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CHANGES IN CORTICOSTEROID SYNTHESIS OF THE HUMAN ADRENAL CORTEX *IN VITRO*, INDUCED BY TREATMENT WITH o,p'-DDD FOR CUSHING'S SYNDROME: EVIDENCE FOR THE SITES OF ACTION OF THE DRUG

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SUMMARY

The *in vitro* synthesis of cortisol, cortisone, corticosterone, 18-hydroxycorticosterone, aldosterone and 11-dehydrocorticosterone has been studied in human adrenals removed from patients with Cushing's syndrome (adrenal adenomas, hyperplasias or carcinoma) treated or not with o,p'-DDD. A very significant fall occurred in the synthesis of cortisol (precursor: 11-deoxycortisol), corticosterone (precursor: 11-deoxycorticosterone), 18-hydroxycorticosterone and aldosterone (precursors: 11-deoxycorticosterone and corticosterone) in the adrenals resected from patients treated with o,p'-DDD, when compared with adrenals from untreated patients. In one patient with Cushing's syndrome, corticosteroidogenesis was studied on both adrenals. The first adrenal was removed after treatment with o,p'-DDD for a month (treated adrenal); the second one was removed four months later when the patient relapsed. No medical treatment was given during this period (control adrenal). Steroids synthesis (cortisol, corticosterone, 18-hydroxycorticosterone, aldosterone) was much lower (40–60%) in treated adrenal as compared to control adrenal. These results clearly establish the inhibitory effect of o,p'-DDD on 11-hydroxylase and 18-hydroxylase activities in human adrenals of treated subjects. In contrast, addition of o,p'-DDD (10^{-2} M), o,p'-DDE (10^{-2} M), p,p'-DDMU (10^{-2} M) and p,p'-DDOH (2.1×10^{-3} M) to incubation media had no effect on steroid synthesis.

INTRODUCTION

o,p'-DDD and aminoglutethimide are two inhibitors of adrenocortical steroidogenesis most currently used for the treatment of hypercorticisms. Aminoglutethimide acts very rapidly, sometimes inducing an adrenal insufficiency within 48-h after ingestion [for review see 1, 2, 3]. o,p'-DDD acts more slowly and is mainly used for the treatment of Cushing's syndrome either as therapeutic or to prepare the patient for surgery[4]. Mechanism and levels of action of aminoglutethimide on adrenocortical steroidogenesis *in vitro* are now well-known[1, 5]; in contrast mechanism and site(s) of action for o,p'-DDD are poorly understood.

The inhibitory effect of this drug on the adrenal steroid biosynthesis has been mostly described after *in vivo* administration of the drug to animals[6–9]. Hart and Straw showed in 1971 that o,p'-DDD inhibited the *in vitro* steroidogenic response to ACTH of adrenal slices from dog treated before adrenalectomy with the drug, when compared with control adrenals[10].

In human hypercorticism, the *in vivo* inhibitory effect of o,p'-DDD on adrenal steroidogenesis has been described by many authors in[4] but to our knowledge, there are very few reports about the site(s) of action of this inhibitor on adrenocortical steroid synthesis.

The present paper reports *in vitro* studies on corticosteroidogenesis in human adrenals, obtained at surgery, from patients with the Cushing's syndrome treated for various periods of time with o,p'-DDD prior to surgery.

The results are compared with those obtained in the same conditions with adrenals removed at surgery from untreated patients with Cushing's syndrome and adrenals removed at autopsy from two patients without any endocrine disease.

EXPERIMENTAL

Chemicals

[1, 2-^{3}H]-corticosterone (S.A.: 40–60 Ci/mmol), [1, 2-^{3}H]-deoxycorticosterone (46.8 Ci/mmol), [1, 2-^{3}H]-17α hydroxy-11-deoxycorticosterone (S.A.: 44.1 Ci/mmol), [4-^{14}C]-cortisone (S.A.: 59.8 mCi/mmol), [4-^{14}C]-aldosterone (S.A.: 55.0 mCi/mmol), [4-^{14}C]-corticosterone (S.A.: 59.3 mCi/mmol), [4-^{14}C]-cortisol (S.A.: 55.4 mCi/mmol) were purchased from New England Nuclear Corporation. The radiochemical purity of these labeled steroids was checked by paper chromatography shortly before use.

[4-^{14}C]-18-hydroxycorticosterone was obtained from [4-^{14}C]-deoxycorticosterone by rat adrenal homogenate incubation. The radiochemical purity of the steroid was checked by mixing an aliquot with

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a trace amount of authentic [1, 2-^{3}H]-18-hydroxycorticosterone. After periodic acid oxidation followed by chromic acid oxidation, the derivative isotope ratios remained constant throughout multiple paper chromatographies[5].

[4-^{14}C]-11-dehydrocorticosterone was obtained from the chromic acid oxidation of [4-^{14}C]-corticosterone. The protection of the side chain of corticosterone by acetylation was not necessary[11].

Unlabeled steroids were purchased from Merck and Ikapharm, nicotinamide adenine dinucleotide phosphate ($NADP^+$) and potassium hydroxide from Merck, malic acid from Sigma Corporation and sterile Earle's medium from Institut Pasteur, Paris. The solvents were analytical grade from Merck. PPO (2, 5-diphenyloxazole) and POPOP [1, 2-di (2,5-diphenyloxazole)] were purchased from Merck.

Drugs

o,p'-DDD: 2(o-chlorophenyl)-2 (p-chlorophenyl)-1, 1-dichloroethane. o,p'-DDE: 2(o-chlorophenyl)-2(p-chlorophenyl)-1, 1-dichloroethylene. p,p'-DDMU: 2,2-bis (p-chlorophenyl)-2-chloroethylene. p,p'-DDOH: 2,2-bis (p-chlorophenyl)-ethanol.

o,p'-DDD and o,p'-DDE were generously supplied by Roussel Pharmaceutical Corp., Paris; p,p'-DDOH was a gift of Pr J. E. Sinsheimer (University of Michigan, U.S.A.) and p,p'-DDMU was a gift of Drs G. E. Westlake and P. J. Bunyan (Ministry of Agriculture, Fisheries and Food, England).

Human adrenals

1. *Normal human adrenal glands.* Adrenals were removed from two male subjects (age: 17 and 30 years) deceased in the hospital after a road accident, within one hour after death. The adrenals were immediately transferred, in ice, to the laboratory and frozen at $-20°C$ until processed.

2. *Human adrenal glands from patients with Cushing's syndrome.* Adrenals were resected from patients suffering from Cushing's syndrome whatever its origin: either dysfunction of the hypothalamo-pituitary axis (Cushing's disease) resulting in an adrenocortical hyperplasia, or adrenocortical adenoma, or adrenocortical carcinoma. All the patients were females; most of them were 40–45 years old except two patients (16 and 26 years).

All the patients received, orally, prior to surgery various doses of o,p'-DDD (total dose: 324–2280 g) during various lengths of time (1–12 months). Table 1 displays the scheme of treatment. Treatment was always stopped on the day before surgery.

Two patients (case 6: adrenal hyperplasia and case 7: adrenal cortical carcinoma) were not treated with o,p'-DDD.

One of the patients (case 8) was submitted to bilateral adrenalectomy at 4-months interval. She received o,p'-DDD for one month at a total dose of 324 g just prior to the first adrenalectomy. She was not treated prior to the second surgical operation.

In all 9 adrenals removed from patients with hypercorticism were studied *in vitro*. Three of them were adenomas removed from women treated with o,p'-DDD (cases 1–3). Four other adrenals removed from women treated with o,p'-DDD showed a fasciculata-reticularis hyperplasia (cases 4, 5, 8, 8 bis), three of them showing a micronodular hyperplasia (cases 4, 8, 8 bis). Glands were brought to the laboratory in ice-filled containers and frozen at $-20°C$ until processed.

Table 2 displays the main morphological findings of the adrenals studied.

Experimental procedure

1. *Tissue preparation and incubation.* The adrenal glands were cleared of fatty and connective tissues. The adrenal tissue was weighed, trimmed and homogenized with a Teflon–glass homogenizer in Earle medium buffer [Na^+: 150 mM, K^+: 5.3 mM, Ca^{++}: 1.32 mM, Mg^{++}: 0.79 mM, Cl^-: 125 mM, HCO_3^-: 26 mM, $H_2PO_4^-$: 1 mM, SO_4^{--}: 0.81 mM, glucose: 1 g and phenolsulphonaphtaleine: 0.02 g]. Calcium concentration of the medium was adjusted to 4 mM by adding $CaCl_2$. This improved steroid hydroxylations[12]. The incubations were performed with a 200 mg wet weight of tissue, except where indicated.

Incubation flasks contained exactly measured amounts of radioactive precursors—tritiated deoxycorticosterone, corticosterone or 11-deoxycortisol—solutions which were evaporated to dryness, then taken up in 2.0 ml Earle's solution about one hour just before the incubation time. To facilitate the *in vitro* hydroxylations an NADPH generating system made up of $NADP^+$ (1 mM) and malic acid (5 mM) neutralized with a 0.1 N potassium hydroxide solution (pH: 7.3) was added to each incubation flask[13]. The final total volume of each incubation flask was 10.0 ml.

Aerobic incubations were performed in a Dübnoff metabolic shaking incubator at 37°C for 2 h.

2. *Extraction and isolation of steroids.* After incubation 15.0 ml acetone was added to stop enzymatic reactions by precipitating the proteins. In order to account for procedural losses during subsequent extraction and identification of synthesized steroids, a trace amount of [14-^{14}C]-18-hydroxycorticosterone, [4-^{14}C]-aldosterone, [4-^{14}C]-corticosterone, [4-^{14}C]-11-dehydrocorticosterone, [4-^{14}C]-cortisone, [4-^{14}C]-cortisol was added to the incubation flasks to be used as internal standard according to the tritiated steroid precursor.

Incubation mediums were filtered through a glass cotton layer into a Büchi vessel and the filter washed several times with aqueous acetone. The organic solvent was then removed by vacuum distillation with a Büchi rotavapor at a temperature below 40°C and the final aqueous phase was partitioned first, three times with chloroform (v/v) then again three times with ethyl acetate (v/v). After evaporating the organic extracts to dryness by vacuum distillation, the total

radioactivity recovered in the extracts was evaluated by radioassay.

Radioactivity was measured in a POPOP–PPO toluene scintillation solution with a 3-channel liquid scintillation spectrometer (Tricarb, model 3255, Packard Instrument Company). Isotope contents were expressed as disintegrations per minute (d.p.m.). The results were calculated as percentage conversion of radioactivity added as a substrate. The data were corrected for losses.

The following solvent systems were used for descending paper chromatography (PC) of labelled steroids and conversion products:

PC1: Dichloroethane–ethylene glycol, PC2: Toluene–propylene glycol, PC3: Benzene–formamide, PC4: Methylcyclohexane–toluene–formamide (10:10:1, by vol.), PC5: Benzene–heptane–methanol–water (67:33:80:20, by vol.), PC6: Benzene–methanol–water (10:5:5, by vol.).

Individual metabolites isolated were further run in suitable chromatographic systems in which their isopolarity with carbon-14 internal standards was established. A conversion product was considered pure when constancy of $^{3}H/^{14}C$ ratios was established in successive chromatographic systems.

The dried extracts were first chromatographed in PC1 system for 4 h which allowed the separation either of 18-hydroxycorticosterone and aldosterone (precursors:deoxycorticosterone or corticosterone), or cortisol and cortisone (precursor:11-deoxycortisol). Radioactive steroids located in different areas of the paper chromatograms were then purified and characterized. Throughout the purification and characterization of the steroids, the carbon-14 steroids added as standards behaved exactly as the synthesized steroids. Moreover the isotope ratios of the purified steroids and their conversion products remained stable throughout multiple chromatographies.

18-Hydroxycorticosterone was purified in PC1 system for 17 h, characterized by periodic acid oxidation into 18-hydroxycorticosterone $20 \rightarrow 18\gamma$ lactone the polarity of which was checked in PC3 and PC5 system, then, after chromic acid oxidation into the 11-keto derivative i.e. 18-hydroxy-11-dehydrocorticosterone $20 \rightarrow 18\gamma$ lactone the polarity of which was checked in PC3, PC4 and PC5 systems [5].

Aldosterone was purified in PC2 or PC3 system, respectively for 72 h and 48 h. The steroid was characterized by acetylation with unlabelled acetic anhydride into a compound, the polarity of which in PC3 and PC5 systems was identical to that of the derivative obtained from acetylation of [4-^{14}C]-aldosterone. The 18, 21-diacetate thus obtained was further characterized by chromic acid oxidation into the $11 \rightarrow 18\gamma$ lactone, 21-monoacetate of aldosterone[14] the polarity of which was checked in PC3 and PC5 systems.

Corticosterone and 11-dehydrocorticosterone were not separated in PC1 system and were located in the same area. To separate them, PC2 system for 7 h was used.

Corticosterone was acetylated with unlabelled acetic anhydride into its 21-acetate the polarity of which was checked in PC4 system for 14 h and PC5 for 4 h. Corticosterone 21-acetate was either oxided with chromic acid oxidation into 11-dehydrocorticosterone 21-acetate (polarity checked in PC4 system for 7 h), either hydrolysed one hour with a 2.5% solution of Na_2CO_3 into corticosterone (polarity checked in PC2 system for 14 h). In some experiments corticosterone was first characterized by chromic acid oxidation, then acetylated with unlabelled acetic anhydride.

11-dehydrocorticosterone was characterized by acetylation and the derivative product was characterized as described just before.

Cortisol was purified in PC1 system for 17 h, then characterized by chromic acid oxidation into 11-keto androstenedione, the polarity of which was checked in PC5 and PC6 systems.

Cortisone was purified in PC3 system for 48 h and characterized by chromic acid oxidation into 11-keto androstenedione, the polarity of which was checked in PC5 and PC6 systems.

3. *Drug additions in the medium.* o,p'-DDD and one of its unsaturated metabolites o,p'-DDE were added directly as a powder to the incubation medium (10^{-2} M). Two other drugs were assayed: p,p'-DDOH (2.1×10^{-3} M) and p,p'-DDMU (10^{-2} M). The last two drugs are derived from the metabolism of DDD (dichlorodiphenyldichloroethane).

Abbreviations and trivial names

Aldosterone: 11β, 21-dihydroxy 4-pregnene 3,20-dione 18-al; Corticosterone (compound B): 11β, 21-dihydroxy 4-pregnene 3,20-dione; Cortisol (compound F): 11β, 17α, 21-trihydroxy 4-pregnene 3,20-dione; 11-dehydrocorticosterone (compound A): 21-hydroxy 4-pregnene 3,11, 20-trione; Deoxycorticosterone (DOC): 21-hydroxy 4-pregnene 3,20-dione; 11-deoxycortisol (compound S): 17α, 21-dihydroxy 4-pregnene 3,20-dione; 18-hydroxycorticosterone (18-OHB): 11β, 18, 21-trihydroxy 4-pregnene 3,20-dione;18-hydroxy-11-deoxycorticosterone(18-OH DOC): 18, 21-dihydroxy 4-pregnene 3,20-dione. Adrenosterone: 4-androstene 3,11, 17-trione.

RESULTS

In this work the biosynthesis of cortisol and cortisone (from 11-deoxycortisol), corticosterone, 18-hydroxycorticosterone, aldosterone and 11-dehydrocorticosterone (from deoxycorticosterone or corticosterone) was studied.

Incubations were realized with 2 adrenals from subjects deceased at hospital without any known endocrine disease (control), 2 adrenals from patients with Cushing's syndrome (1 hyperplasia and 1 adrenocortical carcinoma) untreated with o,p'-DDD, 5 adrenals

Table 1. Scheme of the treatment with o,p'-DDD of patients with Cushing's syndrome

Patients	Age	Daily dose (g)	Treatment duration (month)	Total dose (g)
Cushing's syndrome				
Treated patients				
1—Ag.	46	12	3	1140
2—Bo.	42	12	1	360
3—Or.	38	12	3	1180
4—Mi.	16	6	12	2340
5—Le.	45	6	11	2280
Untreated patients				
6—Gr.	41	—	—	—
7—Ma.	26	—	—	—
Patient alternately treated and untreated				
8—Ja. treated	44	12	1	324
8 bis—Ja. untreated	44	—	—	—
Patients without Cushing's syndrome				
9—Di.	17	—	—	—
10—Ti.	30	—	—	—

Patient [8—Ja.] was treated one month just prior to adrenalectomy (left adrenal). The right adrenal was then removed after a 4-month period without any treatment. The adrenals of patients [9—Di.] and [10—Ti.] (human adrenals of control) were removed at autopsy, after a road accident.

removed from patients treated with o,p'-DDD for Cushing's syndrome (3 adenomas and 2 hyperplasias) and 2 adrenals removed from the same patient, the first after treatment with o,p'-DDD and the second one without any treatment.

Tables 1 and 2 show the age of patients, the scheme of treatment and the main morphological findings of the adrenals.

All the data expressed in the tables are the mean of duplicate incubations.

1. *Synthesis of cortisol and cortisone from 11-deoxycortisol as precursor*

Five adrenals removed at surgery from patients with Cushing's syndrome treated with o,p'-DDD showed a low biosynthesis of both cortisol: mean ± 1 S.D. = 1.85% ± 0.90 (1.13–3,36%) and cortisone: mean ± 1 S.D. = 0.60% ± 0.21 (0.39–0.89%). .In contrast adrenals from untreated patients with Cushing's syndrome showed a synthesis of 34.1 and

Table 2. Main morphological findings of the adrenals removed from patients with Cushing's syndrome

Patients	Cushing's type	Localization	Adrenal weight (gm)	Histological changes	Medulla
Cushing's syndrome					
Treated patients					
1—Ag.	Adenoma	Left	18	Spongiocyte cells	N
2—Bo.	Adenoma	Left	22	Spongiocyte cells	N
3—Or.	Adenoma	Left	12	Spongiocyte cells	N
4—Mi.	Hyperplasia	Right	7.50	Moderate hyperplasia	N
5—Le.	Hyperplasia	Left	5	Micronodules	Narrow
Untreated patients					
6—Gr.	Hyperplasia	Left	9.60	1 extracapsular nodule	N
7—Ma.	Carcinoma	Left	700	Hemorrhagic and necrosis foci	
Patient alternately treated and untreated					
8—Ja. treated	Hyperplasia	Left	8	1 extracapsular nodule	N
8 bis—Ja. untreated	Hyperplasia	Right	8	Micronodules	N
Patients without Cushing's syndrome					
9—Di.	—	Left	4	—	—
10—Ti.	—	Left	4.5	—	—

Table 3. *In vitro* biosynthesis of cortisol and cortisone from tritiated 11-deoxycortisol by adrenals removed from patients with Cushing's syndrome with or without presurgical treatment with o,p'-DDD

Adrenals	Per cent of cortisol	Per cent of cortisone
Treated patients		
1—Ag.	1.13	0.58
2—Bo.	1.76	0.53
3—Or.	3.36	0.89
4—Mi.	1.18	0.39
5—Le.	1.81	—
Mean ± 1 S.D.	1.85 ± 0.90	0.60 ± 0.21
Untreated patients		
6—Gr.	34.1	2.52
7—Ma.	64.4	7.33
Control adrenals		
9—Di.	10.7	2.15
10—Ti.	11.7	1.48

Two adrenals were removed from two males without any known endocrine disease, at autopsy, after a road accident. Each flask contained: $NADP^+$ (1 mM), malate (5 mM), and 200 mg homogenized human adrenal tissue except case Ma. (1000 mg) in a total volume of 10.0 ml. Incubation lasted for 2 h at 37°C in air. Data are the mean of duplicate incubations.

64.4% of cortisol, while synthesis of cortisone reached 2.52% and 7.33%.

Cortisol and cortisone synthesis was respectively about 11% and 2% in adrenals from two patients without any endocrine disease.

The biosynthesis of cortisol and cortisone from these nine adrenals are summarized in Table 3.

2. *Synthesis of 18-hydroxycorticosterone, aldosterone and 11-dehydrocorticosterone from corticosterone as a precursor*

Five adrenals from patients with Cushing's syndrome treated with o,p'-DDD showed a very low biosynthesis of 18-hydroxycorticosterone: mean ± 1 S.D. = 0.20% ± 0.03 (0.15 – 0.24%). Aldosterone synthesis was also poor: mean ± 1 S.D. = 0.14% ± 0.03 (0.11–0.18%), while 11-dehydrocorticosterone production was more important (9.5–27.2%) with a mean ± 1 S.D. = 18.4 ± 8.1.

In one "normal" human adrenal a greater synthesis of these three steroids occurred.

Table 4 displays the data obtained from corticosterone as precursor with these adrenals.

Table 4. *In vitro* biosynthesis of 18-hydroxycorticosterone (18-OHB), aldosterone (Aldo) and 11-dehydrocorticosterone (A) from tritiated corticosterone as a precursor by adrenals removed from patients with Cushing's syndrome presurgically treated with o,p'-DDD

Adrenals	Per cent of 18-OHB	Per cent of Aldo	Per cent of A
Treated patients			
1—Ag.	0.19	0.14	27.2
2—Bo.	0.20	0.18	23.0
3—Or.	0.23	0.11	—
4—Mi.	0.15	0.12	13.8
5—Le.	0.24	0.16	9.5
Mean ± 1 S.D.	0.20 ± 0.03	0.14 ± 0.03	18.4 ± 8.1
Control adrenal			
10—Di.	0.98	0.32	35.3

One adrenal was removed, at autopsy, from a male after a road accident. Experimental conditions were identical to those described in Table 3. Incubation lasted two h at 37°C in air. Data are the mean of duplicate incubation.

3. *Synthesis of corticosterone, 18-hydroxycorticosterone, aldosterone and 11-dehydrocorticosterone from 11-deoxycorticosterone as precursor*

Table 5 displays the data obtained from the incubation of the adrenals of two patients with Cushing's syndrome not treated with o,p'-DDD. The biosynthesis of corticosterone with the adrenal of case 7 (untreated adrenocortical carcinoma) was about twice that obtained with the adrenal of case 6 (untreated adrenal hyperplasia). By contrast, there were no marked differences in the biosynthesis of 18-hydroxycorticosterone and aldosterone by both adrenals.

Table 5. *In vitro* biosynthesis of corticosterone (B), 18-hydroxycorticosterone (18-OHB), aldosterone (Aldo) and 11-dehydrocorticosterone (A) from tritiated 11-deoxycorticosterone as a precursor by adrenals removed from untreated patients with Cushing's syndrome

Adrenals	Per cent of B	Per cent of 18-OHB	Per cent of Aldo	Per cent of A
Untreated patients				
6—Gr.	19.9	0.32	0.13	6.52
7—Ma.	45.2	0.31	0.23	3.66

Experimental conditions were identical to those described in Table 3. Incubation lasted two hours at 37°C in air. Data are the mean of duplicate incubation.

4. *Synthesis of cortisol and cortisone (precursor: 11-deoxycortisol), 18-hydroxycorticosterone, aldosterone, corticosterone and 11-dehydrocorticosterone (precursors: corticosterone or deoxycorticosterone)*

Table 6 displays the data obtained with both adrenals from a patient suffering from Cushing's syndrome (adrenocortical hyperplasia). The first adrenal (case 8) was removed after treatment with o,p'-DDD. The second one (case 8 bis) was removed after a four months period, without any treatment; This adrenal can be considered as a control when compared to the first one. The biosynthesis of cortisol (from 11-deoxycortisol), corticosterone (from 11-deoxycorticosterone), 18-hydroxycorticosterone and aldosterone (from corticosterone as from 11-deoxycorticosterone) was markedly higher in the control adrenal, thus establishing an effect of o,p'-DDD on 11-hydroxylase and 18-hydroxylase activities.

Table 6. *In vitro* biosynthesis of cortisol (F), cortisone (E), corticosterone (B), 11-dehydrocorticosterone (A), 18-hydroxy-corticosterone (18-OHB), aldosterone (Aldo) from tritiated 11-deoxycortisol, deoxycorticosterone or corticosterone from both adrenals of the same patient (Cushing's syndrome)

Precursor = S-^{3}H	Per cent of cortisol		Per cent of cortisone	
8—Ja. treated	22.4		2.33	
8 bis—Ja. untreated	64.0		2.79	
Precursor = DOC-^{3}H	Per cent of B	Per cent of A	Per cent of 18-OHB	Per cent of Aldo
8—Ja. treated	26.1	5.53	2.60	—
8 bis—Ja. untreated	62.7	5.52	6.71	1.19
Precursor = B-^{3}H	Per cent of 18-OHB		Per cent of Aldo	
8—Ja. treated	2.81		0.56	
8 bis—untreated	4.66		0.94	

The first adrenal (left) was removed after a treatment period of one month with o,p'-DDD. The right adrenal was removed four months later. There was no treatment during this four month-period. Experimental conditions were identical to those described in Table 3. Incubation lasted 2 h at 37°C in air. Data are the mean of duplicate incubation.

5. *Synthesis of cortisol and cortisone (precursor: 11-deoxycortisol) by a "normal" human adrenal with and without addition of o,p'-DDD, o,p'-DDE and other metabolites of DDD*

Table 7 shows the data obtained from incubation of the left adrenal removed at autopsy from a patient (case 9) after a road accident. Four drugs were investigated: o,p'-DDD (10^{-2} M), o,p'-DDE (10^{-2} M), p,p'-DDMU (10^{-2} M) and p,p'-DDOH (2.1×10^{-3} M); they all were added as powders directly in the incubation flasks. The data obtained showed no differences in biosynthesis of either cortisol or cortisone in the incubation with o,p'-DDD when compared to the control and a slight inhibition of cortisol biosynthesis with o,p'-DDE, DDMU and DDOH.

DISCUSSION

o,p'-DDD, a structural analog of the insecticide DDT [1,1-bis (p-chlorophenyl)-2,2,2-trichloroethane] is a strong inhibitor of adrenocortical steroidogenesis.

Table 7. *In vitro* incubation of a human adrenal removed at autopsy after a road accident (case 9—Di.), with and without addition of o,p'-DDD, o,p'-DDE, p,p'-DDOH and p,p'-DDMU

Addition	Per cent of cortisol	Per cent of cortisone
None	10.7	2.15
o,p'-DDD	10.9	2.17
o,p'-DDE	9.70	2.08
p,p'-DDMU	9.50	1.84
p,p'-DDOH	9.81	2.29

Every drug was added directly in the incubation medium at a concentration of 10^{-2} M, except p,p'-DDOH (2.1×10^{-3} M). Experimental conditions were identical to those obtained in Table 3. Incubation lasted 2 h at 37°C in air. Data are the mean of duplicate incubation.

Several reports have emphasized the action of o,p'-DDD in the treatment of hypercorticisms: adrenocortical carcinoma[16–20] and Cushing's syndrome[21–23].

o,p'-DDD is unanimously recognized as causing a chemical adrenalectomy when the treatment is instituted for a long-time (6 to 12 months, an average).

The exact mechanism by which o,p'-DDD exerts its antiadrenocortical activity is not yet fully understood. The drug does not act by preventing the formation of cyclic-AMP in response to ACTH[24]. Moreover, the step in ACTH-mediated steroidogenesis which is postulated as involving protein synthesis is not affected in adrenal slices of treated dogs as their is a failure of o,p'-DDD to inhibit the incorporation of ^{14}C-labeled amino acids into adrenocortical proteins[10]. o,p'-DDD blocks dibutyryl cyclic AMP-induced steroidogenesis indicating that the drug acts after the generation of cyclic-3',5'-AMP[24].

The goal of our present work is to locate the inhibitory effect of o,p'-DDD on adrenal steroid synthesis pathways. In this purpose adrenals removed from patients suffering from Cushing's syndrome and treated with o,p'-DDD were studied. From the results, the following comments might be made:

(1) Cortisol synthesis was markedly reduced in adrenals from treated patients (conversion rate of 11-deoxycortisol to cortisol: 1.85% ± 0.90). It was obviously impossible to obtain individual control adrenals. However, we were able to study adrenals from two untreated patients with Cushing's syndrome: there was a 30-fold increase in cortisol biosynthesis in one case (Cushing's disease) and a 60-fold increase in the second case (adrenocortical carcinoma). In addition, 11-deoxycortisol conversion rate to cortisol was 11% in "normal" adrenals obtained, post mortem, from two young adult males, age 17 and 30.

A similar decrease in cortisone synthesis was shown in treated patients, relative to the other groups. It is of interest to note that a high *in vitro* cortisone synthesis (7.33%) was obtained with the adrenocortical carcinoma. The ratio of cortisol to cortisone synthesis [F/E] was on the average of 3 in the treated adrenals and higher in "normal" adrenal [F/E = 5] and untreated adrenal [F/E = 13.5].

The data show that after *in vivo* administration of o,p'-DDD an inhibitory effect on 11-β-hydroxylation of 11-deoxycortisol can be demonstrated in human adrenals *in vitro*. This is consistent with the report by Hart *et al.*[25] and Nelson and Woodard[26] of a partial blockade of cortisol synthesis in dogs treated with o,p'-DDD.

(2) Synthesis of aldosterone and 18-hydroxycorticosterone synthesis from corticosterone was five times lower in treated adrenals when compared with normal adrenals. These data demonstrate a very potent effect of o,p'-DDD on adrenocortical 18-hydroxylase activity. This is, to our knowledge, the first report of such an effect, it indicates that o,p'-DDD can act not only on adrenal fasciculata-reticularis zona but also on glomerulosa zona where aldosterone synthesis occurs. These results concur with the report of Bergenstal *et al.*[16] of zona glomerulosa alterations in patients with adrenocortical carcinoma treated with o,p'-DDD.

For the sake of completeness, it is necessary to point out that Temple *et al.*[27] reported in four patients with Cushing's disease, a correction of hypercortisolism after several months of treatment with o,p'-DDD without aldosterone deficiency. In contrast, in a series of 46 subjects aldosterone secretion was low in most treated patients[22, 23]. Our data clearly demonstrate an alteration of aldosterone biosynthetic pathway in o,p'-DDD-treated subjects.

(3) Corticosterone, 18-hydroxycorticosterone and aldosterone synthesis from 11-deoxycorticosterone could only be performed with adrenals removed from two untreated patients with Cushing's syndrome (one hyperplasia and one carcinoma). The high level of corticosterone synthesis in both cases demonstrates an important activity of deoxycorticosterone 11β-hydroxylation *in vitro* and parallel rates of deoxycorticosterone and 11-deoxycortisol hydroxylation (Tables 3 and 5). The synthesis of 18-hydroxycorticosterone was not important in these adrenals, however, it remained higher than in treated adrenals, despite the fact that in the latter case the precursor was corticosterone. It should be mentioned that there was no difference in the synthesis of 18-hydroxycorticosterone regardless of the type of tissues.

(4) In our study, we were able to incubate the two adrenals of the same subjects; one was considered the control of the second. A 44-year-old woman was treated during eight months with o,p'-DDD (total dose: 3240 g) with good results. Four months later, she again showed clinical and biological signs of hypercorticism. She was treated afresh for another 8 months-period (total dose: 3060 g). Similarly, four months later the patient presented the same signs of relapse. Surgical treatment was then considered and a one month preoperative treatment with o,p'-DDD (total dose: 364 g) was instituted. The left adrenal was removed at surgery. Four months after surgery, in the absence of treatment with o,p'-DDD, a third relapse was observed. The right adrenal was then resected. Therefore, one can consider the left adrenal as a treated adrenal and the right as a control or non-treated adrenal.

This original case study permitted us to show the difference on adrenocortical steroid synthesis in treated and untreated adrenal glands.

Cortisol synthesis from 11-deoxycortisol showed approximately a 2/3 decrease in the treated adrenal. Cortisone synthesis was not found to be very different between the two adrenals thus explaining that the ratio F/E was very high in the untreated adrenal.

The synthesis of corticosterone from 11-deoxycorticosterone as a precursor was also decreased in the same proportion. It should be emphasized that deoxycorticosterone 11β-hydroxylation and deoxycortisol 11β-hydroxylation were parellely decreased with o,p'-DDD. Aldosterone and 18-hydroxycorticosterone biosynthesis from corticosterone or deoxycorticosterone as precursors decreased in the order of 40–60% in treated adrenal when compared with untreated adrenal.

The incubation of these adrenals permitted us to demonstrate the inhibitory effect of o,p'-DDD on 11β-hydroxylase and 18-hydroxylase activities on human adrenal glands. To our knowledge these experimentally established results have not been reported.

(5) Our previous work showed the inefficacy of o,p'-DDD on sheep adrenal steroid synthesis when added in the incubation medium either directly or after solubilization in organic solvents. o,p'-DDD was only active at very high concentration, beginning with 10^{-2} M[28].

In this work we looked for the effect of o,p'-DDD (10^{-2} M) and one of its unsaturated metabolites, o,p'-DDE (10^{-2} M) *in vitro*, on the deoxycortisol 11-hydroxylation using "normal" human adrenal gland. The results did not show any apparent difference in the synthesis of cortisol with respect to the control. Furthermore, when two metabolites of DDD i.e p,p'-DDMU and p,p'-DDOH were added to the incubation media as powders there was no important changes in the synthesis of cortisol.

In conclusion, this work clearly demonstrates the inhibitory effect of o,p'-DDD on the 11β-hydroxylase and 18-hydroxylase activities in human adrenals.

The possibility of investigating both adrenals from the same patient before and after treatment with o,p'-DDD allowed us to locate adrenal sites of action of the drug. This drug did not prove to be active when added directly to the incubation flasks *in vitro*.

However, the fact that it noticeably decreased the steroid synthesis in adrenals removed from subjects that underwent treatment suggest that o,p'-DDD is not directly active but could be active through its peripheral metabolism. This o,p'-DDD peripheral metabolite does not appear to be o,p'-DDE as we showed that this compound did not interfere in corticosteroidogenesis *in vitro*.

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