

RAPID CORTICOTROPIN TEST WITH 1-24 PEPTIDE*

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The rapid corticotropin test with synthetic corticotropin (1-24 peptide) was studied. A single intravenous injection of the corticotropin was performed in 26 human subjects. The increment of plasma 11-OHCS concentration by the administration was less than that of NH-corticotropin dripping infusion or synthetic corticotropin intramuscular injection. Intramuscular rapid corticotropin test was considered to be an inaccurate method. No allergic reaction occurred in this series of patients.

INTRODUCTION

The need for a simplified test for the pituitary and adrenocortical function has been increased, because of an increase of patients treated with corticoid. The following tests have been described for this purpose: 1) corticotropin test, 2) corticotropin suppression test, and 3) metyrapone test. The corticotropin tests previously described were to determine the urinary or plasma corticoid increment caused by an intravenous infusion of plain corticotropin or intramuscular injections of zinc corticotropin. So the trouble of continuous infusion or urine collections was indispensable. The large dose of corticotropin is liable to cause an increase in the weight of adrenal glands¹⁾. The response of the adrenal cortex might be affected by this phenomenon. From the test requiring a long time, we can not recognize the delicate adrenocortical function changing time by time. The methods of intramuscular administration are considered simple, but the results are inaccurate²⁾.

In 1965, Wood *et al.* reported the rapid synthetic corticotropin test. They determined the increment of plasma 11-OHCS following intramuscular administration of synthetic corticotropin³⁾.

This paper deals with the results of the intravenous rapid test using synthetic 1-24 peptide in 26 persons.

MATERIALS AND METHODS

Synthetic corticotropin used in this experiments was 1-24 peptide (Res. P.

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Received for publication December 4, 1967.

* Part of this study was presented at the 40th meeting of the Japan Endocrinological Society at Nagoya in April 1967.

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Thirteen normal adults, 7 long term corticoid treated patients and 6 patients with Cushing's syndrome were studied.

Each 5 ml venous blood collection was made at time 0 (9 a.m.), 30 and 60 minutes. A single i.v. or i.m. injection of the synthetic 1-24 peptide (0.25 mg sol. in 5 ml manitol) was done immediately after the first blood sampling. Six hour infusion tests with NH-corticotropin (50 I.U.) or synthetic 1-24 peptide (0.25 mg) was initiated at 6 A.M. In this test, 7 blood collections were made at 90 minutes intervals.

From each heparinized blood sample, 2 ml of plasma was separated and kept in the refrigerator till next procedures. De Moor's method was employed to determine the plasma 11-OHCS concentration⁴⁾.

RESULTS

All the results are illustrated in the tables.

The results using the intramuscular method are shown in Table 1. The mean increment in six controls was 24.6 ± 5.1 (S.E.) $\mu\text{g}/100$ ml. This was

TABLE 1. Effects of Intramuscularly Administered Synthetic Corticotropin on Plasma 11-OHCS Concentration

Subjects (Age, Sex)	Plasma 11-OHCS Concentration ($\mu\text{g}/100$ ml)			Increment
	Before	30 min	60 min	
Controls				
T. (22, f)	8.8	26.3		17.5
O. (42, m)	7.0	14.1	20.5	13.5
S. (50, f)	17.9	46.4	45.7	28.5
M. (57, f)	13.7	25.3	24.0	11.6
H. (62, f)	10.9	56.2	32.2	47.1
T. (42, f)	8.3	11.6	37.5	29.2
Mean \pm S. E.	11.1 ± 1.8	33.0 ± 3.3	31.8 ± 4.6	24.6 ± 5.8
Cushing's Patients (adrenocortical hyperfunction)				
H. (24, m)	24.1	35.2	39.0	14.9
S. (22, f)	10.4	15.4	18.3	10.1
K. (18, f)	18.6	35.0	40.7	20.1
Mean \pm S. E.	$17.7 \pm 3.4^*$	$29.6 \pm 6.6^*$	$32.6 \pm 7.2^*$	$15.9 \pm 2.9^*$
Long-term Corticoid Treated Patients				
T. (5, f)	7.3	12.6		5.3
N. (25, m)	5.8	18.3		12.5
Mean \pm S. E.	$6.6 \pm 0.8^*$	$15.5 \pm 2.8^*$		$8.9 \pm 3.6^*$

* $p > 0.05$ vs. controls.

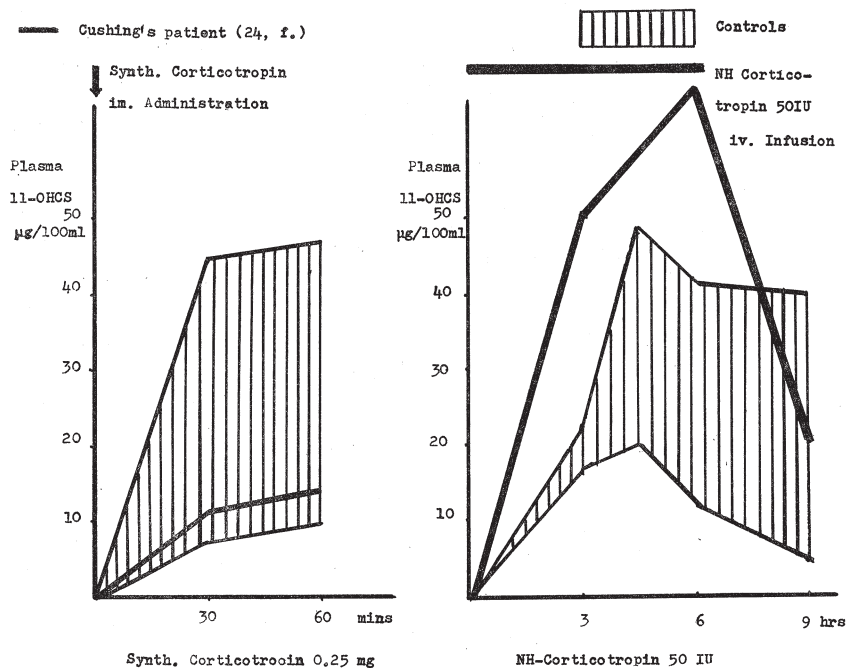


FIG. 1. A male with Cushing's syndrome due to adrenocortical hyperplasia, showing lower response of plasma 11-OHCS to intramuscular injection of 1-24 peptide.

moderately large response. Three patients with Cushing's syndrome due to adrenocortical hyperplasia showed the mean increment of $15.9 \pm 5.0 \mu\text{g}/100 \text{ ml}$. The mean increment in two patients treated with corticoid for long time was $8.9 \pm 5.1 \mu\text{g}/100 \text{ ml}$.

A man with Cushing's syndrome, whose response to the intramuscular injection was rather lower within normal limits, showed larger increment by NH-corticotropin infusion (Fig. 1). Another female patient with Cushing's syndrome had only smaller response to the intramuscular injection of the synthetic peptide and larger response to the infusion (Fig. 2).

Attaching importance to the simplicity, a single intravenous test was performed. As seen in Table 2, the mean increment were $17.9 \pm 1.6 \mu\text{g}/100 \text{ ml}$ in normal controls, $8.5 \pm 2.9 \mu\text{g}/100 \text{ ml}$ in Cushing's syndrome with adrenocortical adenoma, $7.3 \pm 5.7 \mu\text{g}/100 \text{ ml}$ in corticoid treated patients.

The mean increment during infusion of NH-corticotropin to normal controls was $37.2 \pm 5.7 \mu\text{g}/100 \text{ ml}$ (Table 3).

By the intramuscular method, no significant differences in the mean increments between Cushing's syndrome and controls, and between corticoid treated patients and controls were observed (Table 1).

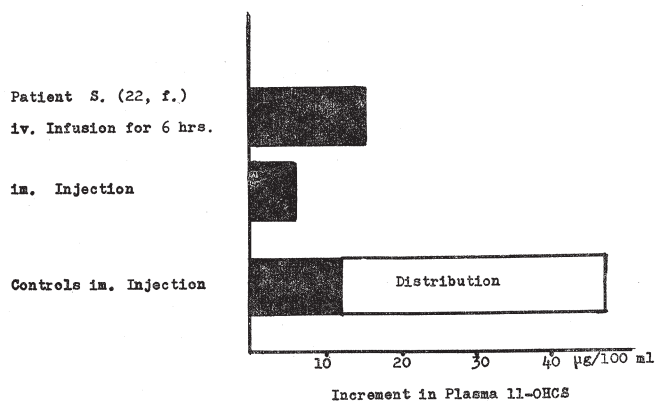


FIG. 2. A female with Cushing's syndrome due to adrenocortical hyperplasia, showing different responses to im. injection and iv. infusion.

TABLE 2. Effects of Intravenously Administered Synthetic Corticotropin on Plasma 11-OHOS Concentration

Subjects (Age, Sex)	Plasma 11-OHCS Concentration			Increment
	Before	30 min	60 min	
Controls				
A. (59, m)	8.4	11.6	21.4	13.0
W. (66, m)	13.8	21.3	30.0	16.2
Y. (19, f)	3.8	28.1		24.3
S. (37, f)	8.9	24.1	26.4	17.5
T. (26, f)	11.7	28.8	31.1	19.4
T. (42, f)	12.5	28.8	30.0	17.5
K. (42, f)	12.5	30.0	23.3	17.5
Mean ± S. E.	10.2 ± 1.3	24.7 ± 2.5	27.0 ± 1.6	17.9 ± 1.6
Cushing's Patients (Adrenocortical Adenoma)				
F. (42, f)	20.3	13.0	23.7	3.4
A. (26, f)	36.0	39.3	54.7	18.7
C. (14, f)	34.3	37.8	30.7	3.5
Mean ± S. E.	30.2 ± 4.8***	30.0 ± 8.5*	36.7 ± 9.5*	8.5 ± 2.9**
Long-term Corticoid Treated Patients				
N. (29, f)	0.6	4.6	4.6	4.0
T. (45, f)	4.6	5.8	7.8	3.2
H. (33, m)	10.0	17.6	18.2	8.2
I. (45, m)	17.6	18.8	28.2	10.6
T. (23, f)	9.7	20.9	20.2	10.5
Mean ± S. E.	8.5 ± 2.9*	13.5 ± 3.5**	15.8 ± 4.0**	7.3 ± 1.6***

* $p > 0.05$, ** $p > 0.05$ and *** $p < 0.01$ vs. controls.

TABLE 3. Effects of Intravenously Infused (for 6 hrs)
NH-Corticotropin on Plasma 11-OHCS
Concentration

	Before	Maximum	Increment
Mean \pm S. E.	15.8 \pm 1.8*	52.9 \pm 3.3**	37.2 \pm 5.7***

* $p > 0.05$, ** $p < 0.05$ and *** $p < 0.01$ vs. controls (F.).

By the intravenous method, corticoid treated patients had significant differences in the 30 min, 60 min values ($p > 0.05$) and the increment ($p > 0.01$). The mean increment in 3 Cushing's syndrome patients with adrenocortical adenoma was lower with significant difference than that of controls ($p < 0.05$).

In control subjects, the mean increment of plasma 11-OHCS levels was less than those of infusion tests (NH-corticotropin) and of intramuscular injection tests (synthetic corticotropin).

The mean response in the intravenous method had less variation than did that in the intramuscular or NH-corticotropin infusion method (F: $p < 0.01$).

No allergic reaction occurred in this experiment.

DISCUSSION

The 1-24 peptide used in this experiment, has no immunological terminal and chain structure of 1 to 24 amino acids as seen in natural corticotropin.

Differences were noted in the results of the rapid corticotropin test, between the methods of intramuscular and intravenous administrations. Such differences have been reported in the results of i.m. administration of NH-corticotropin depot and dripping i.v. administration of plain NH-corticotropin¹⁾. When intramuscular injection is attempted in patients with Cushing's syndrome, it might be difficult to make sure whether the injection was made into muscle or fat tissue, because of well development of the fat tissues and atrophy of the muscle in such patients.

The results of a series using intramuscular administrations showed larger variances or standard errors than did those of intravenous injections in normal controls. This would indicate the disadvantage in the method of intramuscular administration. The intramuscularly injected corticotropin must have two steps: transport into the blood and inactivation, before stimulation of adrenal glands. It is more simple for the intravenously injected corticotropin.

Smaller response of plasma corticoid to the intravenously administered corticotropin could be explained by the short period of influence because of rapid inactivation and by no supply from muscle, by an intravenous single injection.

According to the rapid test by Moncloa. The estimation of plasma corticoids

was made 1 hour after administration of synthetic corticotropin⁵). We have estimated both levels of 30 min and 60 min after administration of corticotropin to find larger response. In 6 out of 7 controls, the 60 min values were higher than 30 min values. In 1 case twice injected with synthetic corticotropin at 30 min intervals, the 30 min value was higher than the 60 min value.

It was shown in this study that it would be preferable to compare the increment of plasma corticoid following intravenous injection of corticotropin, rather than to compare the highest value.

The 6 hour infusion of NH-corticotropin would induce maximum response of plasma corticoid, whereas a single intravenous injection caused, in general, less response. This would imply that the latter stimulation is not strong enough to get maximum response of the adrenal glands. In two cases, the method using two single injections were applied at 30 min interval. As seen in Fig. 3, the response at time 60 min was higher in the first case and lower in the second case, than that at time 30 min.

However, when the higher levels of corticotropin in blood are maintained for a long time as seen in cases of intramuscular or dripping administration, all stages of synthesis and release might be affected not only functionally but also morphologically resulting in hypertrophy. On the other hand, a single intravenous administration of corticotropin would induce a response of short period and would not remain further influence on the adrenal gland. According to Vernikos-Danellis *et al.*, the content of adrenal corticosterone reaches maximum levels at 5 to 10 minutes after intravenous administration in the hypophy-

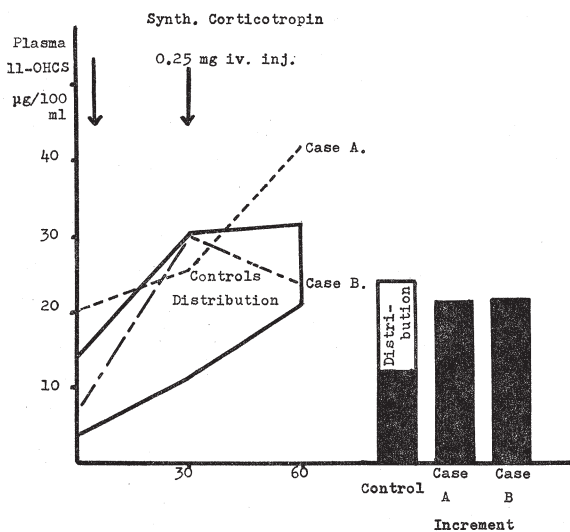


FIG. 3. Results of two time injections of synthetic corticotropin, at 30 min interval, A: patient A, B: patient B.

sectomized rats⁶). Liddle *et al.* reported appearance of the maximum levels of corticosterone in adrenal vein in about 13 min after i.v. administration corticotropin in such rats⁷). Our data revealed the appearance of maximum level in peripheral blood in 30 min or later. The enhanced synthesis of corticoid which occurred shortly after the corticotropin administration would be the results of hypertrophy, vasodilatory effect and other conditions, in addition to the direct effect of functional enhances. It would be ideal to complete all the procedures without complexity.

In two cases of Cushing's syndrome due to adrenocortical adenoma, intravenous dripping infusion of NH-corticotropin and intravenous single injection of synthetic corticotropin were compared, resulting in the same tendency as seen in Fig. 4.

It has been reported that the synthetic corticotropin does not contain immunological terminal and its peptide is small, therefore hypersensitivity hardly occur by its injection⁸). In the present experiment, a case with urticaria and shock was noted by injection of NH-corticotropin or HP-corticotropin. However, no allergic reaction occurred in the same patient who was applied 3 successive tests within one month.

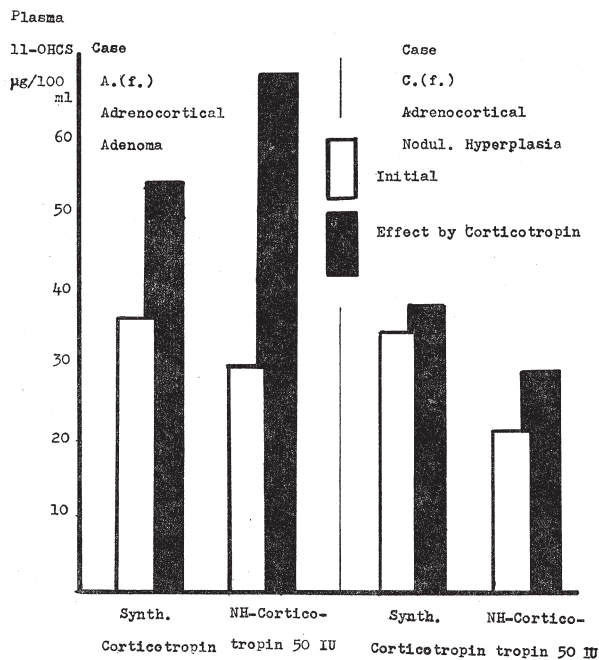


FIG. 4. Comparison of NH-corticotropin infusion and synthetic corticotropin iv. injection,

CONCLUSION

The rapid corticotropin test was clinically available for detecting the adrenocortical function.

The type of responses was the same as seen in cases of injection of NH-corticotropin.

It is preferable to collect 2 blood samples, at 30 and 60 min after administration of corticotropin rather than the single collection.

It is better to compare the increments of plasma corticoid, rather than the highest value. No allergic reactions were noted during the treatments.

ACKNOWLEDGEMENT

We are grateful to Prof. Shin Hoshikawa and Prof. Ryoji Nagai, Drs. Mitsuhiro Sengoku and Mitsuo Ukai for their kind guidance. We debt to Mrs. Ikue Mizuno for her technical assistance in this study.

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