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ACTH stimulated adrenocortical function in obese women: a gas chromatographic study

by
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In order to interpret the behaviour of any hormone it is necessary to examine a few concepts of physiopathology. Firstly, the preferential importance of the determination of the hormonal content of urine instead of plasma levels represent a given moment in the functional activity of an endocrine gland ([1, 2]), whereas the total amount present in the urine over a given period of time represents the secretion rate over that period. Furthermore, as Baulieu ([3]) has clearly pointed out, it is necessary to consider that determination of a single hormone is hardly representative of the secretory activity of an endocrine gland and also quite inadequate to interpret the mechanism of action of that same hormone. This is because the peripheral effect on the target organ is determined by the activity of specific enzymes on a group of hormonal metabolites, whereas data relating to a single hormone, even a hormone such as cortisol, aldosterone or testosterone, offers only a partial and static picture. Further, an androgenic hormone which is considered inactive, such as androstenedione, may be converted in the peripheral tissues into 5-α-metabolites of testosterone, which are responsible, at the level of the target organ, for the androgenic effect which is observed clinically in cases where the blood levels of androstenedione are high ([4]).

Gas chromatographic methods which allow the analysis of several fractions of the principal groups of steroid hormones, providing a complete and dynamic picture of the functional activity of the endocrine system under examination, are therefore important. In addition it is possible to obtain an indirect indication of the activity of certain enzymatic systems at the site of production of the hormone as well as the peripheral tissue level and this allows a more correct interpretation of physiopathological aspects of a given endocrine disorder ([3, 4]). By employing this type of investigation in a group of obese women with obesity of the hypotonic variety we have been able to disprove previously reported observations ([5, 6, 7, 8, 9]) by providing evidence for the existence of a basal hyperactivity of the adrenal cortex ([10]).

Since the adrenal cortex is directly controlled by the pituitry, its increased activity could also be secondary to an increased production of corticotropin. This would be difficult to demonstrate just by determining the level of ACTH is plasma, not only because this determination is not well standardised, but also as this would, as already pointed out, provide a static type of finding ([11]).

We have therefore considered it of interest to determine the response of the

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adrenal cortex to a stimulus by corticotropin in obesity, making the dose as near as possible (in amount and duration) to that which is physiologically effective.

MATERIALS AND METHOD

Our investigation has been carried out on 18 obese women and 7 normal control subjects ranging in age between 18 and 40 years. None of these was affected by other types of endocrine or metabolic disturbance and they all showed normal hepatic, cardiac and renal functions. During the course of the investigations any pharmacological treatment and any dietary restriction was avoided. In all cases a baseline determination was performed on the urine of the 12 hour night specimen (6pm - 6am). Stimulation tests have been carried out during the same period, ie. 6pm - 6am, since this period is least influenced by endogenous corticotropic activity.

Stimulation has been performed by slow intravenous infusion of synthetic ACTH (Synacthen-Ciba) in a dose of 5μg of corticotropin in a volume of 250 ml physiological saline at a rate of about 30 drops/min. Urines were collected from the beginning of the stimulus for the following 12 hours. 5 urinary samples were collected, three under baseline conditions and two after the stimulus. The determination of the steroid hormones was carried out on all samples.

This was carried out by the gas chromatographic technique, by employing a Carlo Erba Fractovap-GI using the method of « urinary profiles » suggested by Horning and Gardiner ([12]) as modified by Sommerville and Ros ([13]) through a capillary colum and the use of trimethylsilyl and enoltrimethylsilyl ethers.

The activity of the two enzyme systems (dehydrogenase and 21-hydroxylase) has been calculated. The first was obtained from the ratio between THF (tetrahydrocortisol) and THE (tetrahydrocortisone) and is representative of peripheral hydrogenase activity in sites such as the liver; the second from the ratio PT: PT + THF + THE (PT = pregnantriol) which expresses the 21-hydroxylase activity of the adrenal cortex ([14]).

All the results have been subjected to statistical analysis by applying the Student's test to the data obtained for control and obese subjects.

RESULTS

In Table I the level of steroids in the urine before and after stimulus with corticotropin in normal and obese women is reported.

From the statistical analysis it is seen that in control subjects the stimulus with 5μg of synthetic ACTH produced a significant increase only in tetrahydrocortisol and tetrahydrocortisone (Table I). In the obese patients, on the other hand, statistical significance was obtained for the increase in all of the glucocorticoid and mineralocorticoid metabolites, while the androgenic metabolites and the pregnan- and pregnen-derivatives, with the exception of 11-OH-androsterone, did not show any significant increase in the urine after corticotropin stimulus (Table I).

In Table II the statistical analyses of the changes in the indices of enzymatic function (dehydrogenase and 21-hydroxylase activity) after stimulus with ACTH in the two groups are compared. The dehydrogenase activity is significantly decreased in normal women and almost unchanged in obese patients; the 21-

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Table I. *Comparison between basal values and values after corticotropic stimulus of various steroids considered (mg/12 hr).*

Steroid	Obese			Normal		
	basal	stimulus	P	basal	stimulus	P
Androsterone	0.56	0.56	n. s.	0.98	1.10	n. s.
Etiocolanolone	0.55	0.55	n. s.	0.62	0.73	n. s.
Etiocolandiol	0.11	0.11	n. s.	0.11	0.13	n. s.
11-OH-androsterone	0.30	0.29	n. s.	0.42	0.55	<0.01
Pregnantriol	0.25	0.27	n. s.	0.47	0.48	n. s.
Δ-5-pregnantriol	0.14	0.19	n. s.	0.19	0.24	n. s.
Tetrahydrocortisone	0.70	0.92	<0.05	1.25	2.05	<0.001
Tetrahydrocortisol	0.27	0.49	<0.05	0.52	1.11	<0.01
Tetrahydrodehydrocorticosterone	0.33	0.38	n. s.	0.38	0.54	<0.005
Allo-tetrahydrocorticosterone	0.20	0.30	n. s.	0.29	0.64	<0.02
Cortolone	0.50	0.61	n. s.	0.65	1.05	<0.01
β-cortolone	0.26	0.28	n. s.	0.26	0.34	<0.02

Table II. *Changes in indices of dehydrogenase and 21-hydroxylase activity after stimulus with corticotropin.*

Index of enzyme activity	Normal			Obese		
	basal	stimulus	p<	basal	stimulus	p<
$\frac{THF}{THE}$	0.43	0.53	0.05	0.52	0.52	n. s.
$\frac{PT}{PT+THF+THE}$	0.21	0.18	n. s.	0.20	0.13	0.005

hydroxylase activity, on the other hand, is not noticeably changed in control subjects but is significantly higher in obese women.

DISCUSSION

From examination of our data it appears, first of all, that in normal women stimulus with 5μg tetracosactide (ACTH) produced a statistically appreciable adrenal response only for tetrahydrocortisol and tetrahydrocortisone. This fact shows the dose of corticotropin used is the minimum which is effective, as a significant response was only obtained for glucocorticoid metabolites, production of which is known to be totally dependent on corticotropic activity.

The results obtained in obese women are of great interest. As is seen in Table I, all the steroids belonging to the glucocorticoid and mineralocorticoid metabolic pathways have responded very significantly to the stimulus with ACTH. As was expected, the levels of pregnan- and pregnen-derivatives and of the androgens were not significantly altered with the exception of 11-OH-androsterone. The significant increase in the level of this latter steroid, known especially as a catabolite of androsterone, is not surprising; on the contrary, it confirms the validity of our method and shows the precision of our results, since it has been established that it is also a direct metabolite of cortisol ([15]).

One can therefore state that the adrenal corted of obese women, in addition to having a larger basal production of hormones (in comparison with normal subjects), responds excessively to a stimulus with corticotropin which appears to be somewhat similar to the physiological response.

The changes in the indices of enzyme activities also require careful analysis. The ratio THF: THE, index of the dehydrogenase activity, has shown a significant decrease in normal women after stimulus, as was to be expected ([14, 16]). In obese patients, on the other hand, the index remained the same. This phenomenon is probably related to the fact that excess cortisol produced by the gland after stimulation with ACTH finds in the normal subject a relative insufficiency of the dehydrogenase system and so cannot be converted into cortisone. In obesity, on the other hand, this enzyme system shows considerable activity, even in basal conditions, and therefore is capable of metabolizing the large quantity of cortisol adequately.

It should be pointed out that cortisone is a metabolite of adrenocortical steroids; its formation takes place at hepatic level by the action of a dehydrogenase on cortisol. The ratio THF: THE therefore expresses the activity of a peripheral enzyme system; it is not considered likely that this can be influenced by the action of ACTH, and therefore, in conditions of maximal functional activity, it remains unchanged.

The behaviour of the 21-hydroxylase was very different: in normal women changes after stimulation were not significant but in obese women there was a highly significant increase in activity. It is worth remembering that the 21-hydroxylase has a position of great importance in the intraglandular metabolism of steroid hormones, controlling the synthesis of the glucocorticoids (2-13). The significant increase in its activity after corticotropic stimulus, in obese women as opposed to controls, might possibly be related to the general hyperactivity that the adrenal cortex shows even under basal conditions ([10]).

Therefore, if one accepts that any endocrine-metabolic hyperactivity is inevitably linked to the functional capacity of various enzyme systems, then the action of corticotropin must express itself at the very level of the adrenal enzyme systems which are hyperactive in obesity.

CONCLUSIONS

It is possible to state that the basal functional activity of the adrenal cortex is greater in obese women that in normal women and that this increased activity concerns the whole of the cortex. On stimulation with a minimum effective dose of corticotropin, obese women again behave differently from normal women, with a significantly increased production of the mineralocorticoids and especially of the glucocorticoid hormones.

Finally the response in the indices of enzyme activities (the dehydrogenase and the 21-hydroxylase) has provided further evidence of hyperactivity of the cortex at the level of cortical enzymes, of which the 21-hydroxylase appears to be most significant.

It is not yet clear whether the increased enzymatic activity is in any way related to the pathogenesis of obesity of whether it merely correlates with the increase in body mass: however, we favour the first possibility, analagous examples of which can also occur in endocrinemetabolic areas other than the adrenocortical one.

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SUMMARY

Having pointed out in a previous work that the adrenal cortex of obese women in basal conditions shows a greater activity with respect to normal women, the authors have tested the functional response of this gland to stimulus with minimal effective doses of corticotropin. It was shown that, while in control subjects only the metabolites of cortisol were significantly increased, in obese women all mineralocorticoids and particularly glucocorticoids respond in a statistically significant way. Also the indices of enzyme activities (hydrogenase and particularly 21-hydroxylase) have confirmed the functional hyperactivity of the adrenal cortex in obesity.

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Cytogenetics of cervical dysplasias

by
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Dysplasias of the uterine cervix are difficult to classify cytogenetically for the following reasons: the most interesting and useful study method by far is the one involving direct hypotonic « squash » which, more than cultures, follows the real karyotypic arrangement of the cell.

However, it is well known that in relation to the tissues, this method has various

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