Simultaneous Determinations of Plasma ACTH and GH Levels after an Oral Metyrapone*

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Synopsis

The levels of plasma ACTH and GH were simultaneously measured in normal subjects every 30 min for 4 hr after an oral administration of metyrapone, either 1.0 g or 1.5 g. Both doses of metyrapone caused a significant decrease in plasma cortisol levels 90 min later, when the mean values of plasma cortisol levels were not significantly different between both groups. An administration of 1.0 g of metyrapone raised plasma ACTH levels in 2 out of 6 subjects within 90 min, while an increase in plasma GH levels of 5.0 ng/ml above the base line levels was observed in 2 subjects. In one of the subjects in whom the levels of plasma GH increased, plasma GH peak was preceded by that of plasma ACTH, but no rise in plasma ACTH levels was observed in another subject. When 1.5 g of metyrapone was given, plasma ACTH levels increased in all 6 subjects. The response of plasma ACTH was quite different in each individual and there appeared one or two peaks within 240 min. The mean value of plasma ACTH was 69 ± 17 pg/ml at 0 time and increased to 165 ± 18 pg/ml at 60 min ($p < 0.01$ vs 0 time). An increment in plasma GH levels was observed in 5 out of 6 subjects. The mean peak value was reached at 90 min and the difference between the values at 0 and 90 min was significant. There was also no correlation between the secretion of ACTH and that of GH. These results may indicate that a higher dose of metyrapone causes both ACTH and GH secretions within a short period not by a negative feedback mechanism of cortisol but presumably by acting directly on the pituitary and/or on the hypothalamus as a nonspecific stimulus.

Metyrapone is known to stimulate the release of ACTH by a negative feedback mechanism of cortisol and widely used for testing the hypothalamo-pituitary axis. In the previous study (Kunita *et al.*, 1970), we reported an oral administration of 1.5 g of metyrapone induced an increase in plasma GH levels in normal subjects. Since the rise in plasma GH was not suppressed in the subjects pretreated with dexamethasone, it was considered that the response of GH might not be related to a negative feedback mechanism through which metyrapone stimulated

ACTH release. In order to conclude the possibility, however, a relationship between ACTH and GH responses to metyrapone should be elucidated. On the other hand, ACTH levels within a short period following an oral administration of relatively large dose of metyrapone have not been well understood. It seems, therefore, to be of interest to measure simultaneously both hormones in plasma after metyrapone.

Materials and Methods

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Ten healthy male college students, aged 19–22 were studied. Their body weights ranged 53–61 kg. After an overnight fast 6 subjects were given 1.0 g of

metyrapone orally at 9:00 a.m. One week later, 2 out of the 6 subjects and other 4 subjects received 1.5 g of metyrapone in the same manner. They were at bed rest for 30 min before and throughout the study. Thirty min prior to the initiation of blood sampling, an indwelling needle was inserted into a forearm vein. Blood samples for the estimation of plasma ACTH, GH and cortisol were obtained immediately before and every 30 min for 4 hr following. Plasma ACTH was measured by a radioimmunoassay according to Berson and Yalow (1968). The minimal detectable dose of ACTH was as low as 2.5 pg/tube in this assay system. Plasma GH was determined by a double antibody radioimmunoassay (Schalch and Parker, 1964) and plasma cortisol by the method of Rudd *et al.* (1963). For the evaluation of plasma GH response, an increase in plasma GH levels of 5.0 ng/ml above the base line considered to be a positive response. Statistical calculations were made by Student *t* test.

Results

Plasma cortisol levels, when a single dose of metyrapone, either 1.0 or 1.5 g was administered, are shown in Figure 1. The mean basal levels of plasma cortisol at 9:00 a.m. were 14.1 ± 0.8 $\mu\text{g}/100$ ml (1.0 g group) and 13.3 ± 1.9 $\mu\text{g}/100$ ml (1.5 g group). After 90 min the levels decreased sharply to 2.1 ± 0.6 $\mu\text{g}/100$ ml and to 0.8 ± 0.3 $\mu\text{g}/100$ ml, respectively. Plasma cortisol levels at 90 min was not significantly different between both groups. The similar side effects appeared after 1.5 g of metyrapone, as we observed previously (Kunita *et al.*, 1970), but they were less or none after 1.0 g of metyrapone.

Each individual data of plasma ACTH and GH levels after either 1.0 or 1.5 g of metyrapone is reported in Tables 1 and 2. The responses of plasma ACTH to 1.0 g of metyrapone were not uniform. In Subject 1 an initial rise in plasma ACTH appeared at 60 min and a second small peak at 180 min. A similar sharp peak was observed in Subject 2 at 90 min and then rapidly decreased at 150 min. On the contrary, plasma ACTH levels decreased in Subjects 3 and 5 during the first 90 min, and increased at 240 min in Subjects 3. No clear changes in plasma ACTH

Fig. 1. Changes in plasma cortisol levels after administration of metyrapone orally at 0 time (9:00 a.m.), either 1.0 g (dashed line) or 1.5 g (solid line). Brackets indicate standard errors.

levels were observed in Subjects 4 and 6. Summarizing these individual observations, there is no consistent tendency in the levels of plasma ACTH after 1.0 g of metyrapone (Table 1). The response of plasma GH to 1.0 g of metyrapone was seen in Subjects 2 and 5 (Table 2). In Subject 2 plasma GH peak was preceded by that of plasma ACTH, but no rise in plasma ACTH was observed in Subject 5.

When 1.5 g of metyrapone was given, plasma ACTH levels increased in all subjects (Table 1). The magnitudes and the patterns of ACTH responses were different in each subject. An initial sharp rise in plasma ACTH was seen within 90 min in all subjects except Subject 8 in whom the level increased gradually to a maximal level at 120 min. The responses of plasma ACTH to 1.5 g of metyrapone were similar in fashion to those to 1.0 g of metyrapone in Subjects 1 and 2, to whom the both doses of metyrapone were administered. However, when 1.5 g of

Table 1. Effect of an oral administration of either 1.0 or 1.5 g of metyrapone on the levels of plasma ACTH

Subject	Dose of metyrapone total (g) mg/kg		Plasma ACTH levels (pg/ml)							
			0	30	60	90	120	150	180	240 min
1. MA	1.0	17.2	50	45	185	150	55	—	105	80
2. HO	1.0	16.7	10	25	45	270	135	25	50	90
3. KI	1.0	16.9	190	45	80	55	115	115	55	335
4. WA	1.0	17.5	65	65	90	45	120	65	45	80
5. IK	1.0	18.9	135	65	80	10	80	45	10	90
6. SA	1.0	18.1	90	90	115	90	115	90	80	90
Mean ± S.E.			90 ±26	56 ±9	99 ±19	103 ±39	103 ±12	68 ±16	58 ±13	128 ±42
1. MA	1.5	25.9	90	210	225	80	150	150	205	135
2. HO	1.5	25.0	10	80	135	245	205	245	115	—
7. MO	1.5	23.1	55	55	205	150	150	205	55	150
8. HI	1.5	24.6	135	150	150	170	240	170	135	335
9. NI	1.5	25.9	80	10	170	185	80	25	10	80
10. AR	1.5	27.8	45	55	105	55	25	150	90	—
Mean ± S.E.			69 ±17	93 ±30	165** ±18	148* ±29	142 ±32	158* ±30	102 ±27	175 ±55

* $p < 0.05$ vs 0 time; ** $p < 0.01$ vs 0 time.

Table 2. Effect of an oral administration of either 1.0 or 1.5 g of metyrapone on the levels of plasma GH

Subject	Dose of metyrapone total (g) mg/kg		Plasma GH levels (ng/ml)							
			0	30	60	90	120	150	180	240 min
1. MA	1.0	17.2	<0.5	1.8	3.3	2.0	1.0	1.0	1.3	0.8
2. HO	1.0	16.7	0.5	2.0	1.6	1.2	1.1	5.6	6.2	1.6
3. KI	1.0	16.9	1.1	1.2	1.5	1.2	1.0	0.8	0.8	0.8
4. WA	1.0	17.5	0.5	0.5	1.0	0.5	<0.5	<0.5	<0.5	<0.5
5. IK	1.0	18.9	0.6	5.6	6.5	5.4	3.6	1.4	0.6	<0.5
6. SA	1.0	18.1	0.6	3.6	4.4	1.8	0.6	<0.5	<0.5	0.7
Mean ± S.E.			0.6 ±0.12	2.5 ±0.8	3.1 ±0.9	2.0 ±0.7	1.3 ±0.5	1.6 ±0.8	1.7 ±0.9	1.0 ±0.2
1. MA	1.5	25.9	0.5	0.5	41.4	49.5	25.8	9.6	5.5	1.2
2. HO	1.5	25.0	0.8	<0.5	0.5	<0.5	2.9	2.7	1.2	—
7. MO	1.5	23.1	<0.5	<0.5	7.2	12.4	3.8	1.6	1.1	<0.5
8. HI	1.5	24.6	0.7	0.6	6.0	7.4	5.4	1.4	0.5	<0.5
9. NI	1.5	25.9	<0.5	<0.5	4.0	13.4	3.4	0.6	<0.5	<0.5
10. AR	1.5	27.8	0.6	0.6	0.9	3.9	5.7	2.8	0.7	—
Mean ± S.E.			0.6 ±0.1	0.5	10.0* ±6.4	14.5** ±6.4	7.8 ±3.6	4.4 ±1.5	1.6 ±0.8	0.7 ±0.1

* $p < 0.05$ vs 0 time; ** $p < 0.01$ vs 0 time.

Values below 0.5 ng/ml were considered as 0.5 ng/ml for statistical calculation.

metyrapone was given, the level of the second ACTH peak was higher in Subject 1 and the duration of high levels of ACTH was longer in Subject 2 than when 1.0 g of metyrapone was given. A statistical analysis indicated that the levels of plasma ACTH increased significantly 60, 90 and 150 min after 1.5 g of metyrapone (Table 1). The GH response to 1.5 g of metyrapone was observed in 5 out of 6 subjects (Table 2). The mean peak value was reached at 90 min. The differences between the values at 0 and 90 min and between those at 0 and 60 min were statistically significant. No relationship was observed between the changes in plasma ACTH and GH.

Discussion

Recently Sawin *et al.* (1971) reported that a rise in plasma GH levels was not induced 120 min after 1.5 g of metyrapone under the similar condition as we previously described (Kunita *et al.*, 1970). The GH response to metyrapone was reconfirmed in the present study, so that it is difficult to explain their negative results. Two explanations, however, could be possible. First, their subjects must have weighed more than ours, so 1.5 g of metyrapone might have not been enough to evoke GH release. The dose of metyrapone per kg body weight in each subject is shown in Tables 1 and 2. Second, since in the present study a statistically significant increment in plasma GH levels was observed 60 and 90 min after 1.5 g of metyrapone, and the value at 120 min was not significantly different. This may suggest that measuring once at 120 min is not enough to evaluate the action of metyrapone.

The present study clearly indicates that GH response to metyrapone is not related to ACTH secretion. This may also exclude the possibility that an increase in endogenous ACTH stimulates GH release, though Zahnd *et al.* (1969) have shown that the exogenous ACTH simulated the release of GH. With

regard to the mechanism of metyrapone action on GH secretion, Bruno *et al.* (1971) found a significant negative correlation between the cortisol disappearance half time and the maximal increment in plasma GH after metyrapone. Since in the present study plasma cortisol levels were not measured 30 and 60 min after metyrapone, an exact pattern of the disappearance of plasma cortisol was not clear. However, despite the fact that the levels of plasma cortisol at 90 min were similar between the groups of 1.0 g and 1.5 g of metyrapone, the responses of plasma GH and ACTH in 1.0 g group were not significant, whereas the responses in 1.5 g group were significant. It is, therefore, unlikely that the releases of both GH and ACTH induced by metyrapone are due to a fall of plasma cortisol concentration.

Considering the mechanism of GH response to metyrapone, the "early rise" in plasma ACTH seen in the present study is quite interesting. Liddle (1960) originally suggested that metyrapone stimulated the secretion of ACTH through its inhibitory action on the 11 β -hydroxylase activity in the adrenal cortex. Since then, numerous studies have indicated that when plasma ACTH levels were measured directly after metyrapone, it took at least 3–6 hr (Berson and Yalow, 1968) or longer (Berson and Yalow, 1968; Liddle *et al.*, 1962; Strott *et al.*, 1969; Jubiz *et al.*, 1970a, and b) to stimulate the release of ACTH by a negative feedback mechanism. Therefore, the "early rise" in plasma ACTH levels after 1.5 g of metyrapone may not be caused by the negative feedback mechanism. There are a few studies measuring the changes in plasma ACTH within a short period when given a single dose of metyrapone orally. Jubiz *et al.* (1970a) gave a dose of 750 mg of metyrapone at 8:00 a.m. and found no change in plasma ACTH levels 3 hr later. A similar observation was made by Berson and Yalow (1968), who showed that when the same dose of metyrapone was given at 9:00 a.m., plasma ACTH concentration changed little or continued to

drop for 2–3 hr. Since only 2 out of 6 subjects responded to 1.0 g of metyrapone in this study, a smaller amount of this drug could not evoke the “early rise” in plasma ACTH levels. Considering together with GH and ACTH responses to metyrapone, it is strongly indicated that a higher dose of metyrapone may stimulate the release of both hormones by acting directly on the pituitary and/or on the hypothalamus within a short period as a non-specific stimulus. The direct effect of metyrapone has been suggested by Ganong and Gold (1960).

A question may arise as to whether or not an increase in plasma ACTH levels after metyrapone reflects the periodic diurnal variation of plasma ACTH reported by Krieger *et al.* (1971). To exclude this possibility the control experiment should have been performed. We, however, started our experiments at 9:00 a.m., when they reported plasma ACTH levels tended to decrease. A similar tendency was observed in 2 subjects who did not respond to 1.0 g of metyrapone. Moreover, our results indicate that the stimulatory effect of metyrapone on ACTH release was dose dependent. Therefore, it seems to be possible that metyrapone itself stimulated the release of ACTH.

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