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EFFECT OF NATURAL AND SYNTHETIC β^{1-24}- AND β^{1-18}-CORTICOTROPHINS ON THE EXTRA-ADRENAL METABOLISM OF CORTISOL AND ALDOSTERONE IN PATIENTS WITH ADDISON'S DISEASE

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SUMMARY

The effect of natural ACTH and synthetic β^{1-24}- and β^{1-18}-corticotrophins on the extra-adrenal metabolism of cortisol and aldosterone was studied in patients with Addison's disease. ACTH enlarged the distribution volume of the steroids and slowed down their rate of disappearance from the plasma. In the case of cortisol, ACTH augmented its urinary excretion and percentage conversion into unconjugated 4-ene-3-oxometabolites, and diminished the conversion into metabolites with an oxogroup at C-11 and 11-oxygenated 17-oxosteroids. These effects were observed in a wide range of administered doses of cortisol (20–400 mg daily) and persisted after prolonged administration of ACTH for three to six weeks. At this time, a distinct reduction of the transcortin binding capacity for cortisol occurred in two out of three experiments. The results suggest that ACTH enhances cellular uptake of cortisol and aldosterone, and inhibits the reduction of ring A of the steroid molecule. In the case of cortisol, ACTH inhibits to some extent also the 11β-OH dehydrogenation and the cleavage of the side chain, and possibly reduces the binding capacity of transcortin.

INTRODUCTION

ACTH, besides its role in the regulation of corticosteroidogenesis, influences also the extra-adrenal metabolism of cortisol. Firschein *et al.*[1] observed a significant decrease in the rate of disappearance of radioactivity from blood and tissues of stressed rats injected with [^{14}C]-cortisol. Berliner *et al.*[2] demonstrated a retention of cortisol and its metabolites in blood, liver, kidney and thymus of adrenalectomized mice after the administration of ACTH. A decrease of the disappearance rate of exogenous cortisol from the plasma after ACTH administration was observed in dogs [3] and in patients with Addison's disease [4]. However, the mechanism of this extra-adrenal effect of ACTH has not so far been satisfactorily elucidated. On the basis of their *in vitro* experiments in stressed and ACTH-treated adrenalectomized mice, Dougherty and Berliner[5] believe ACTH to inhibit the conjugation of the steroid metabolites in the liver. Another explanation could be the inhibition of hydrogenation of the A ring as suggested by De Moor *et al.*[4]. In our earlier study of the metabolism of exogenous cortisol in patients with Addison's disease short-term administration of natural ACTH was seen to enhance the excretion of unmetabolized cortisol and its 4-ene-3-oxometabolites, together with some fall of 11-oxygenated 17-oxosteroids [6]. Later, we demonstrated that ACTH not only slows down the disappearance rate of cortisol from the plasma, as reported by De Moor *et al.*[4], but also enlarges its distribution volume in the body [7, 8]. Similar extra-adrenal effects were observed with synthetic β^{1-24}-corticotrophin [9]. Our findings were confirmed by the work of Kornel and Meador[10, 11] and Kumasa *et al.*[12]. However, Kumasa did not find any significant difference in the urinary excretion of cortisol and its metabolites between control studies and after ACTH.

In the present work the effect of a short- and long-term administration of natural and synthetic corticotrophins on the plasma disappearance curves of [^{3}H]-cortisol and on the urinary excretion of cortisol and its metabolites was studied in patients with Addison's disease, maintained on a constant dose of unlabelled cortisol. Data on the transcortin binding capacity and on the kinetics of [^{3}H]-aldosterone are also presented.

MATERIAL AND METHODS

Ten patients with Addison's disease, five males and five females, mean age 44 years (range 30–54), were selected for the study. The diagnosis was confirmed by near zero levels of plasma cortisol and urinary 17-hydroxycorticosteroids and by their unresponsiveness to ACTH administration for 3 days. None showed clinical or laboratory signs of altered hepatic, renal or thyroid functions.

In eight patients, the effect of short-term administration of ACTH on urinary excretion of metabolites of administered cortisol was studied. Patients received 30 mg of cortisol orally, divided in three equal doses at 8-h intervals, for 7 days. As this dosage of cortisol does not fully block the endogenous secretion of ACTH [13], we added 1 mg of dexamethasone every 6 h on days 4 to 7. On days 6 and 7, 80 IU of natural

ACTH* (patients Nos. 1–5) or 1 mg of synthetic β^{1-24}-ACTH† (patients Nos. 6–8) in depot form were given intramuscularly. The urine was collected for steroid analyses on days 5 (control) and 7 (ACTH). Four weeks later the test was repeated with a daily dose of 400 mg cortisol (100 mg every 6 h). In this case the administration of cortisol was restricted to four days, that of dexamethasone omitted, and ACTH was given on days 3 and 4. The urine was then collected on the 2nd (Control) and 4th day (ACTH). In two of these subjects (A.S., J.K.) the urine was also analysed after the adminstration of β^{1-24}-ACTH depot 0·5 mg daily for 6 weeks.

In two subjects (A.S., J.K.) the effect of short-term and prolonged administration of β^{1-24}-ACTH on the disappearance rate of [1,2-^{3}H]-cortisol from the plasma and its cumulative excretion in the urine, together with its metabolites, was studied as follows: patient A.S. received orally 20 mg and J.K. 60 mg of cortisol daily, divided in four equal doses at 6-h intervals. On the fifth day, the morning dose of cortisol was substituted by an intravenous injection of a priming dose of unlabelled cortisol, followed by its constant infusion for the next 6 h. Two hours after the beginning of the infusion, 50 μCi of [1,2-^{3}H]-cortisol (The Radiochemical Centre, Amersham, England, S.A. > 40,000 mCi/mmol) chromatographically purified before application was rapidly injected. Blood samples were drawn 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 and 90 min after the injection of labelled cortisol. Urine was collected in six periods for a total of 48 h (0–3, 3–6, 6–9, 9–12, 12–24, 24–48 h). The test was repeated after 2 days and 3 weeks in the case of J.K. and after 6 weeks of continued administration of ACTH in the case of A.S. The daily dose of β^{1-24}-ACTH was 1 mg for the first 2 days and 0·5 mg for the remainder of the study. In these two subjects and in patient M.W. from the previous study the transcortin binding capacity for cortisol was measured before and after prolonged application of ACTH.

In two other patients (M.M., P.H.) the [1,2-^{3}H]-cortisol disappearance tests were performed similarly as in subject A.S. However, 1 mg of β^{1-18}-ACTH‡ was infused for 4 h instead of β^{1-24}-ACTH. At the mid-point of the infusion labelled cortisol was injected.

Finally, the rate of disappearance of aldosterone from the plasma was studied before and after administration of β^{1-24}-ACTH for 2 days in patients K.P. and J.K. To approach physiological corticosteroid levels in the plasma, a mixture of unlabelled aldosterone (10 μg) and cortisol (1·5 mg) was given in a single injection followed by a continuous infusion of the two steroids at a rate of 5 μg and 0·75 mg/h respectively. These doses correspond to mean daily secretion rates of aldosterone and cortisol in healthy unstressed subjects on unrestricted diet (120 μg and 18 mg respectively). Plasma cortisol levels measured during the infusion ranged between 20–23 μg/100 ml in K.P. and between 16 and 18 μg/100 ml in J.K. Two hours after the start of the infusion, 10 μCi of [1,2-^{3}H]-aldosterone (Radiochemical Centre, Amersham, England, S.A. 35,000 mCi/mmol) was rapidly injected. Before application [1,2-^{3}H]-aldosterone was purified by paper chromatography on ethanol-washed Whatman No. 1 in benzene–cyclohexane–methanol–water (9:1:6:4 by vol.). This method separates aldosterone and isoaldosterone. Blood samples were obtained at the same intervals as in the test with [1,2-^{3}H]-cortisol.

The [^{3}H]-cortisol and [^{3}H]-aldosterone disappearance curves were subjected to a mathematical analysis in terms of a two-compartment model described by Tait *et al.*[14]. In this model V_1 represents the calculated volume of the inner pool, V_2 the calculated volume of the outer pool, k_1 is the rate constant for the distribution of steroid between the inner and outer pools, k_2 the rate constant for the metabolic removal of the steroid from the inner pool. MCR represents the metabolic clearance rate of aldosterone.

Chemical methods. All reference steroids, except 6β-OH-F and 6β-OH-E, were commercial preparations (L. Light and Co., Colnbrook, England). 6β-OH-E was a USP Steroid Reference Substance and 6β-OH-F was isolated and purified in our laboratory from pooled urine extracts of patients receiving high doses of cortisol, using the procedure described by Frantz *et al.*[15].

All solvents were reagent grade and redistilled before use. Following solvent systems were used for chromatography: (I) benzene–methanol–water (2:1:1 by vol.); (II) benzene–ethylacetate–methanol–water (10:1:10:10 by vol.); (III) petroleum–ether (70–110°C)–methanol–water (10:8:2 by vol.) [16]; (IV) cyclohexane–benzene–methanol–water (10:10:10:5 by vol.); (V) cyclohexane–benzene–methanol–water (10:9:6:4 by vol.); (VI) benzene–tert. butylalcohol–water (70:43:86 by vol.) [15].

The chromatography was carried out at 20–22 C; the papers were equilibrated for 3–6 h. Whatman papers Nos. 1, 2 or 3 mm were used.

Volumes of urine representing 20 or 5% of the 24 h output were chosen for the analysis. After saturation with sodium sulphate, the unconjugated steroids were extracted from the urine samples by twice their volume of ethyl-acetate (Extract A). The conjugated metabolites of cortisol were hydrolysed enzymatically with the gastric juice of the snail *Helix pomatia*. The 11-oxygenated 17-oxosteroids were extracted with tetrachloromethane (Extract B), the more polar corticosteroids with ethyl acetate (Extract C).

The dried extract A was dissolved in methanol and divided into several equal parts. One half of them was chromatographed on Whatman No. 1 paper in system I for 12–16 h with cortisol, cortisone, THF, THE, 20β-OH-F and 20β-OH-E as reference standards; the second half in system II with 20α-OH-F, 20β-OH-F, 6β-OH-F and 6β-OH-E as reference standards for 48–50 h. The detection of spots location was performed using absorption of short-wave U.V. light. Alkaline blue tetrazolium reaction combined with the sodium

* Corticotrophin SPOFA.

† Synacthen CIBA-GEIGY, Basel, Switzerland.

‡ From Research Laboratories of CIBA-GEIGY, Basel, Switzerland.

fluorescence test was applied to one of the test strips and to the standards run in lanes neighbouring the unknowns on the paper chromatogram. The unstained areas of the steroids were eluted overnight chromatographically with 90% ethanol. Suitable aliquots were taken for quantitation. The 20-OH steroids were measured by means of INH reaction [17], 6β-OH-F by Porter–Silber reaction [18]. For the estimation of cortisol and cortisone, both reactions were used. The values obtained by the two methods were in good agreement. The purity of cortisol, cortisone and 20-OH-E eluted from the chromatogram was checked by re-running in system IV for 5 days, 6β-OH-F in system VI for 4–5 h. 6β-OH-F, 20α-OH-F and 20β-OH-F were acetylated in pyridine-acetic anhydride overnight and the acetates chromatographed in system IV. As in the initial experiments with unlabelled cortisol, the separation of the two isomers of 20-OH-F in the first chromatography was incomplete; the sum is stated in the results.

The dried extract B was chromatographed with reference steroids (11β-hydroxy- and 11-oxoetiocholanolone, 11β-hydroxy- and 11-oxoandrosterone) on ethanol washed Whatman No. 2 paper in system III for 48 h. After detection of the standards and of one of the test strips with alkaline m-dinitrobenzene solution, the unstained areas of individual steroids were eluted with benzene–ethanol (10:1) overnight. For quantitation, the Zimmermann reaction was used. Since the chromogenicity of the 11β-hydroxy- and 11-oxo-17-ketosteroids differs from that of dehydroepiandrosterone used for the calibration curve, the values were corrected by multiplication by a factor suitable for the measured steroid. These factors were obtained by dividing the amount of the 11-oxygenated 17-oxosteroid studied by the amount of dehydroepiandrosterone producing identical colour intensity in the Zimmermann reaction.

Extract C was divided in a similar manner as extract A. One half was chromatographed in system I on Whatman No. 1 or 3 mm with overrunning (24–28 h) along with standard THF, ATHF, THE, 20β-OH-F, 20β-OH-E and cortisol. After detection with alkaline blue tetrazolium, the unstained areas of THF, ATHF and THE were eluted with ethanol. An aliquot of the eluate was taken for quantitation and the remainder rechromatographed in system IV for 7–8 days. For quantitation of the steroids eluted after the first and second chromatographies, Porter–Silber reaction was used. The loss in the second chromatography (in comparison with the first chromatography) ranged from 10 to 15%. In cases where separation after the first chromatography was imperfect, values obtained after the second chromatography were taken and corrected for the loss.

The remaining portions of the extract C were chromatographed on Whatman No. 1 paper in system II for 28–32 h [19] with β-cortol, α- and β-cortolone as standards. The standards and one of the test strips were stained with phosphomolybdic acid [20]. Under the conditions used C-20 epimers of cortol and cortolone were not separated. The unstained "cortol" and "cortolone" areas were eluted with ethanol and the dried eluates oxidized with HIO_4 to the corresponding 11-oxygenated 17-oxosteroids [21]. Samples of authentic β-cortol and β-cortolone were handled in the same fashion and used for the calibration curves. After the side-chain cleavage the products were extracted with chloroform and chromatographed on paper in system III for 48 h. The areas of 11β-hydroxy- and 11-oxoetiocholanolone were eluted and quantitated by means of Zimmermann reaction.

The urine samples collected after the injection of [1,2-^{3}H]-cortisol were handled similarly. Radioactivity of the isolated metabolites were determined after addition of a POPOP–toluene scintillation fluid (SLT61, SPOLANA) in a liquid scintillation counter (ABAC-Intertechnik, France).

The plasma samples were extracted with 5 vol. of methylene chloride, 5 μg of unlabelled cortisol or aldosterone were added. After evaporation, the extracts were chromatographed at room temperature in the solvent system I (cortisol) or V (aldosterone). After drying for a few minutes, the paper was briefly scanned under 254 nm light to locate the U.V. absorbing spots. The cortisol (aldosterone) areas were eluted chromatographically with ethanol and the eluates transferred quantitatively to counting vials. Radioactivity was measured after evaporation and addition of the POPOP–toluene scintillation fluid.

The transcortin binding capacity was measured at 20–22°C using the gel-filtration method described by de Moor *et al.*[22]. The plasma was overloaded with unlabelled cortisol (0·5 μg/ml) and 10,000 d.p.m. of [1,2-^{3}H]-cortisol was added. The cortisol level was determined fluorometrically on an Aminco–Bowman spectrophotofluorometer according to the procedure described by Spencer-Peet *et al.*[23]. The amount of the protein bound and unbound cortisol was quantitated by radioactivity measurements.

RESULTS

The effect of a 2-day administration of ACTH on the urinary excretion of cortisol metabolites in eight Addisonian patients receiving orally 30 and 400 mg of cortisol daily is presented in Table 1. After ACTH, the excretion of steroids with the unreduced 4-ene-3-oxo-group (mainly cortisol, 6-hydroxycortisol and 20-dihydrocortisol) rises to an almost two-fold level ($P < 0{\cdot}001$). In the case of substitution with 30 mg of cortisol, the rise of all 4-ene-3-oxosteroids was statistically significant. When high doses of cortisol were given, only the excretion of the 4-ene-3-oxosteroids with a hydroxyl group at C-11 was significantly increased. No significant difference was found in the excretion of tetrahydro- and hexahydrometabolites of cortisol, while the excretion of 11-oxygenated 17-oxosteroids decreased.

After ACTH, the proportion of 4-ene-3-oxosteroids to the individual groups of ring A reduced metabolites of cortisol was significantly increased in all cases

Table 1. The effect of a 2 day administration of ACTH on the excretion of cortisol metabolites in eight Addisonian patients receiving orally 30 and 400 mg of cortisol daily

	Control			ACTH			
	Mean	S.D.	Range	Mean	S.D.	Range	P*
Cortisol 30 mg daily							
Cortisol	0·32	0·06	0·26–0·42	0·56	0·15	0·38–0·82	<0·005
Cortisone	0·33	0·13	0·18–0·54	0·49	0·34	0·18–1·15	<0·05
6-OH-F	0·98	0·48	0·34–1·71	1·96	0·77	0·58–3·13	<0·005
20-OH-F	0·67	0·16	0·43–0·89	1·16	0·47	0·83–2·18	<0·01
20-OH-E	0·45	0·15	0·23–0·64	0·71	0·24	0·29–0·93	<0·01
Sum	2·7	0·4	2·2–3·3	4·8	1·7	3·3–8·2	<0·001
THF	7·1	2·0	3·9–9·7	8·3	3·1	4·1–13·0	<0·05
ATHF	3·8	0·9	2·4–4·9	3·5	0·7	2·5–4·6	NS
THE	8·0	2·1	5·1–11·3	7·5	1·8	4·7–10·9	NS
Sum	18·8	3·4	13·7–23·5	19·3	3·5	13·8–23·6	NS
Cortols	3·7	0·7	2·6–4·2	4·0	0·3	3·5–4·2	NS
Cortolones	6·0	1·2	4·1–7·0	5·8	1·2	4·3–7·0	NS
Sum	9·7	1·9	6·8–11·2	9·8	1·6	7·8–10·9	NS
11-OH-Etio	2·6	1·1	1·4–4·1	2·1	1·2	0·8–3·9	<0·025
11-OXO-Etio	1·3	1·4	0·5–4·1	1·0	1·3	0·3–3·5	<0·01
11-OH-Andro	2·2	1·8	1·1–5·7	1·2	0·4	0·8–1·8	<0·01
Sum	6·1	3·7	3·4–13·8	4·3	2·8	2·0–9·2	<0·05
Cortisol 400 mg daily							
Cortisol	0·77	0·22	0·45–1·20	1·30	0·60	0·80–2·52	<0·005
Cortisone	0·21	0·10	0·10–0·39	0·24	0·12	0·11–0·45	NS
6-OH-F	1·58	0·34	0·99–1·92	2·52	0·36	1·86–3·02	<0·001
20-OH-F	0·96	0·56	0·45–1·11	1·39	0·48	0·77–2·14	<0·025
20-OH-E	0·33	0·24	0·11–0·79	0·39	0·26	0·12–0·86	NS
Sum	3·8	1·0	2·5–5·3	5·8	1·3	4·5–8·3	<0·001
THF	8·2	2·2	5·6–11·9	8·3	1·6	6·1–10·6	NS
ATHF	3·6	1·4	1·4–5·5	3·5	1·6	1·8–6·3	NS
THE	5·0	1·8	3·4–8·5	4·9	1·3	3·4–7·0	NS
Sum	16·8	3·5	11·3–21·2	16·7	1·8	13·4–19·6	NS
Cortols	2·9	0·8	1·9–4·1	3·3	0·8	2·3–4·6	NS
Cortolones	2·7	0·8	1·6–3·9	2·5	0·9	1·1–3·8	NS
Sum	5·6	1·6	3·5–8·0	5·8	1·4	4·0–7·9	NS
11-OH-Etio	3·9	2·7	1·1–10·2	2·8	1·9	0·9–6·4	<0·05
11-OXO-Etio	1·1	1·1	0·2–3·4	1·0	1·2	0·1–3·5	NS
11-OH-Andro	1·3	1·0	0·4–3·4	0·9	0·8	0·3–2·7	<0·05
11-OXO-Andro	0·18	0·16	0·1–0·5	0·15	0·12	0·1–0·4	NS
Sum	6·5	4·8	1·8–15·6	4·9	3·3	1·4–10·8	NS

* Statistical significance of differences between Control and ACTH.

Table 2. The effect of a 2 day administration of ACTH on the ratios 4-ene-3-oxo/ring A reduced, 11-OH/11-OXO and 5α/5β between metabolites of administered cortisol in eight Addisonian patients

	Control			ACTH			
Ratio	Mean	S.D.	Range	Mean	S.D.	Range	P*
Cortisol 30 mg daily							
4-ene-3-oxo/ring A reduced							
Unconjug. 4-ene-3-oxo/Tetrahydro	0·15	0·04	0·10–0·20	0·25	0·08	0·16–0·38	<0·01
Unconjug. 4-ene-3-oxo/Tetra + Hexahydro	0·10	0·01	0·08–0·11	0·19	0·05	0·13–0·25	<0·025
Unconjug. 4-ene-3-oxo/Total reduced†	0·08	0·01	0·07–0·09	0·16	0·03	0·12–0·20	<0·01
11-OH/11-OXO							
Unconjug. cortisol/cortisone	1·0	0·4	0·6–1·5	1·3	0·5	0·7–2·2	<0·025
Unconjug. 20-OH-F/20-OH-E	1·6	0·7	1·1–3·1	1·8	0·8	1·1–3·3	<0·025
THF/THE	0·9	0·3	0·6–1·5	1·2	0·7	0·7–2·7	NS
Cortols/Cortolones	0·6	0·1	0·6–0·6	0·7	0·1	0·6–0·8	NS
11-OH-Etio/11-OXO-Etio	2·8	1·0	1·0–3·6	3·6	1·9	1·1–7·3	NS
5α/5β							
ATHF/THF	0·57	0·22	0·36–0·98	0·46	0·20	0·26–0·83	<0·025
11-OH-Andro/11-OH-Etio	0·77	0·35	0·50–1·39	0·66	0·25	0·36–1·00	NS
Cortisol 400 mg daily							
4-ene-3-oxo/ring A reduced							
Unconjug. 4-ene-3-oxo/Tetrahydro	0·22	0·07	0·15–0·31	0·35	0·10	0·22–0·48	<0·005
Unconjug. 4-ene-3-oxo/Tetra + Hexahydro	0·17	0·03	0·12–0·20	0·26	0·06	0·20–0·36	<0·005
Unconjug. 4-ene-3-oxo/Total reduced†	0·13	0·02	0·09–0·16	0·22	0·06	0·16–0·34	<0·005
11-OH/11-OXO							
Unconjug. cortisol/cortisone	3·9	1·3	2·3–6·0	5·9	1·7	2·9–7·7	<0·025
Unconjug. 20-OH-F/20-OH-E	3·2	0·8	2·6–4·8	4·2	1·6	2·3–6·8	NS
THF/THE	1·8	0·8	0·7–3·2	1·8	0·6	1·0–2·8	NS
Cortols/Cortolones	1·0	0·2	0·7–1·4	1·4	0·6	0·9–2·5	<0·05
11-OH-Etio/11-OXO-ETio	4·3	1·6	1·4–6·4	4·4	2·5	1·5–9·0	NS
5α/5β							
ATHF/THF	0·45	0·18	0·25–0·77	0·45	0·20	0·23–0·79	NS
11-OH-Andro/11-OH-Etio	0·42	0·32	0·08–1·00	0·36	0·35	0·08–1·07	NS

* Statistical significance of differences between Control and ACTH.
† Tetrahydro + Hexahydro + 11-oxy-17-KS.

Table 3. The effect of prolonged administration of ACTH on the excretion of cortisol metabolites in two Addisonian patients receiving orally 30 and 400 mg of cortisol daily

	Patient M.W.						Patient A.S.					
	Cortisol 30 mg daily ACTH			Cortisol 400 mg daily ACTH			Cortisol 30 mg daily ACTH			Cortisol 400 mg daily ACTH		
	Control	2 days	6 weeks	Control	2 days	6 weeks	Control	2 days	6 weeks	Control	2 days	6 weeks
Cortisol	0·27	0·39	0·42	0·56	1·34	2·37	0·30	0·63	0·48	0·62	1·10	0·93
Cortisone	0·18	0·18	0·23	0·15	0·21	0·23	0·25	0·37	0·31	0·14	0·16	0·18
6-OH-F	1·71	2·79	1·89	1·92	2·35	3·38	0·75	2·70	1·20	0·99	2·55	2·25
20-OH-F	0·72	0·97	0·92	1·11	1·71	2·96	0·60	0·97	0·60	0·62	1·00	1·12
20-OH-E	0·23	0·29	0·24	0·38	0·31	0·35	0·47	0·77	0·50	0·13	0·20	0·22
Sum	3·11	4·62	3·70	4·12	5·92	9·29	2·37	5·44	3·09	2·50	5·01	4·70
THF	8·5	11·4	10·7	7·1	6·1	8·6	6·1	6·6	7·1	9·3	10·3	10·5
ATHF	4·6	3·6	3·4	4·5	3·3	3·0	2·7	2·5	1·8	2·5	2·3	1·0
THE	5·4	5·2	6·9	4·8	4·4	3·8	5·1	5·2	5·2	5·1	6·0	6·3
Sum	18·5	20·2	21·0	16·4	13·8	15·4	13·9	14·3	14·1	16·9	18·6	17·8
Cortols	4·2	4·0	3·7	4·1	4·6	2·7	2·6	3·5	3·0	2·4	3·1	2·5
Cortolones	7·0	6·0	4·2	3·9	3·3	1·5	4·1	4·3	4·5	2·2	2·1	1·7
Sum	11·2	10·0	7·9	8·0	7·9	4·2	6·7	7·8	7·5	4·6	5·2	4·2
11-OH-Etio	2·1	1·4	1·5	2·2	1·5	1·4	2·9	2·1	1·9	1·7	1·5	1·2
11-OXO-Etio	0·7	0·3	0·2	0·4	0·2	0·2	1·0	0·7	0·7	0·7	0·7	0·6
11-OH-Andro	1·4	1·2	0·6	1·8	0·3	0·4	1·9	1·4	0·4	1·7	1·6	0·5
11-OXO-Andro	—	—	—	—	—	—	0·4	0·3	0	0·1	0·1	0
Sum	4·2	2·9	2·3	4·4	2·0	2·0	6·2	4·5	3·0	4·2	3·9	2·3

Table 4. The effect of prolonged administration of ACTH on the ratios 4-ene-3-oxo/ring A reduced, 11-OH/11-OXO and 5α/5β between metabolites of administered cortisol in two Addisonian patients

	Patient M.W.						Patient A.S.					
	Cortisol 30 mg daily ACTH			Cortisol 400 mg daily ACTH			Cortisol 30 mg daily ACTH			Cortisol 400 mg daily ACTH		
Ratio	Control	2 days	6 weeks	Control	2 days	6 weeks	Control	2 days	6 weeks	Control	2 days	6 weeks
4-ene-3-oxo/ring A reduced												
Unconjug. 4-ene-3-oxo/Tetrahydro	0·17	0·23	0·18	0·25	0·43	0·60	0·17	0·38	0·21	0·15	0·27	0·27
Unconjug. 4-ene-3-oxo/Tetra + Hexahydro	0·10	0·15	0·12	0·17	0·27	0·47	0·11	0·25	0·14	0·12	0·21	0·22
Unconjug. 4-ene-3-oxo/Total reduced*	0·09	0·14	0·12	0·14	0·25	0·43	0·09	0·20	0·12	0·10	0·18	0·19
11-OH/11-OXO												
Unconjug. cortisol/cortisone	1·5	2·2	1·8	3·7	6·4	10·3	1·2	1·7	1·5	4·7	7·1	5·2
Unconjug. 20-OH-F/20-OH-E	3·1	3·3	3·8	2·9	5·5	8·4	1·3	1·3	1·2	4·8	5·0	5·1
THF/THE	1·6	2·2	1·6	1·5	1·4	2·4	1·2	1·3	1·4	1·8	1·7	1·7
THF + ATHF/THE	2·4	2·9	2·0	2·4	2·1	3·0	1·7	1·7	1·7	2·3	2·1	1·8
Cortols/Cortolones	0·6	0·7	0·9	1·1	1·4	1·9	0·5	0·8	0·7	1·1	1·4	1·5
11-OH-Etio/11/OXO-Etio	3·0	4·7	7·5	5·5	7·5	7·0	2·9	3·0	2·7	2·4	2·1	2·0
11-OH-17-KS/11-OXO-17-KS	5·1	8·1	10·5	9·4	11·2	9·0	3·3	3·4	2·6	3·9	3·6	2·8
5α/5β												
ATHF/THF	0·57	0·32	0·32	0·65	0·55	0·35	0·45	0·38	0·25	0·27	0·23	0·10
11-OH-Andro/11-OH-Etio	0·66	0·85	0·40	0·82	0·20	0·17	0·65	0·66	0·21	1·00	1·06	0·42

* Tetrahydro + Hexahydro + 11-oxy-17-KS.

Table 5. The effect of prolonged administration of ACTH on the percentage conversion of [1.2-^{3}H]-cortisol into its metabolites in two Addisonian patients

	Patient A.S.*			Patient J.K.†		
		ACTH			ACTH	
	Control	2 days	6 weeks	Control	2 days	3 weeks
Cortisol	0·15	0·99	0·32	0·89	1·94	1·73
Cortisone	0·29	0·51	0·44	0·53	0·60	0·51
6-OH-F	0·85	1·90	1·20	1·82	3·44	3·29
20α-OH-F	0·27	1·01	0·43	1·34	1·86	1·97
20β-OH-F	0·08	0·12	0·09	0·83	1·06	1·10
20-OH-E	0·22	0·40	0·15	0·90	1·15	1·03
Sum	1·86	4·93	2·63	6·31	10·05	9·63
THF	4·9	5·0	5·8	8·8	9·1	10·7
ATHF	3·1	2·9	2·1	5·6	5·3	4·0
THE	7·0	5·2	7·3	7·0	6·6	4·8
Sum	15·0	13·1	15·2	21·4	21·0	19·5
Cortols	2·2	3·3	2·4	5·0	5·1	5·6
Cortolones	3·9	3·8	3·4	6·9	5·5	5·7
Sum	6·1	7·1	5·8	11·9	10·6	11·3
11-OH-Etio	0·82	0·65	0·43	0·84	0·86	0·74
11-OXO-Etio	0·32	0·30	0·15	0·34	0·39	0·33
11-OH-Andro	0·60	0·45	0·12	0·86	0·83	0·20
Sum	1·74	1·40	0·70	2·04	2·08	1·27

* A.S. received during the study 20 mg of unlabelled cortisol daily.
† J.K. received during the study 60 mg of unlabelled cortisol daily (for details see text).

(Table 2). A small but consistent rise occurred also in the ratio 11-OH/11-OXO. ACTH lowered the ratio ATHF/THF (5α/5β), but only when 30 mg of cortisol daily were given.

In two experiments with prolonged administration of ACTH, the excretion of 4-ene-3-oxosteroids remained increased after six weeks of ACTH application, while the excretion of ATHF and 11-oxygenated 17-oxosteroids, especially that of 11β-hydroxyandrosterone, continued to decrease (Tables 3 and 4).

Similar results were obtained in two experiments with [^{3}H]-cortisol (Tables 5 and 6), although in A.S. the changes in the excretion of individual 4-ene-3-oxosteroids after 6 weeks of ACTH administration were less marked than in the acute experiment. On the other hand, the decrease of 11-oxygenated 17-oxosteroids was noted to appear only after prolonged exposure to ACTH. The cumulative excretion of the individual metabolites of [^{3}H]-cortisol in one of these patients (J.K.) is shown in Figs 1 and 2. Unlike other metabolites, cortisol and cortisone are excreted almost entirely in the first 3–6 h and their increase after ACTH is also limited to this period. Similar cumulative excretion curves were observed in the second patient (A.S.).

Table 6. The effect of prolonged administration of ACTH on the ratios 4-ene-3-oxo/ring A reduced, 11-OH/11-OXO and 5α/5β between metabolites of [1.2-^{3}H]-cortisol in two Addisonian patients

	Patient A.S.*			Patient J.K.†		
		ACTH			ACTH	
Ratio	Control	2 days	6 weeks	Control	2 days	3 weeks
4-ene-3-oxo/ring A reduced						
Unconjug. 4-ene-3-oxo/Tetrahydro	0·12	0·37	0·17	0·29	0·48	0·49
Unconjug. 4-ene-3-oxo/Tetra + Hexahydro	0·09	0·28	0·13	0·19	0·32	0·31
Unconjug. 4-ene-3-oxo/Total reduced‡	0·08	0·23	0·12	0·18	0·30	0·30
11-OH/11-OXO						
Unconjug. cortisol/cortisone	0·5	1·9	0·8	1·7	3·0	3·4
Unconjug. 20-OH-F/20-OH-E	1·6	2·8	3·5	2·4	2·7	3·0
THF/THE	0·7	1·0	0·8	1·3	1·4	2·2
THF + ATHF/THE	1·1	1·5	1·1	2·0	2·2	3·1
Cortols/Cortolones	0·6	0·8	0·7	0·8	0·9	1·0
11-OH-Etio/11-OXO-Etio	2·6	2·2	2·9	2·5	2·2	2·2
5α/5β						
ATHF/THF	0·63	0·58	0·36	0·64	0·58	0·37
11-OH-Andro/11-OH-Etio	0·74	0·69	0·28	1·00	0·97	0·27

* A.S. received during the study 20 mg of unlabelled cortisol daily (for details see text).
† J.K. received during the study 60 mg of unlabelled cortisol daily (for details see text).
‡ Tetrahydro + Hexahydro + 11-oxy-17-KS.

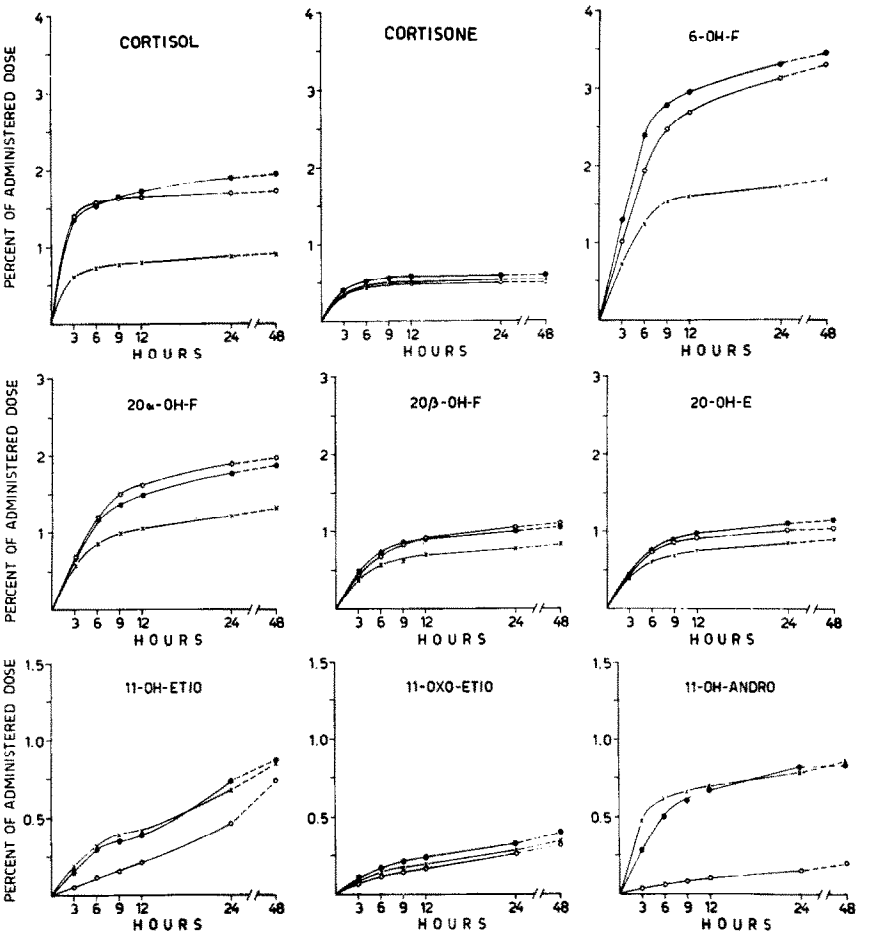

Fig. 1. Cumulative excretion of individual 4-ene-3-oxo- and 11-oxygenated 17-oxometabolites of [^{3}H]-cortisol before (× —— ×) and after β^{1-24}-ACTH administration for two days (●——●) and three weeks (○——○) in patient J.K. with Addison's disease receiving orally 60 mg of unlabelled cortisol daily.

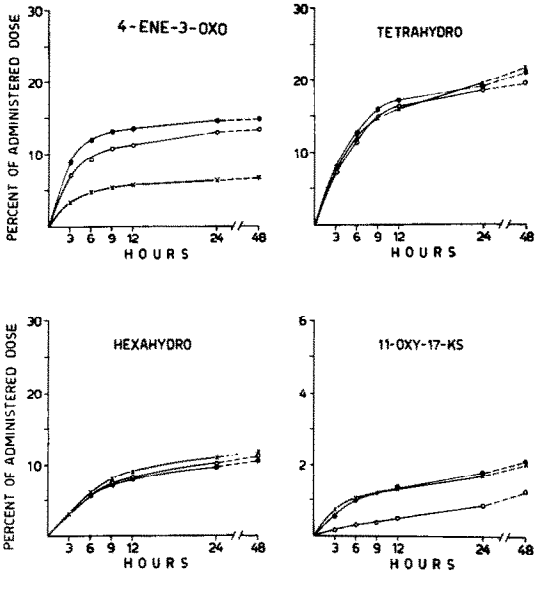

Fig. 2. Cumulative excretion of the sum of 4-ene-3-oxo-, tetrahydro-, hexahydro and 11-oxygenated 17-oxo-metabolites of [^{3}H]-cortisol before (× —— ×) and after β^{1-24}-ACTH administration for 2 days (●——●) and three weeks (○——○) in patient J.K.

The prolonged administration of ACTH affected the binding capacity of transcortin for cortisol in two of three cases. In J.K., it decreased after 3 weeks from 18·2 to 10·1 μg/100 ml, in M.W. from 19·1 to 12·7 μg/100 ml after 6 weeks. In A.S., the control value of transcortin binding capacity (18·1 μg/100 ml) remained unchanged after ACTH administration for 6 weeks.

The results obtained in the studies of the extra-adrenal action of β^{1-24}- and β^{1-18}-corticotrophins on the kinetics of [^{3}H]-cortisol in the plasma are summarized in Table 7. In all cases, ACTH prolonged the half-life of cortisol in the plasma and decreased the value of the metabolic removal constant (k_2). Except for one case (J.K.), all distribution volumes of cortisol in the body (V_1, V_2, $\overline{V}$) were increased after ACTH.

Similar results were obtained in the two experiments with [^{3}H]-aldosterone. Here too, ACTH enlarged the distribution volumes of aldosterone, prolonged its half-life in the plasma and reduced the value of k_2. The plasma disappearance curve of aldosterone in one of these subjects (K.P.) is shown in Fig. 3.

DISCUSSION

The results of the present study demonstrate that natural and synthetic corticotrophins profoundly

Table 7. The effect of the administration of ACTH on the kinetics of [1,2-^{3}H]-cortisol in patients with Addison's disease

Patient	Dosis of unlabelled cortisol (mg/day)	ACTH				V_1	$V_1 + V_2$ (liters)	$\bar{V}$	k_1 (units/day)	k_2	MCR (l/day)	$t_{\frac{1}{2}}$ (min)
		Type	Mode of application	Dosis (mg/day)	Duration							
J.K.	30	0	0	0	0	4·1	13·6	28·5	122·7	94·3	385·7	50
	30	β^{1-24}-ACTH dep.	i.m.	1	2 days	5·1	16·8	28·6	137·9	63·4	323·3	69
A.S.	20	0	0	0	0	3·4	8·3	11·0	117·0	51·2	170·0	57
	20	β^{1-24}-ACTH dep.	i.m.	1	2 days	6·4	19·3	21·0	197·0	29·0	185·6	110
	20	β^{1-24}-ACTH dep.	i.m.	0·5	6 weeks	4·5	10·0	12·5	94·2	32·7	148·5	84
J.K.	60	0	0	0	0	21·7	44·4	49·8	96·7	20·1	436·2	107
	60	β^{1-24}-ACTH dep.	i.m.	1	2 days	28·5	40·9	44·4	56·4	10·4	296·4	140
	60	β^{1-24}-ACTH dep.	i.m.	0·5	3 weeks	29·4	55·9	66·0	60·3	14·7	432·1	145
M.M.	20	0	0	0	0	5·8	10·9	12·5	105·5	37·9	218·7	54
	20	β^{1-18}-ACTH	i.v.	1	4 hours	6·7	12·2	17·7	65·4	31·2	208·1	75
F.H.	20	0	0	0	0	7·4	16·6	27·0	71·5	46·7	345·6	65
	20	β^{1-18}-ACTH	i.v.	1	4 hours	9·5	26·5	32·2	143·9	34·2	324·9	87

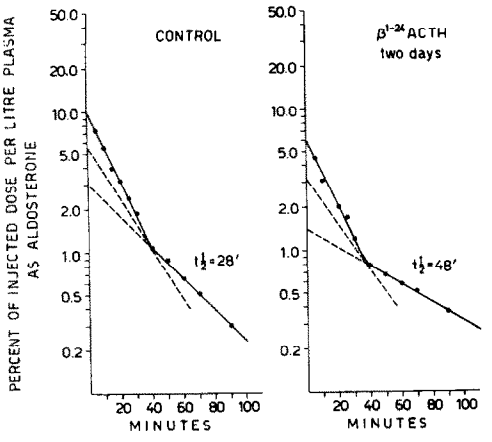

Fig. 3. The effect of a two day administration of β^{1-24}-ACTH on the kinetics of [^{3}H]-aldosterone in patient K.P. with Addison's disease.

affect the extra-adrenal metabolism of cortisol. Changes in the rate of urinary excretion of cortisol and its metabolites indicate ACTH to inhibit the reduction of ring A of the steroid molecule. The most affected seems to be the 5α-reductase, as indicated by the lowering of the ATHF/THF and 11-OH-Andro/11-OH-Etio ratios after ACTH, especially after its prolonged administration. A small increase in the 11-OH/11-OXO ratio and decrease in the excretion of C_{19} metabolites suggests further that ACTH inhibits to some extent also the 11β-OH dehydrogenation and the cleavage of the side chain of cortisol.

The inhibition of the reduction in ring A and 11β-OH dehydrogenation may account for the prolongation of cortisol disappearance from the plasma and the decrease of its metabolic removal rate from the inner pool. On the other hand, the enlargement of the distribution volumes suggests that ACTH enhances cellular uptake of cortisol. This explanation is supported by the results of Berliner *et al.*[2], who found an increased concentration of cortisol in various tissues of adrenalectomized mice after the administration of ACTH.

As indicated by the present study, the extra-adrenal effects of ACTH does not depend on the amount of cortisol given and persist after ACTH administration for several weeks. In this case, the reduction of the transcortin binding capacity may be also at play. Moreover, the extra-adrenal effect of ACTH is not limited to cortisol, but affects also aldosterone and probably other 4-ene-3-oxosteroids as well. The reproducibility of this effect with synthetic β^{1-24}- and β^{1-18}-corticotrophins excludes the possibility of a nonspecific reaction. It is an inherent property of the active part of the corticotrophin molecule.

The findings of an increased urinary excretion of unconjugated cortisol and decreased transcortin binding capacity after long-term administration of ACTH in Addisonian patients help explain the alteration in the steroid metabolism in Cushing's disease. This is particularly true of patients with the recurrence of the disease after subtotal adrenalectomy who have a distinctly elevated plasma level of ACTH. In these subjects, we regularly found a disproportionately high excretion of unconjugated cortisol compared to its actual secretion rate [24, 25]. Most patients also display a decreased transcortin binding capacity, which is not affected by total adrenalectomy, but increases (mostly to normal values) after successful pituitary implantation with ^{90}ytrium [26].

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