholesterol-cleavage enzyme' by plasma concentrations of its enzymatic product pregnenolone. In fact, PL decreased in a dose related manner after onset of metyrapone infusion (Fig. 2 A). If, however, correlating plasma PL with plasma ACTH, the fall of plasma PL reveals being secondary to the lowering of plasma ACTH. It is conceivable that a
more pronounced decrease of PL may be masked by the blocking effect of metyrapone on the 11-monoxygenase and a consequent, slight accumulation of all plasma steroids preceding this biosynthetic blockade.

An ‘inhibitory’ effect of metyrapone on the initial step of adrenal steroidogenesis, however, may be indirectly deduced from the following findings: a) Immediately after onset of metyrapone infusion, the sums of all plasma steroids measured distinctly decreased in both drug experiments, b) in spite of increasing plasma ACTH levels, they remained lowered up to the end of the infusion, and c) in the high-dose experiment, the sum markedly increased after stopping the infusion (Fig. 4 D). However, even under the conditions of high plasma concentrations of metyrapone, considerable amounts of steroids were still secreted (Fig. 4 Dc). Hence, ‘inhibition’ of total adrenal steroidogenesis cannot – in contrast to the 11-monoxygenase blockade – be complete under the in vivo conditions of metyrapone application in humans. This finding of an ‘inhibition’ of total adrenal steroidogenesis is compatible with the in vitro data of Cheng et al. (1974) and Carballéira et al. (1976), who described an inhibition of the ‘cholesterol-cleavage-enzyme’ being ‘competitive’ to ACTH, and of Lye & Challis (1984), who observed an inhibition by metyrapone of the corticotrophin 1–24 induced accumulation of cAMP by ovine adrenal cells.

Theoretically, the lowering of all plasma steroids measured during metyrapone infusion may be caused by a metyrapone-induced activation of the biosynthetic pathway towards the C19-steroids. Plasma concentrations of C19-steroids during iv application of metyrapone have not been studied so far. However, de Lange et al. (1980) as well as Fiet et al. (1980) observed only moderate increases of plasma dehydroepiandrosterone and androstenedione in the classical, oral metyrapone test, i.e. after metyrapone concentrations had already fallen to lower levels. These increases were comparable with those of plasma PL, 17-PL, P and 17-OHP after the end of the high-dose metyrapone infusion (Fig. 2 A–2 D). Thus, it is unlikely that metyrapone may induce a shift of adrenal biosynthesis towards C19-steroids.

However, by the present data it cannot be excluded that lowering of the sum of adrenal steroids – at least partially – is caused by a metyrapone induced acceleration of the metabolic clearance of plasma S and DOC as is the case for cortisol (Levin et al. 1978).

11- and 18-monoxygenase

The time courses of plasma concentrations of the steroids involved in 11- and 18-hydroxylation, DOC and S, on the one side, as well as F, B and 18-OH-DOC on the other side, are in accordance with the known inhibitory effects of metyrapone on both enzymes (Liddle et al. 1959; Schönshöfer et al. 1980; Sonino et al. 1981).

Other adrenal enzymes

No metyrapone induced alterations of the adrenal enzymes, 3β-hydroxysteroid dehydrogenase/Δ5-isomerase, 17-monoxygenase and 21-monoxygenase are recognizable by the present data. A potential influence of metyrapone on adrenal 18-hydroxysteroid dehydrogenase catalyzing the dehydrogenation of 18-OH-B to aldosterone is not seriously discernible by the present findings (Fig. 4 B and 4 C), since the enzyme substrate itself, 18-OH-B, is already effectively lowered by the preceding 11- and 18-hydroxylation blockade (Sonino et al. 1981).

Practical implications

The inhibitory effect of metyrapone on 18-monoxygenase is not relevant for evaluating pituitary reserve of ACTH by the metyrapone test. The ‘inhibition’ of total adrenal steroidogenesis, however, represents a great challenge for the correct interpretation of the metyrapone test, particularly if the secondary parameters of pituitary ACTH release, i.e. total corticosteroid excretion (Liddle et al. 1959) or exclusively 11-deoxycorticisol (Mahajan et al. 1972), are used as response in the metyrapone test. This inhibitory effect is probably involved in the observations of false negative results claimed in the literature (Schneider 1964). It obviously becomes negligible when metyrapone concentrations are lowered to levels sufficient for 11-monoxygenase blockade but insufficient for ‘inhibition’ of total adrenal steroidogenesis. Considering in addition the lowering effect of metyrapone on plasma ACTH (Fig. 1), interpretation of the metyrapone test, using either corticotrophin or steroid response, is particularly critical in all situations, where metyrapone is present at high plasma concentrations, e.g. in the 3 h metyrapone test proposed by Leisti (1977).
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