ADRENOCORTICAL RESERVE IN HYPERTHYROIDISM

KOJI NAKAGAWA

*Second Department of Internal Medicine,
Hokkaido University School of Medicine, Sapporo*

Recent investigations on the functional status of the adrenal cortex in hyperthyroidism have shown that there is an increased production of cortisol (Peterson, 1958), possibly in an effort to maintain the normal plasma levels (Perkoff *et al.*, 1954; Levin and Daughaday, 1955; Brown *et al.*, 1958; Schwarz, 1958; Peterson, 1958; Takebe, 1961) in the face of the accelerated removal of this hormone from the plasma (Levin and Daughaday, 1955; Peterson *et al.*, 1955; Brown *et al.*, 1958; DiRaimondo *et al.*, 1958; Jakobson *et al.*, 1961; Takebe, 1961). This increased rate of disappearance of cortisol from the plasma can not be observed in patients with euthyroidal hypermetabolism (Garren and Lipsett, 1961) and is considered to be due to specific effects of the thyroid hormones directly and/or indirectly on the enzyme systems involved in the steroid metabolism in the liver (Yates *et al.*, 1958; McGuire and Tomkins, 1959; Hellman *et al.*, 1961). It is conceivable that long duration of such compelled hyperactivity of the adrenal cortex in patients with hyperthyroidism might result in impairment of the adrenal cortex in the ability to secrete the hormones. This "decompensated" adrenocortical activity has been considered to be the cause of so-called "thyroid crisis".

Mikulaj and Németh (1958a, b) found that in hyperthyroidism the urinary 17-hydroxycorticosteroids (17-OH-CS) response to an 8-hr. intravenous infusion of adrenocorticotrophin (ACTH) repeated for 3 consecutive days decreased gradually from the 1st to the 3rd day. Felber *et al.* (1959) also observed that in hyperthyroidism the response to an 8-hr. intravenous infusion of ACTH on the 2nd day showed no further increase than that of the 1st day, presuming as a sign of impairment of adrenocortical reserve. The findings of Sugiyama (1961) and Takebe (1961) were also essentially the same as these results.

It might be considered to be more reasonable, however, that the impairment of adrenocortical reserve, if it exists in hyperthyroidism, can be shown more clearly under the condition where ACTH is administered so as to stimulate the adrenal cortex continuously to its maximum level than under the condition where the adrenal cortex is stimulated only intermittently. The present studies were undertaken to demonstrate the adrenocortical responses in patients with hyperthyroidism to the continuous maximum stimulation and to the intermittent stimulation with repository ACTH for 5 days. Since Brown *et al.* (1958) pointed out that urinary 17-OH-CS values after ACTH administration were more reliable index for the adrenocortical secretion of cortisol than the levels of free 17-OH-CS in plasma, urinary 17-OH-CS were determined throughout these studies.

MATERIALS AND METHODS

Continuous maximum stimulation with ACTH-Z

Five patients with hyperthyroidism were studied in the first series of these studies. Five patients without hepatic, renal or endocrine abnormalities served as controls. In each patient three 24-hr. urine collections were obtained to determine the basal excretion of total and free 17-OH-CS. Thereafter each patient received 30 I.U. of ACTH-Z (Organon) intramuscularly every 12 hrs. (at 6 a.m. and 6 p.m.) for 5 days and 24-hr. urine collections from 6 a.m. to next 6 a.m. were obtained for these 5 days and 2 following days in order to determine total and free 17-OH-CS.

Intermittent stimulation with ACTH-Z

Five patients with hyperthyroidism and 3 control patients served as the subjects of this study. The program of investigation was different from that of the former study only in that each patient received 30 I.U. of ACTH-Z intramuscularly only every 24 hrs. (at 6 a.m.) for 5 days. In this study only total 17-OH-CS were determined.

Four-hr. urine collection following a single injection of ACTH-Z

In each of the 4 hyperthyroid and control patients, urine were collected in serial 4-hr. period for 24 hrs. following a single injection of 30 I.U. of ACTH-Z at 6 a.m. Each urine specimen was served for the determination of total 17-OH-CS.

Four-hr. urine collection during and after the serial oral administration of cortisol

In each of the 3 hyperthyroid and control patients, 5 mg of cortisol was administered orally every 2 hrs. from 6 a.m. to 10 p.m. and urine were collected in the same fashion as that of the former study. Each urine specimen was served for the determination of total 17-OH-CS.

Diurnal variations of total 17-OH-CS excretion

In the patients served as the subjects in the third series of the studies, urine were collected in serial 4-hr. periods for 24 hrs. prior to the injection of ACTH-Z. Each urine specimen was served to determine the basal diurnal rhythm of total urinary 17-OH-CS excretion.

Fig. 1. Correlogram of total urinary 17-OH-CS values measured by butanol extraction and β -glucuronidase hydrolysis

Determinations of 17-OH-CS

Total urinary 17-OH-CS were determined by the modification described by DiRaimondo *et al.* (1955) of the Raddy-Jenkins-Thorn method for the estimation of 17-OH-CS in urine (Reddy *et al.*, 1952).

Total urinary 17-OH-CS were determined also by a modification of the Glenn-Nelson method (Glenn and Nelson, 1953), after incubation of the urine samples with 300 Fishman units of calf β -glucuronidase (Tokyo Zoki-Kagaku Co.) per ml of urine, which was undiluted or diluted so as to contain less than 2 μ g/ml of 17-OH-CS, and extraction with dichloromethane.

In most cases total urinary 17-OH-CS were determined by these two methods, and the values were significantly correlated ($r=0.92$) as shown in Figure 1.

Free urinary 17-OH-CS were determined by a method after the Peterson's modification (Peterson *et al.*, 1957) of the Silber-Porter method for the estimation of free 17-OH-CS in plasma (Silber and Porter, 1954).

Urine collections were checked by creatinine determinations.

The diagnosis of hyperthyroidism was established by clinical evaluation and confirmed by basal metabolic rate, the 3-hr. and the 24-hr. thyroidal uptake of radioactive iodine and plasma protein-bound iodine levels.

RESULTS

Continuous maximum stimulation with ACTH-Z

The results obtained with the stimulation of the adrenal cortex with 30 I.U. of ACTH-Z injected every 12 hrs. for 5 days are summarized in Figures 2, 3 and 4.

Fig. 2 Total urinary 17-OH-CS before, during and after the 5-day continuous maximum stimulation with ACTH-Z (30 I.U. every 12 hrs.)

a) measured by butanol extraction, $n=5$ in each group

b) measured by β -glucuronidase hydrolysis, $n=4$ in hyperthyroids, $n=3$ in controls

The vertical lines represent the standard deviation about the mean. The means are connected by a solid (in hyperthyroidism) or a broken (in normal) line.

Fig. 3. Free urinary 17-OH-CS before, during and after the 5-day continuous maximum stimulation with ACTH-Z (30 I.U. every 12 hrs.), $n=5$ in each group. The vertical lines represent the standard deviation about the mean. The means are connected by a solid (in hyperthyroidism) or a broken (in normal) line.

Fig. 4. Ratios of free urinary 17-OH-CS to total urinary 17-OH-CS (measured by butanol extraction), $n=5$ in each group. The vertical lines represent the standard deviation about the mean. The means are connected by a solid (in hyperthyroidism) or a broken (in normal) line.

Baseline values for total urinary 17-OH-CS in hyperthyroidism were significantly ($P<0.01$) higher than in normal subjects.

The total urinary 17-OH-CS response to ACTH-Z injection in hyperthyroidism increased more quickly and to much higher levels than that in normal subjects; the values for 17-OH-CS appeared to have reached a plateau on the 3rd or 4th day of the stimulation in the normal group, whereas those in hyperthyroidism continued to increase even on the 5th day of the stimulation; the values on the 3rd, the 4th and the 5th day of the stimulation were significantly ($P<0.01$) higher in hyperthyroidism than in normal subjects.

There was no significant difference between baseline values for free urinary 17-OH-CS in two groups. The response to the stimulation with ACTH-Z showed gradual increase both in hyperthyroidism and in normal subjects. The slope of increase and the value on each day, however, showed no significant difference in two groups.

The ratios of free 17-OH-CS to total 17-OH-CS (measured by butanol extraction) were lower in hyperthyroidism than in normal subjects throughout the period of this study. However, these differences in two groups were merely statistically significant in those on the 3rd, the 4th ($P<0.01$) and the 5th ($P<0.05$) days. The ratios on either of the 2 following days after cessation of ACTH-Z injection were markedly lower than those on the baseline days, without any statistical significance due to wide individual variations.

Fig. 5. Total urinary 17-OH-CS before, during and after the 5-day intermittent stimulation with ACTH-Z (30 I.U. every 24 hrs.)

a) measured by butanol extraction, $n=3$ in each group

b) measured by β -glucuronidase hydrolysis, $n=5$ in hyperthyroids $n=3$ in controls

The vertical lines represent the standard deviation about the mean. The means are connected by a solid (in hyperthyroidism) or a broken (in normal) line.

Fig. 6. Total urinary 17-OH-CS responses to the 5-day continuous maximum stimulation (30 I.U. every 12 hrs.) and intermittent stimulation (30 I.U. every 24 hrs.) in two patients with hyperthyroidism, measured by β -glucuronidase hydrolysis

Intermittent stimulation with ACTH-Z

The results obtained with the stimulation of the adrenal cortex with ACTH-Z injected every 24 hrs. for 5 days are given in Figure 5.

Baseline values for total urinary 17-OH-CS were again significantly ($P<0.05$) higher in hyperthyroidism than in normal subjects.

The total urinary 17-OH-CS response to ACTH-Z injection in hyperthyroid-

Fig. 7. Total urinary 17-OH-CS in 4-hr. urine samples following a single injection of 30 I.U. of ACTH-Z at 6 a.m., measured by β -glucuronidase hydrolysis, $n=4$ in each group

Fig. 8. Total urinary 17-OH-CS in 4-hr. urine samples during and after the serial oral administration of cortisol (every 2 hrs. from 6 a.m. to 10 p.m.), measured by β -glucuronidase hydrolysis, $n=3$ in each group

ism showed no further rise, or only showed rise after the 2nd or 3rd day of the stimulation, whereas in the normal group there was an essentially similar tendency observed in the stimulation with ACTH-Z injected every 12 hrs. the values for total urinary 17-OH-CS on the 5th day of the stimulation were significantly ($P < 0.05$) lower in hyperthyroidism than in the normal group.

In 2 patients with hyperthyroidism in this study, the response to the stimulation with ACTH-Z injected every 24 hrs. was compared to that with ACTH-Z injected every 12 hrs. As shown in Figure 6, the results indicate that the remarkable difference between the responses in these two stimulation methods was not due to the individual difference of the patients.

Duration of the effects of a single injection of ACTH-Z

The results obtained from the estimation of total urinary 17-OH-CS in 4-hr. urine samples after a single injection of 30 I.U. of ACTH-Z are shown in Figure 7.

In hyperthyroidism the response of 17-OH-CS to ACTH-Z injection appeared to decline more rapidly, as compared with that in the normal group.

Total urinary 17-OH-CS excretion during and after the serial oral administration of cortisol

In order to learn if this rapid decline of 17-OH-CS excretion after a single injection of ACTH-Z in hyperthyroidism might be accounted for only by the accelerated rate of steroid metabolism and the rapid excretion in urine in this disease, cortisol was given orally so as to make a similar pattern of urinary 17-OH-CS excretion to that observed in a single injection of 30 I.U. of ACTH-Z in normal group. The results are shown in Figure 8. The accelerated rate of cortisol metabolism and the rapid excretion of the metabolites in hyperthyroidism

Fig. 9. Diurnal variations of total urinary 17-OH-CS, measured by β -glucuronidase hydrolysis, $n=4$ in each group

were shown as higher values for 17-OH-CS in the 1st and the 2nd 4-hr. urine. However, the slope of decline of 17-OH-CS in the 3rd to the 5th 4-hr. urine was essentially similar in two groups, indicating that the difference in the slope of decline of 17-OH-CS observed in ACTH-Z injection was not due to the post-adrenal factors.

Diurnal variations in total urinary 17-OH-CS excretion

The results are given in Figure 9. The diurnal variations in total urinary 17-OH-CS demonstrated that the magnitude of variation, *i.e.* difference between the urinary 17-OH-CS excretion of 6 to 10 a.m. and that of 10 p.m. to 2 a.m., was significantly ($P<0.05$) greater in hyperthyroidism than in normal subjects. The minimal values (10 p.m.~2 a.m.) in hyperthyroidism were 1.20 times higher than in normal subjects, whereas the maximal values (6 a.m.~10 a.m.) in hyperthyroidism were 1.85 times higher in the normal group.

DISCUSSION

The studies of numerous investigators have shown the elevated baseline values for urinary 17-OH-CS in patients with hyperthyroidism. The results of the present studies confirmed this finding.

The adrenocortical response in hyperthyroidism to a single 6- or 8-hr. intravenous infusion of ACTH or to a single injection of repository ACTH has been reported to be within normal range, although in the upper limit. The responses on the 1st day of the stimulation in the present studies were in agreement with these reports.

The response of total urinary 17-OH-CS to the continuous maximum stimulation with ACTH-Z (30 I.U. every 12 hrs.) in the present study was, however, significantly higher in hyperthyroidism. DiRaimondo *et al.* (1958) reported that the recovery of total urinary 17-OH-CS, when cortisol was administered intrave-

nously, was the same for both normals and hyperthyroids, whereas Levin and Daughaday (1955) reported lesser amounts of recovery in hyperthyroidism. On the basis of these reports, the results obtained with the continuous maximum stimulation with ACTH-Z in the present study demonstrate that the adrenal cortex in hyperthyroidism has a greater capacity than in normals.

The findings in the continuous maximum stimulation (30 I.U. every 12 hrs.) were in remarkable contrast with previous reports by other investigators suggesting the impairment of adrenocortical reserve in hyperthyroidism in intravenous ACTH drip test or intramuscular ACTH-gel injection repeated for 2 or 3 consecutive days. However, the results obtained with the intermittent stimulation (30 I.U. every 24 hrs.) in this study were in agreement with those reports. It would be concluded, therefore, from the results in these two stimulation methods that the intermittent stimulation (30 I.U. every 24 hrs.) could not potentiate the adrenal secretory capacity in hyperthyroidism, in contrast with clear potentiation in normal subjects, and that the failure to potentiate the adrenal secretory capacity in hyperthyroidism in the intermittent stimulation does not necessarily mean an existence of the impairment of adrenocortical reserve and rather suggests strongly a possibility of the increased disappearance of the effects of exogenous ACTH in patients with hyperthyroidism.

The study on the 4-hr. urine samples following a single injection of 30 I.U. of ACTH-Z was undertaken to make clear a possibility that the effects of a single injection of ACTH-Z disappear more rapidly in hyperthyroidism than in the normal group. The results, being superimposed on the data in serial cortisol administration, showed that the effects of exogenous ACTH declined more rapidly in hyperthyroidism than in normal subjects.

The shortened duration of the effects of ACTH in hyperthyroidism may be a reflection of 1) shortened duration of ACTH activity itself or 2) some abnormality of the adrenal cortex to respond to ACTH.

The shortened duration of ACTH activity may be due to the enhanced destruction of ACTH or the enhanced excretion of ACTH.

There have been only two reports about the estimation of the duration of ACTH activity in blood compartment of man (Sayers *et al.*, 1949; Meakin *et al.*, 1959). However, no report has been presented in patients with hyperthyroidism.

It has been reported that in Cushing's syndrome with bilateral adrenal hyperplasia, the return of adrenocortical function to prestimulation levels following the administration of ACTH is markedly delayed (Dyrenfurth *et al.*, 1960). From this observation, it is obvious that the increased destruction of ACTH is not a general property of hypertrophied adrenal cortex.

On the urinary excretion of ACTH, Ibayashi *et al.* (1961) reported in a case of hyperthyroidism slightly low values for ACTH activity in urine, although the significance is not yet clarified.

Also no definite report has been presented on the sensitivity of the adrenal cortex to ACTH in hyperthyroidism. Therefore the precise mechanism involved in this phenomenon remains to be elucidated.

SUMMARY

The responses of urinary 17-hydroxycorticosteroids (17-OH-CS) to two stimulation methods with repository ACTH were investigated in patients with hyperthyroidism.

Baseline values for total urinary 17-OH-CS were significantly elevated in hyperthyroidism. The study on the diurnal variations in total urinary 17-OH-CS revealed the greater magnitude of variation in hyperthyroidism than in the normal group.

The urinary 17-OH-CS response to 30 I.U. of ACTH-Z injected every 12 hrs. for 5 days increased more quickly and to much higher levels in hyperthyroidism than in normal subjects, whereas the response to 30 I.U. of ACTH-Z injected only every 24 hrs. for 5 days showed no further rise or much slower rise after the 2nd or 3rd day of the stimulation. The effects of a single injection of 30 I.U. of ACTH-Z had a tendency to decline more rapidly in hyperthyroidism than in euthyroid subjects.

These results appear to indicate that in hyperthyroidism 1) there is no sign of impairment of adrenocortical reserve, at least under the continuous maximum stimulation of the adrenal cortex for 5 days, 2) the capacity of the adrenal cortex in the ability to secrete corticosteroids is greater than in normal subjects and 3) the effects of ACTH disappear more rapidly than in normal subjects.

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