

MULTIPLE STEROID HORMONE LEVELS IN DEPRESSED PATIENTS AND NORMAL CONTROLS BEFORE AND AFTER EXOGENOUS ACTH

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SUMMARY

Forty depressed patients and 36 age- and sex-matched controls were given 250 µg ACTH_{1–24} by IV bolus. Plasma steroid hormone levels were measured prior to and 60 min after ACTH administration. The depressed patients had significantly greater cortisol (F), 11-deoxycortisol (S), androstenedione (AD), and 17α-hydroxyprogesterone (17α-OHP) responses (delta; $p < 0.05$) and a marginally greater 11β-hydroxyandrostenedione (11β-OHAD) response (delta; $p = 0.091$) than the controls. There was no significant difference in the corticosterone (B) response between the two groups.

With the exception of 11β-OHAD, all the steroid hormones were significantly negatively correlated with age in the controls, but only S and AD marginally demonstrated this relationship in the depressed patients. F, S, AD, 17α-OHP, and B, but not 11β-OHAD, were significantly positively correlated with each other in the controls, but only F was significantly correlated with AD in the depressed patients. These data suggest that the hypercortisolemia found in some depressed patients involves increased precursor and metabolite levels both at baseline and in response to exogenous ACTH, compared to controls. Furthermore, variability in these precursors is greater in depressed patients, and their relationship to age is lost. These findings are consistent with the hypothesis that adrenal products other than cortisol also could be related to affective symptoms.

INTRODUCTION

REVIEW OF THE LITERATURE reveals a substantial amount of evidence of hypercortisolism in some populations of depressed patients (Stokes, 1987; Arana & Mossman, 1988). While much of the research on hypercortisolemia in depressed patients has centered on the limbic and hypothalamic systems, few studies have investigated adrenal gland activity, despite the fact that reversal of corticosteroid production by adrenal resection or ablation in patients with Cushing's disease is an effective means of treating the high incidence of depression in this population (Kathol, 1985); i.e., there appears to be something in adrenal output that effects emotion.

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The adrenocorticotropin (ACTH) stimulation test has been used to investigate peripheral abnormalities of the hypothalamo-pituitary-adrenal axis by assessing adrenal cortex responsiveness. Some investigators have reported a higher cortisol response to exogenous ACTH in actively depressed patients compared to normal controls (Amsterdam *et al.*, 1983; 1986; Jaeckle *et al.*, 1987) or to those who have recovered from their depression (Endo *et al.*, 1974; Amsterdam *et al.*, 1987a; Fang *et al.*, 1988a). Others (Carpenter & Bunney, 1971; Sclare & Grant, 1972) have not observed this difference.

When using the ACTH stimulation test to study adrenal responsiveness in depressive illness, most investigators have concentrated only on the adrenal steroid, cortisol. The secretion of the cortisol precursors progesterone (Strott *et al.*, 1969), 17α -hydroxyprogesterone (Strott *et al.*, 1969), and 11-deoxycortisol (Liddle *et al.*, 1962), as well as corticosterone (Peterson & Pierce, 1960) its precursor, deoxycorticosterone (Crane & Harris, 1966; Harris *et al.*, 1967; Biglieri *et al.*, 1969;), and some adrenal androgens, dehydroepiandrosterone (Vaitukaitis *et al.*, 1969), androstenedione (Rivarola *et al.*, 1966) and 11β -hydroxyandrostenedione (Lejeune-Lenain *et al.*, 1980), also are stimulated by ACTH in man. These steroid hormones, however, have received little or no attention in assessments of adrenal responsiveness to endogenous or exogenous ACTH in depressed patients.

Since biologic activity is largely determined by the chemical structure and stereotaxis of steroid molecules, precursors and metabolites of cortisol could play a significant role in producing changes in CNS activity, i.e., in influencing the development of affective symptoms. It is known, for instance, that cortisol can adversely affect neuronal function (Sapolsky, 1985) and that the receptors which mediate glucocorticoid effects are sensitive to the structure of the compound (McEwen *et al.*, 1986). Studies on rats have identified two distinct classes of corticosteroid receptors in the brain which resemble corticosteroid receptors found in the kidney (type I) and the liver (type II) (reviewed in McEwen *et al.*, 1986; 1988; De Kloet *et al.*, 1987; De Kloet & Reul, 1987). The type I receptor is primarily localized in the hippocampus and has a high affinity for aldosterone and the major glucocorticoids (corticosterone in the rat and cortisol in other species) (McEwen *et al.*, 1986; 1988; De Kloet *et al.*, 1987; De Kloet & Reul, 1987). The type II receptor has a lower affinity for glucocorticoids and does not bind mineralocorticoid steroids (McEwen *et al.*, 1986; 1988; De Kloet *et al.*, 1987; De Kloet & Reul, 1987). This receptor is more widely distributed, occurring not only in the hippocampus but also in the lateral septum, central amygdala, supraoptic nucleus, paraventricular nucleus, and nucleus tractus solitarii (McEwen *et al.*, 1986; 1988; De Kloet *et al.*, 1987; De Kloet & Reul, 1987). Because the type II receptors have lower glucocorticoid affinity it is thought that these receptors are only occupied during the corticosteroid stress response, while the type I receptors are occupied during the basal state (McEwen *et al.*, 1986; 1988; De Kloet *et al.*, 1987; De Kloet & Reul, 1987). In turn, because of the widespread distribution of the type II receptors, the regulation of the CNS response to stress is, at least in part, likely affected by high levels of corticosteroids. It is possible that steroid compounds other than cortisol, even when produced in much smaller amounts, could be more closely related to the production of depressive symptoms than would be cortisol.

In this study we investigated the basal hormonal state and the responses of cortisol, 11-deoxycortisol, corticosterone, 17α -hydroxyprogesterone, androstenedione, and 11β -hydroxyandrostenedione to exogenous ACTH₁₋₂₄ administration in patients with major depression and in normal controls.

SUBJECTS AND METHODS

Subjects

Sixty-four patients were recruited from the inpatient psychiatric service at the University of Iowa Hospitals and Clinics (UIHC). The patients were screened by a research assistant with the National Institute of Mental Health's Diagnostic Interview Schedule (DIS) (Robins *et al.*, 1981) ($n=39$) or by clinical interview by a psychiatrist ($n=25$), and all diagnoses were confirmed by consensus among the investigators. Of the 64 patients recruited, 50 met DSM-III (American Psychiatric Association, 1980) criteria for major depressive disorder (MDD). Ten of these 50 patients were excluded for the following reasons: one patient also met criteria for anti-social personality disorder, three were receiving carbamazepine, three were taking oral contraceptives, one was using hydrocortisone cream, one was receiving estrogen replacement, and one had recently received ECT. Therefore, 40 patients with MDD (13 male and 27 female) were studied before and after the administration of exogenous ACTH₁₋₂₄. The mean age (\pm SD) was 35.0 ± 14.3 yr (range 16–70 yr) (men: 32.9 ± 14.1 yr; women: 36.0 ± 14.6 yr). Only one patient was not receiving a psychotropic medication. The medications and doses were unchanged from admission and throughout the testing, to avoid the effects of drug withdrawal (Devanand *et al.*, 1984; Dilsaver & Greden, 1984; Krause *et al.*, 1987). The 17-item Hamilton Rating Scale for Depression (HRSD) (Hamilton, 1960) and the Carroll Rating Scale for Depression (CRSD) (Carroll *et al.*, 1981) were administered to 36 patients. All patients gave informed written consent prior to entering the study.

Thirty-six age- and sex-matched healthy control subjects were identified by advertisement or contacted from a list of research participants obtained from the Clinical Research Center at the UIHC. There were 15 men and 21 women; their average age was 35.8 ± 12.6 yr (range 21–62 yr) (men: 32.9 ± 11.9 yr; women: 38.0 ± 12.9 yr). All were free of psychiatric symptoms, were on no medications at the time of the study, and gave informed written consent. Twenty-eight completed an HRSD, and 32 completed a CRSD. All 36 control subjects were studied before and after administration of ACTH₁₋₂₄.

Procedures

Each participant received 250 μ g ACTH₁₋₂₄ by IV bolus between 1300h and 1700h to take advantage of the relatively steady basal afternoon state. An IV butterfly catheter was routinely placed 30 min before testing began. Blood samples for steroid analysis were drawn before and 60 min after the administration of ACTH₁₋₂₄ into chilled tubes containing EDTA. All specimens were centrifuged and the plasma removed and frozen at -20° C within 1 hr of collection. Some of the subjects (35 patients and 30 controls) also completed a dexamethasone suppression test (DST) by taking orally 1 mg of dexamethasone at 2330h. The DST was performed at least 48 hr after the ACTH test. Blood samples for post-DST cortisol levels were drawn at 0800h and 1600h the following day. DST nonsuppressors were identified as those with post-DST cortisol levels > 5 μ g/dl at either 0800h or 1600h.

Hormone Assays

Cortisol (F) was measured in duplicate unextracted plasma samples by a standard ¹²⁵I double-antibody radioimmunoassay (RIA) developed at the Medical Research Facility at the UIHC as described previously (Jaekle *et al.*, 1987). Intra-assay coefficients of variation were 8.3%, 6.6% and 5.1%, and interassay coefficients of variation were 14.6%, 13.4% and 16.7%, for cortisol levels of 2.7 μ g/dl, 11.7 μ g/dl and 23.6 μ g/dl, respectively.

11-deoxycortisol (S), 17 α -hydroxyprogesterone (17 α -OHP), androstenedione (AD), and corticosterone (B) were measured by RIA kits purchased from ICN Biomedicals, Los Angeles, CA (formerly Radioassay Systems Laboratories, Carson, CA). 11 β -hydroxyandrostenedione (11 β -OHAD) was measured by RIA kits purchased from Inter Sci Diagnostics, Los Angeles, CA.

S and 17 α -OHP levels were measured in duplicate unextracted plasma samples. Both assays used ¹²⁵I-labelled steroids as the ligand and goat anti-rabbit γ -globulin to separate the bound from the unbound steroid. The S intra-assay coefficients of variation were 7.4% and 3.3%, and the interassay coefficients of variation were 4.1% and 14.1%, for levels of 0.21 μ g/dl and 2.41 μ g/dl, respectively. Cross-reactivity with 17 α -OHP was 3.9%, deoxycorticosterone 0.22%, progesterone 0.16%, and cortisol 0.09%. All other cross-reactivities were $< 0.03\%$. 17 α -OHP intra-assay coefficients of variation were 0.0%, 4.9% and 3.3%, and interassay coefficients of variation were 18.2%, 11.2% and 8.9%, for levels of 0.11 ng/ml, 0.73 ng/ml and 3.99 ng/ml,

respectively. The 17 α -OHP antiserum cross-reacted 0.58% with 17 α -hydroxypregnenolone, 0.18% with progesterone, 0.06% with deoxycorticosterone, and 1.48% with 11-deoxycortisol. All other related steroid cross-reactivities were <0.02%.

Samples for AD levels were extracted with ethyl acetate:hexane (3:2), as per the manufacturer's procedure, prior to RIA. The radioligand was ¹²⁵I-labelled AD, and the bound and unbound steroid was separated by goat anti-rabbit γ -globulin. The intra-assay coefficients of variation were 8.1%, 8.3% and 7.5%, and the interassay coefficients of variation were 12.6%, 7.4% and 9.7%, for levels of 0.69 ng/ml, 2.20 ng/ml and 6.14 ng/ml, respectively. The AD antiserum cross-reacted 1.79% with estrone, 0.64% with testosterone, 0.07% with progesterone, 4.40% with DHEA-S, and 3.50% with DHEA. All other related steroid cross-reactivities were <0.02%.

The 11 β -OHAD RIA also required prior extraction with ethyl acetate:hexane (1:1). The radioligand was ³H-labelled 11 β -OHAD, and the bound and unbound steroid were separated with saturated ammonium sulfate. The intra-assay coefficients of variation were 6.1% and 6.3%, and the interassay coefficients of variation were 7.2% and 8.2%, for levels of 115 and 393 ng/dl respectively (few 11 β -OHAD assays were run; therefore, this information is from Inter Sci Diagnostics). The antiserum is very specific for 11 β -OHAD, with little cross-reactivity with androstenedione (2.64%) and adrenosterone (0.12%). All other related steroids cross-reacted <0.01%.

B determinations required sample extraction and purification prior to the RIA. To 2 ml plasma (pipetted into 40 ml glass centrifuge tubes) was added approximately 500 cpm of ³H-corticosterone, 200 ng d-aldosterone-21-acetate (internal standard), and 2 ml double-distilled H₂O. Following a 30-min incubation period at room temperature, the samples were extracted with 10 ml methylene chloride, gently shaken, and centrifuged at 1500 rpm for 20 min. The organic phase was removed and placed in separate clean glass centrifuge tubes. This was repeated two more times, combining all the organic phase for each sample (approximately 30 ml). The extract then was washed four times with 5 ml 0.1 N NaOH, with removal and discard of the NaOH after each wash. After the final wash, the extract was drawn into a glass syringe, filtered through a 0.22 μ m durapore filtering system (Millipore, Bedford, MA), and blown down to dryness under nitrogen in a 37°C water bath. The sides of the glass tubes then were rinsed with 0.5 ml of ethanol, vortexed, the ethanol transferred to a 1 ml glass test tube, and the tube tightly sealed with parafilm and frozen at -20°C until the purification step.

Purification was done by high-pressure liquid chromatography (HPLC). The HPLC system consisted of a Shimadzu Model LC-4A liquid chromatograph (Shimadzu, Columbia, MD); DuPont UV spectrophotometer detector (DuPont, Wilmington, DE); DuPont Model 860 column compartment; Shimadzu C-RIA Chromatopac integrator; and Pharmacia Model FRAC-100 fraction collector (Pharmacia, Piscataway, NJ). Once the HPLC system was equilibrated, the samples were evaporated to less than 50 μ l, drawn up into a Hamilton syringe, and shot into the HPLC system with a Rheodyne injector. The sample was applied to a 50 mm C-18 guard column (Jones Chromatography, Columbus, OH) and then a 15 cm, 5 μ Apex ODS column (Jones Chromatography) at 45°C with a flow rate of 2 ml/min, resulting in a pressure of about 140 kg/cm². The detector was set at 254 nm with an absorbance range of 0.01 A. The eluent system was methanol:acetonitrile:water (MeOH:ACN:H₂O). The water was double-distilled and deionized, and the methanol and acetonitrile were HPLC grade. All reagents were filtered through a 0.5 μ m filter and degassed with helium for about 20 min prior to use. The eluent gradient began at 38:1:61 (MeOH:ACN:H₂O) and went to 55.5:1:43.5 by 20.5 min, and then the column was washed with 38:55:7 eluent for 5 min and equilibrated again at 38:1:61 for 20 min. The retention time for B under these conditions was about 14 min. The B fraction was collected in 16 \times 125 mm glass tubes via the FRAC-100, and the tubes sealed and frozen at -20°C until all samples were purified.

The samples then were lyophilized in a Labconco Model 75040 Freeze Dryer 8 (Labconco, Kansas City, MO), reconstituted with 2.5 ml assay buffer, and incubated at room temperature for a minimum of 30 min prior to RIA. From each reconstituted sample a recovery % was determined and used in calculating the final sample concentration. The average sample recovery (\pm SD) was 50.9% \pm 14.1%. The radioligand was ³H-corticosterone, and the bound steroid was separated from the unbound by charcoal-dextran. The intra-assay coefficients of variation were 20.7%, 9.2% and 7.2%, and the interassay coefficients of variation were 6.9%, 8.7% and 8.6%, for corticosterone levels of 2.89 ng/ml, 6.57 ng/ml and 29.7 ng/ml, respectively. The antiserum cross-reacted 100% with cortisol, 4.5% with progesterone, 1.7% with 17 α -hydroxyprogesterone, 2.5% with 20 α -dihydroprogesterone, 4.5% with deoxycorticosterone, 3.0% with 11-deoxycortisol, 3.0% with aldosterone, 1.3% with androstenedione, 3.1% with testosterone, and 1.1% with dihydrotestosterone. All other related steroid cross-reactivities were <0.01%.

Statistical Methods

Data are reported as mean \pm SD. Steroid hormone responses to exogenous ACTH within each group were analyzed with paired *t*-tests. Comparisons between the MDD and control groups were done with the Student's unpaired *t*-test. When variances between groups were grossly unequal, approximation for the degrees of freedom was determined according to Satterthwaite's formula (Satterthwaite, 1946). For comparisons of three groups (i.e., comparisons made based on DST status), one-way analysis of variance (ANOVA) was used, and when appropriate a Tukey's *post hoc* test was used to identify between-group differences. Pearson's correlation coefficient was used to estimate the fit between two parameters. Analysis results were considered statistically significant when the confidence level was ≤ 0.05 .

RESULTS

The symptom rating scale scores and comparisons for the MDD patients and controls and for subgroups of the MDD patients are presented in Table I. Ten of the 35 patients (29%) completing the DST were DST nonsuppressors (DST-NS) and 25 were suppressors (DST-S). Two of the 30 controls (7%) were DST-NS.

All steroid hormones demonstrated a marked increase ($p < 0.0006$) following the IV bolus 250 μ g ACTH₁₋₂₄ in both the MDD patients and the controls. The time of day the ACTH stimulation test was performed had no significant affect on the basal levels or the responses of the measured steroid hormones. Table II shows the mean steroid levels at baseline, 60 min after ACTH infusion, and the response (delta) to ACTH infusion for the controls and patients with MDD. The MDD patients had significantly higher baseline F ($t = 2.96$; $df = 74$; $p = 0.004$) and S ($t = 2.63$; $df = 59.8$; $p = 0.011$) than the controls and showed a trend towards higher AD baseline levels ($t = 1.70$; $df = 69$; $p = 0.095$). Sixty minutes following the ACTH infusion the MDD

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TABLE I. MEAN \pm SD HAMILTON RATING SCALE FOR DEPRESSION (HRSD) AND CARROLL RATING SCALE FOR DEPRESSION (CRSD) SCORES AND COMPARISONS FOR THE CONTROLS AND PATIENTS WITH MDD AND SUBGROUPS OF PATIENTS WITH MDD

Group	Total N	HRSD	CRSD
Controls	36	2.4 \pm 1.3 ^a (28)	2.2 \pm 2.7 ^b (32)
MDD	40	17.6 \pm 6.5 (36)	27.0 \pm 10.9 (36)
DST-NS	10	16.8 \pm 4.7 (8)	26.0 \pm 13.6 (9)
DST-S	25	16.9 \pm 7.3 (23)	26.7 \pm 10.5 (22)
Bipolar	7	22.4 \pm 8.1 ^c (7)	32.3 \pm 8.4 (7)
Unipolar	33	16.4 \pm 5.6 (29)	25.8 \pm 11.2 (29)
Psychotic	17	19.6 \pm 17.2 (16)	30.2 \pm 10 (15)
Nonpsychotic	23	15.9 \pm 5.5 (20)	25.8 \pm 11.3 (21)
Melancholic	15	20.3 \pm 7.1 (14)	32.0 \pm 8.6 ^d (14)
Nonmelancholic	8	15.3 \pm 3.3 (7)	19.7 \pm 12.4 (7)
Undetermined	17		

Number of observations for each measure is in parentheses.

^a controls vs. MDD: $t = 12.7$; $df = 38.4$; $p = 0.0001$

^b controls vs. MDD: $t = 13.2$; $df = 39.7$; $p = 0.0001$

^c bipolar vs. unipolar: $t = 2.36$; $df = 34$; $p = 0.024$

^d melancholic vs. nonmelancholic: $t = 2.66$; $df = 19$; $p = 0.016$

TABLE II. MEAN \pm SD STEROID LEVELS AT BASELINE (0) AND 60 MIN POST-250 μ G ACTH (60) AND THE CHANGE IN LEVELS (Δ), IN THE CONTROLS AND PATIENTS WITH MDD

		Controls	MDD	<i>p</i>
S [μ g/dl]		(35)	(39)	
	0	.24 \pm .12	34 \pm .22	0.011
	60	.49 \pm .15	.68 \pm .31	0.001
	Δ	.25 \pm .13	.33 \pm .21	0.04
F [μ g/dl]		(36)	(40)	
	0	10.5 \pm 4.1	13.6 \pm 5.0	0.004
	60	24.5 \pm 6.0	33.7 \pm 12.6	0.0001
	Δ	13.9 \pm 5.0	20.1 \pm 11.7	0.004
AD [ng/ml]		(34)	(37)	
	0	1.45 \pm .67	1.75 \pm .82	0.095
	60	2.48 \pm 1.06	3.27 \pm 1.28	0.006
	Δ	1.02 \pm .69	1.51 \pm .92	0.014
17 α -OHP [ng/ml]		(32)	(24)	
	0	.93 \pm .63	1.10 \pm .62	0.315
	60	2.29 \pm 1.08	3.65 \pm 1.94	0.021
	Δ	1.66 \pm .92	2.55 \pm 1.84	0.036
B [ng/ml]		(28)	(25)	
	0	4.33 \pm 4.38	5.20 \pm 3.89	0.452
	60	31.8 \pm 15.1	32.3 \pm 8.96	0.89
	Δ	27.5 \pm 15.1	27.1 \pm 8.11	0.908
11 β -OHAD [ng/dl]		(9)	(17)	
	0	159.7 \pm 59.8	170.8 \pm 85.5	0.733
	60	253.0 \pm 83.0	317.9 \pm 120.0	0.161
	Δ	87.4 \pm 47.9	147.2 \pm 95.1	0.091

The number measured for each group is in parentheses under the respective columns.

TABLE III. MEAN \pm SD STEROID RESPONSE (DELTA) TO 250 μ G ACTH IN CONTROLS WHO WERE DEXAMETHASONE SUPPRESSORS (CONTROLS) AND PATIENTS WITH MDD WHO ARE SUPPRESSORS (DST-S) AND NONSUPPRESSORS (DST-NS)

	Controls	MDD	
		DST-S	DST-NS
S [mg/dl]	.26 \pm .14 (28)	.31 \pm .20 (25)	.35 \pm .27 (9)
F [μ g/dl]*	14.8 \pm 4.9 ^a (28)	17.5 \pm 6.1 ^a (25)	26.4 \pm 20.5 ^b (10)
AD [ng/ml]	1.12 \pm .71 (28)	1.36 \pm .97 (24)	1.83 \pm .80 (9)
17 α -OHP [ng/ml]	1.72 \pm .98 (26)	2.51 \pm 1.93 (17)	2.94 \pm 2.40 (7)
B [ng/ml]	27.3 \pm 15.9 (25)	25.0 \pm 6.9 (15)	34.1 \pm 7.6 (6)
11 β -OHAD [ng/dl]	87.2 \pm 51.2 (8)	145.8 \pm 108.5 (10)	166.8 \pm 89.8 (5)

Difference among groups with different letters: **p* < 0.001 (Tukey's *p* < 0.05).

The number measured for each group is in parentheses.

TABLE IV. MEAN \pm SD STEROID RESPONSE (Δ) TO 250 μ g ACTH IN THE PATIENTS WITH MDD GROUPED BY MEDICATION AND THE MOST COMMON DRUG COMBINATION TREATMENTS WITH FIVE OR MORE OBSERVATIONS

Medication	S [μ g/dl]	F [μ g/dl]	AD [ng/ml]	17 α -OHP [ng/ml]	B [ng/ml]	11 β -OHAD [ng/dl]
TCA						
Off	.29 \pm .18 (10)	26.1 \pm 19.9 (11)	1.72 \pm .94 (10)	2.55 \pm 1.90 (7)	25.3 \pm 8.9 (6)	162.8 \pm 95.8 (7)
On	.35 \pm .22 (29)	17.8 \pm 5.5 (29)	1.44 \pm .92 (27)	2.54 \pm 1.87 (17)	27.7 \pm 8.0 (19)	136.2 \pm 98.1 (10)
LI						
Off	.33 \pm .21 (25)	20.7 \pm 12.5 (25)	1.47 \pm .81 (24)	2.72 \pm 1.90 (17)	25.8 \pm 7.9 (17)	123.4 \pm 79.9 (10)
On	.35 \pm .22 (14)	19.1 \pm 10.7 (15)	1.60 \pm 1.13 (13)	2.16 \pm 1.73 (7)	29.9 \pm 8.4 (8)	181.1 \pm 110.7 (7)
BNZ						
Off	.33 \pm .23 (26)	22.1 \pm 13.4 (26)	1.43 \pm .86 (24)	2.90 \pm 2.14 (15)	28.6 \pm 7.5 (14)	143.8 \pm 102.8 (10)
On	.34 \pm .18 (13)	16.4 \pm 6.7 (14)	1.68 \pm 1.05 (13)	1.98 \pm 1.05 (9)	25.2 \pm 8.8 (11)	152.1 \pm 90.6 (7)
AP						
Off	.35 \pm .23 (27)	19.9 \pm 13.5 (28)	1.36 \pm .86 (26)	2.83 \pm 2.09 (16)	28.3 \pm 9.1 (16)	138.5 \pm 87.4 (11)
On	.31 \pm .17 (12)	20.5 \pm 6.5 (12)	1.88 \pm 1.01 (11)	2.01 \pm 1.12 (8)	24.9 \pm 5.7 (9)	163.0 \pm 114.9 (6)
TCA + LI	.37 \pm .24 (9)	16.0 \pm 5.7 (9)	1.48 \pm 1.15 (8)	(n<5)	34.9 \pm 4.0 (5)	(n<5)
TCA + BNZ	.34 \pm .19 (8)	15.8 \pm 5.2 (8)	1.53 \pm .89 (8)	1.81 \pm .60 (5)	25.8 \pm 8.0 (7)	(n<5)
TCA + AP	.27 \pm .18 (7)	18.7 \pm 7.4 (7)	1.84 \pm .99 (6)	(n<5)	26.2 \pm 5.2 (5)	(n<5)
LI + BNZ	.33 \pm .18 (7)	18.9 \pm 4.9 (7)	1.88 \pm 1.19 (6)	(n<5)	(n<5)	(n<5)
LI + AP	.38 \pm .15 (6)	15.2 \pm 8.0 (7)	1.87 \pm 1.19 (6)	(n<5)	(n<5)	(n<5)

Abbreviations: TCA=tricyclic antidepressants; LI=lithium; BNZ=benzodiazepines; AP=antipsychotics. Number of observations in parentheses.

TABLE V. PEARSON'S CORRELATION OF AGE WITH MEASURED STEROIDS AT BASELINE

	n	r	p
Controls			
S	35	-.586	0.0002
F	36	-.410	0.013
AD	34	-.650	0.0001
17 α -OHP	32	-.554	0.001
B	28	-.522	0.004
11 β -OHAD	9	+.309	0.419
MDD			
S	39	-.385	0.016
F	40	-.123	0.450
AD	37	-.409	0.012
17 α -OHP	24	-.369	0.076
B	25	-.205	0.326
11 β -OHAD	17	-.055	0.834

TABLE VI. CORRELATION OF STEROID LEVELS WITH EACH OTHER/
P VALUE FOR CORRELATION COEFFICIENT AT BASELINE

	F	AD	17 α -OHP	B	11 β -OHAD
Controls					
S	.586/.0002 (35)	.668/.0001 (34)	.726/.0001 (32)	.534/.0034 (28)	.211/.587 (9)
F		.405/.017 (34)	.521/.0023 (32)	.683/.0001 (28)	.651/.056 (9)
AD			.695/.0001 (31)	.435/.021 (28)	.258/.502 (9)
17 α -OHP				.611/.0009 (26)	.343/.366 (9)
B					.325/.432 (8)
MDD					
S	.021/.901 (39)	.171/.312 (37)	.084/.697 (24)	.228/.272 (25)	-.285/.268 (17)
F		.343/.038 (37)	-.047/.826 (24)	.368/.070 (25)	.073/.781 (17)
AD			.161/.453 (24)	.311/.130 (25)	.350/.168 (17)
17 α -OHP				.196/.393 (21)	-.0092/.973 (16)
B					.407/.132 (15)

Number of observations is in parentheses.

patients had significantly higher F ($t=4.15$; $df=57.0$; $p=0.0001$), S ($t=3.41$; $df=56.2$; $p=0.001$), AD ($t=2.84$; $df=69$; $p=0.006$), and 17 α -OHP ($t=2.43$; $df=33.7$; $p=0.021$) levels than the controls. The MDD patients had a significantly greater response (delta) to exogenous ACTH for F ($t=3.02$; $df=53.8$; $p=0.004$), S ($t=2.10$; $df=63.3$; $p=0.040$), AD ($t=2.53$; $df=69$; $p=0.014$), and 17 α -OHP ($t=2.19$; $df=31.6$; $p=0.036$) than the controls and had a trend toward a greater 11 β -OHAD response ($t=1.76$; $df=24$; $p=0.091$) (Table II & Fig. 1).

When the MDD patients were subcategorized by DST suppression status and compared to only the controls who suppressed normally following dexamethasone, the patients with MDD who were DST-NS had the greatest F response ($F=5.65$; $df=2,60$; $p=0.0057$). In general, the MDD DST-NS patients had the greatest response for all the steroids, followed by the MDD DST-S and the controls except for B where the MDD DST-S had the lowest response (Table III). MDD patients with psychosis had a significantly greater 11 β -OHAD response ($n=6$: 233.8 ± 57.9 ng/dl) than those with nonpsychotic MDD ($n=11$: 99.9 ± 76.4 ng/dl) ($t=3.72$; $df=15$; $p=0.002$). No other significant differences based on the presence of psychosis were noted. No significant hormone differences were observed between patients with

unipolar and bipolar MDD or in those with melancholic versus those without melancholic depression.

The most common treatment medication in the MDD patients was tricyclic antidepressants (29). Other treatment drugs were lithium (15), benzodiazepines (14), antipsychotics (12), and monoamine oxidase inhibitors (4), or a combination of psychotropic medications. The effect that these medications may have on the steroid hormone response to exogenous ACTH is presented in Table IV.

All compounds measured, with the exception of 11β -OHAD, decreased with age in the control subjects; only S and AD demonstrated a similar, but not nearly as robust, relationship in the MDD patients (Table V). Except for 11β -OHAD, all of the steroid hormones were significantly positively correlated with each other at baseline in the controls (Table VI). 11β -OHAD also demonstrated a positive correlation with the other steroids but was measured in fewer subjects and did not reach significance. This relationship is reflected in the MDD patients only between AD and F. Sixty minutes following the infusion of ACTH₁₋₂₄, only S and AD ($r=.438$; $p=0.01$), S and 17α -OHP ($r=.484$; $p=0.005$) and AD and 17α -OHP ($r=.397$; $p=0.027$) remained significantly correlated in the controls. None of the compounds were significantly correlated with each other in the MDD patients 60 min after ACTH₁₋₂₄.

Only the response of S to exogenous ACTH was significantly correlated with the duration of illness in the MDD patients ($r=-.392$; $p=0.014$). Likewise, the severity of symptoms was significantly positively correlated only with the response of 11β -OHAD to ACTH (HRSD: $r=.550$; $p=0.034$; CRSD: $r=.482$; $p=0.059$).

DISCUSSION

The ACTH stimulation test has been used previously in assessing adrenocortical responsiveness in depressed patients (Holsboer *et al.*, 1984), in depressed patients retested following treatment or recovery from their illness (Sclare & Grant, 1972; Endo *et al.*, 1974; Gerken & Holsboer, 1986; Amsterdam *et al.*, 1987a), and in normal controls compared to depressed patients (Carpenter & Bunney, 1971; Amsterdam *et al.*, 1983; 1986; Jaeckle *et al.*, 1987; Fang *et al.*, 1988a). With the exception of a few studies, however, investigators have limited their studies to the cortisol (F) response. In the few studies that have measured steroid hormones other than F (Holsboer *et al.*, 1984; Gerken & Holsboer, 1986), normal controls were not included. In this study we measured F, 11-deoxycortisol (S), corticosterone (B), 17α -hydroxyprogesterone (17α -OHP), androstenedione (AD), and 11β -hydroxyandrostenedione (11β -OHAD) to further address adrenal responsiveness following acute ACTH stimulation and to investigate its effect on the biosynthesis of adrenal steroids in depressed patients. Where these hormones occur in the production and metabolism of corticosteroids is summarized in Fig. 2.

We report augmented responses of F, S, AD, 17α -OHP, and 11β -OHAD, but not of B, to acute stimulation with a supraphysiological dose of ACTH in a group of patients with MDD compared to normal controls. Since some of the subjects in this study were also included in a study by Jaeckle *et al.* (1987) and the methods were the same, the F response is similar to that which Jaeckle *et al.* reported. The increased F response in the depressed patients is also in agreement with the findings of Amsterdam *et al.* (1983) who also used a dose of 250 μ g ACTH but administered it in the morning. However, others have not observed this difference (Carpenter & Bunney, 1971; Fang *et al.*, 1988a). This difference is most likely due to differences in the methods used. Carpenter and Bunney (1971) infused the ACTH continuously over a 4-hr morning period rather than giving an IV bolus. Fang *et al.* (1988a) first suppressed endogenous

ACTH with dexamethasone and then stimulated with a much smaller dose ($<4 \mu\text{g}$), also in the morning.

The studies of Amsterdam *et al.* (1983; 1986; 1987a) and Jaeckle *et al.* (1987) suggested that the differences found in the F response may be due to an increased adrenal sensitivity to ACTH in depressed patients. Fang *et al.* (1988a; 1988b) responded that when using a supra-physiological dose ($250 \mu\text{g}$ ACTH), adrenal secretory capacity is more likely being measured rather than adrenal sensitivity. It is possible, therefore, that the increased steroid response in the depressed patients is due to adrenal hypertrophy, since chronic stimulation with ACTH is known to increase adrenal size. Thus stimulation with a supraphysiological dose of exogenous ACTH to enlarged adrenal glands theoretically would result in greater steroid production. In fact, computed tomography scans of adrenal glands in depressed patients have shown increased adrenal volume in 50% of the patients studied (Amsterdam *et al.*, 1987b). Increased adrenal weight also has been reported in suicide victims compared to persons with no known history of psychiatric illnesses dying from traumatic injuries or sudden medical complications (Dorovini-Zis & Zis, 1987). Because in the present study we also used a supra-physiological dose of ACTH, we cannot conclude that the differences seen between the MDD patients and controls were due to an increase in adrenal responsiveness vs. hypertrophy of the adrenal glands. Only further investigation of steroid responsiveness to more physiological doses of exogenous ACTH, and/or endogenous ACTH stimulated via other provocative tests such as the corticotropin-releasing hormone stimulation test or insulin-induced hypoglycemia, can clarify this point.

B was the only steroid measured that did not show an augmented response compared to the controls. It is possible that, since the measurement of B was the most difficult because it required such extensive sample preparation prior to analysis, differences could be explained on the basis of laboratory error. However, one would have expected the error to have been equal in both the controls and the patients with MDD. Furthermore, while controls were not included in the studies of Holsboer *et al.* (1984) and Gerken and Holsboer (1986), our B levels are in agreement with the levels they reported. An alternative explanation is that the production of B becomes maximally stimulated (approximately 32 ng/ml) with a supra-physiological dose of ACTH. In depressed patients this favors 17α -hydroxylation of Δ^5 -pregnenolone and progesterone. Glucocorticoid and adrenal androgen production would continue to increase, but mineralocorticoid production would not.

Finally, the data in Table III suggest that the augmented hormone responses may be associated with DST status. Previous studies have reported significant correlations of the response of F to $250 \mu\text{g}$ ACTH with post-DST 1600h F levels (Holsboer *et al.*, 1984; Jaeckle *et al.*, 1987). Therefore, had DST-NS patients, i.e., those with the highest production of corticosteroids, been better represented in our study, it is likely that the differences in hormone response between the MDD patients and controls would be more robust, including the response of B. In fact, a greater B response to exogenous ACTH has previously been reported in DST-NS depressed patients, but only when retested following recovery from illness (Gerken & Holsboer, 1986).

Another way that our data could be interpreted is that depressed patients have adrenal hyper-responsiveness by way of enhanced activity or increased synthesis of enzymes involved in the biosynthesis of corticosteroids and adrenal androgens, particularly those under the control of ACTH, resulting in hypercortisolemia. ACTH affects steroidogenesis at two levels. First, acute ACTH stimulation causes a rapid increase in steroidogenesis by increasing the rate of conversion of cholesterol to Δ^5 -pregnenolone, the rate-limiting step in steroidogenesis (Fig. 2), by increasing the amount of mitochondrial cholesterol (substrate) bound to cytochrome

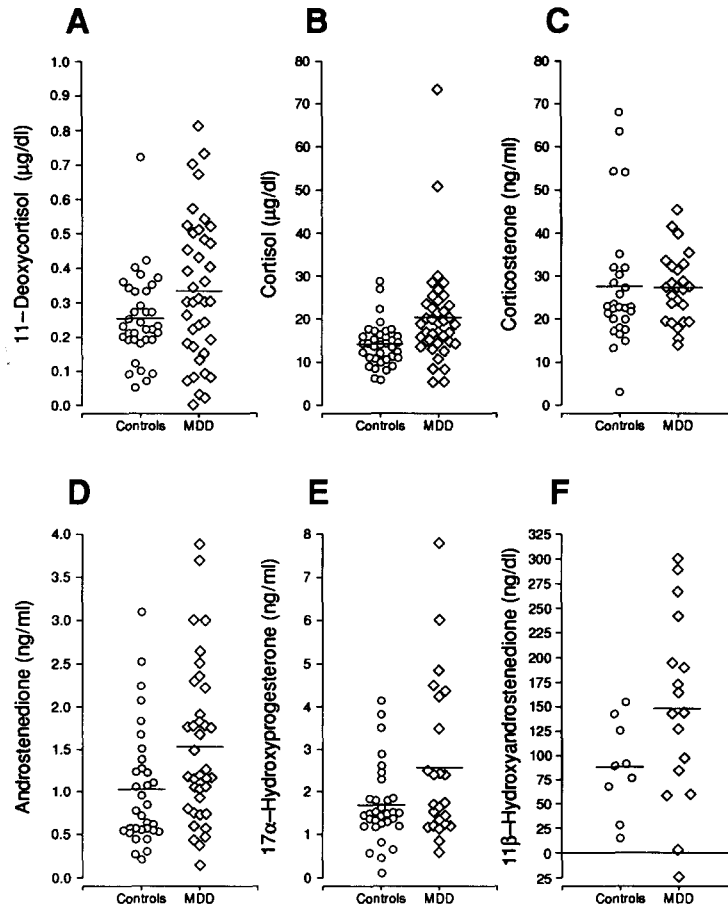


FIG. 1: Scattergram of the change in hormone levels after ACTH₁₋₂₄ in controls and patients with MDD. (A) 11-Deoxycortisol, (B) Cortisol, (C) Corticosterone, (D) Androstenedione, (E) 17 α -Hydroxyprogesterone, (F) 11 β -Hydroxyandrostenedione.

P-450 to be converted to Δ^5 -pregnenolone (Simpson & Waterman, 1983). An acute supraphysiological dose of ACTH therefore would cause a marked increase in the adrenal steroids which was observed in both the controls and depressed patients in this study. Second, ACTH has a tropic affect on steroidogenesis by maintaining adequate levels of steroidogenic enzymes (Simpson & Waterman, 1983). Several of these steroidogenic enzymes require cytochrome P-450, a heme-containing enzyme found in the adrenocortical mitochondria and microsomes, as well as in other organs (Rapp, 1986). Synthesis of mitochondrial cytochrome P-450_{SCC} (cholesterol side chain cleavage) (DuBois *et al.*, 1981) and P-450_{11 β} (11 β -hydroxylase) (Kramer *et al.*, 1983a), and microsomal cytochrome P-450_{C21} (21-hydroxylase) (Funkenstein *et al.*, 1983) and P-450_{17 α} (17 α -hydroxylase) (Zuber *et al.*, 1985) have been shown to increase in ACTH-treated cells. Furthermore, increased 17 α -hydroxylase activity has been reported in *in vitro* studies utilizing cultured bovine adrenocortical cells treated with ACTH (McCarthy *et al.*, 1983; Simpson & Waterman, 1983; Zuber *et al.*, 1985) and in *in vivo* studies with ACTH-

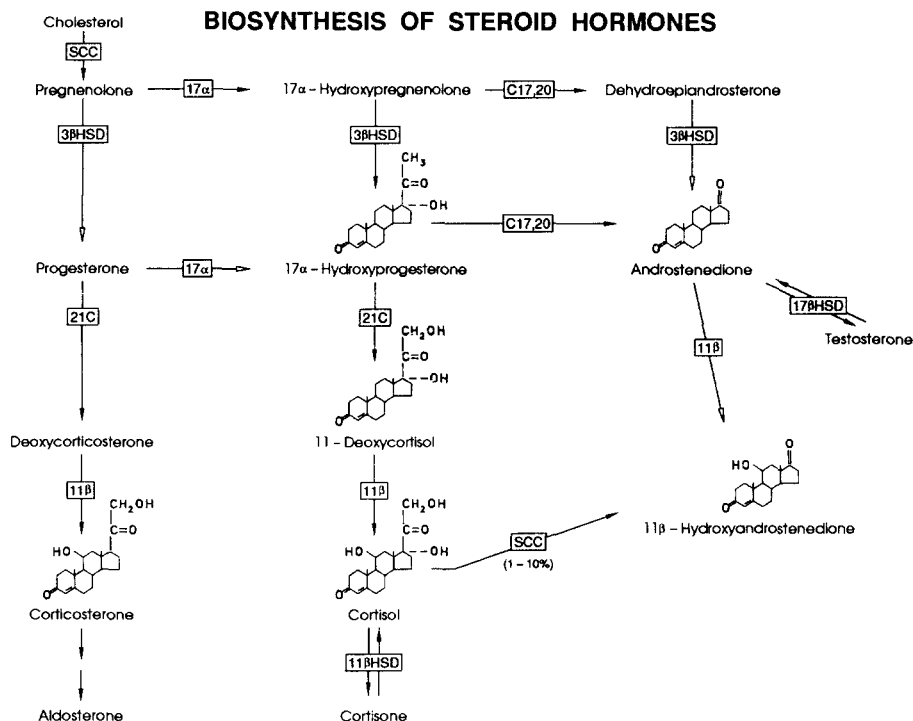


FIG. 2: Biosynthesis of steroid hormones. Major pathways are indicated by the solid arrowheads, minor pathways are indicated by the open arrowheads, and multiple reactions are indicated by two consecutive arrows. Abbreviations: SCC = side chain cleavage; 17 α = 17 α -hydroxylase; 3 β HSD = 5-ene-3 β -hydroxysteroid dehydrogenase and 3-oxosteroid isomerase; C17,20 = 17,20 lyase; C21 = 21-hydroxylase; 11 β = 11 β -hydroxylase; 17 β HSD = 17 β -hydroxysteroid dehydrogenase; 11 β HSD = 11 β -hydroxysteroid dehydrogenase.

treated rabbits (Fevold *et al.*, 1978). ACTH apparently does not have this same effect on 21-hydroxylase (Funkenstein *et al.*, 1983) or 11 β -hydroxylase (Kathol *et al.*, 1987). However, Kramer *et al.* (1983b) reported that secretion of 17 α -hydroxysteroids and C₁₉ steroids (i.e., androgens) was enhanced further by the action of ACTH to increase 17 α -hydroxylase activity and possibly 17,20 lyase. The possibility of 17,20 lyase activity being enhanced by ACTH in conjunction with 17 α -hydroxylase is very likely since 17,20 lyase and 17 α -hydroxylase are now thought to be localized on the same polypeptide chain (Nakajin & Hall, 1981) and associated with one protein (Nakajin *et al.*, 1981). The action of 17 α -hydroxylase lies at an important juncture in the biosynthetic pathway of adrenal steroids. Without it, glucocorticoids and adrenal androgens would be nonexistent. Likewise, 17,20 lyase is necessary for further conversion of 17 α -OHP and 17 α -hydroxypregnenolone to the androgens. Therefore, any enhancement of 17 α -hydroxylase and 17,20 lyase activities or increased production of cytochrome P-450_{C17} (17 α -hydroxylase/17,20 lyase) could potentially favor increased production and secretion of the 17 α -hydroxysteroids and adrenal androgens.

Elevated levels of ACTH in dexamethasone-resistant depressed patients have been reported by several investigators (Fang *et al.*, 1981; Reus *et al.*, 1982; 1983; Kalin *et al.*, 1982). Pfohl *et al.* (1985a; 1985b) reported an increase in ACTH levels in depressed patients over a 24-hr

period, with significant increases at varying times compared to controls. It is possible, then, that in depressed patients chronically higher circulating levels of ACTH are maintaining increased amounts of steroidogenic enzymes by stimulating synthesis of cytochrome P-450 and enhancing the enzyme activity of 17α -hydroxylase and $17,20$ lyase. Therefore, when acute ACTH stimulation occurs in depressed patients, greater amounts of enzymes are available for the necessary conversions in the steroidogenic pathways, causing an increased production and secretion of the steroids. Furthermore, if 17α -hydroxylase and $17,20$ lyase activities are enhanced due to the chronically elevated ACTH while 5-ene- 3β -hydroxysteroid dehydrogenase and 3-oxosteroid isomerase are not, then the steroids converted from 17α -OHP (i.e., F, S, AD, etc.) would demonstrate a greater response, while the mineralcorticoid pathway (i.e., B) would respond less robustly. Enhanced 17α -hydroxylase and $17,20$ lyase activities are consistent with the differences we found in the baseline data. Levels of F, S, and AD were elevated in the depressed patients compared to the controls.

Another explanation of our data could be a question of stimulated thresholds. It is possible that each adrenal steroid has a different responsive threshold to ACTH and that depressed patients have higher thresholds than controls. However, since we only tested a single, supra-physiological dose of ACTH, this explanation cannot not be adequately addressed here.

Our data also suggest that there is a change in the way depressed patients respond to ACTH in other ways besides preferential production of 17α -hydroxysteroids and adrenal androgens. There also was loss of the relationship among the basal levels of the steroids measured. This lost relationship could be ascribed simply to the increased variance in the depressed individuals; however, this in itself is a difference between controls and patients with depression, and found in numerous endocrine evaluations in the literature, which needs an explanation. Both controls and depressed patients in this study had their testing performed in the same structured environment. Is there something about their life circumstances, depressive treatment, or emotional state which has led to a less-coordinated production of adrenal hormones?

Perhaps a more important finding in our study was the consistent negative correlation between age and basal hormone levels in the controls. This negative relationship was largely lost in the patients with depression. These findings support what has been previously reported regarding age and other measures of corticosteroid production. Normal controls had no change, or a reduction, of glucocorticoid excretion with increasing age (Hamburger, 1954; Juselius & Kenny, 1974; Kathol *et al.*, 1989). Findings on post-dexamethasone F levels also suggest either no effect of age or a decrease with age (Tourigny-Rivard *et al.*, 1981; Lewis *et al.*, 1984), although other studies question whether this is the case (Oxenkrug *et al.*, 1983). Patients with depression, on the other hand, have consistently been found to have an increase in urinary glucocorticoid excretion or DST nonsuppression with advancing age (Jacobs *et al.*, 1984; Sharma *et al.*, 1988; Kathol *et al.*, 1989). Many studies also have reported significantly positive correlations of age with post-dexamethasone F levels in depressed patients (Asnis *et al.*, 1981; Lewis *et al.*, 1984; Nelson *et al.*, 1984; Holsboer *et al.*, 1986; Whiteford *et al.*, 1987; Maes *et al.*, 1990) as well as with baseline F levels (Asnis *et al.*, 1981; Whiteford *et al.*, 1987). Still others have reported a significant positive correlation of age with the F response to human corticotropin-releasing hormone stimulation in depressed patients but not in controls (von Bardeleben & Holsboer, 1989). The fact that our patients with depression lost the negative relationship of the hormones with age is in the predicted direction for this group, even though a positive correlation was not found. This suggests that there is a consistent difference between those with depression who, if anything, increase corticosteroid production with age, and those without a history of depression, who generally show a decrease in corticosteroid production with age.

Our study is not without its problems. All but one of our patients were on one or more psychotropic drugs, and we cannot rule out the possibility that these drugs contributed to the differences observed. However, DST-NS depressed patients who are treated with antidepressants have been reported to suppress normally following treatment (Holsboer *et al.*, 1982; Greden *et al.*, 1983). This suggests that if these drugs were affecting the adrenal response, then it is most likely that their effect would have been to diminish the differences by lowering the F, and very likely the other steroid, levels towards those seen in the controls. Since these studies only measured F, however, the possibility of these drugs affecting the other steroid concentrations remains unanswered. Only further research into the effects these drugs have on adrenal responsiveness can clarify this point. Another contributing factor is that our female subjects were not controlled for their menstrual cycle. This may constitute a problem when interpreting the 17α -OHP data, because of the low levels before ovulation and the rise in progesterone and 17α -OHP levels from the corpus luteum following ovulation. However, when the male patients and controls were compared, the differences remained. In any case, this should probably be taken into account when measurements of 17α -OHP are done.

In conclusion, we report increased adrenal steroid responses for S, AD, 17α -OHP, and 11β -OHAD, but not B, similar to those previously reported for F (Jaekle *et al.*, 1987) in depressed patients compared to controls following acute ACTH stimulation. Any of these could be more closely related to the development of depression or characteristic symptoms of depression than F. Interestingly, androgens also have been independently related to depressive symptoms in those taking synthetic androgens (Pope & Katz, 1987; 1988; Perry *et al.*, 1989). Our preliminary findings with 11β -OHAD suggest that it may have a relationship to psychosis in depression. Increased production of 17α -hydroxysteroids and adrenal androgens may be due to an enhanced 17α -hydroxylase and $17,20$ lyase, activity or increased synthesis of cytochrome P-450_{C17}, following chronic higher levels of ACTH in depressed patients. Alternatively, the augmented responses could be related to a defect in the negative feedback mechanism of corticosteroids on pituitary and hypothalamic function, since the DST-NS depressed patients consistently had the greatest steroid responses to exogenous ACTH. However, the possibility that psychotropic drugs influenced the augmented responses cannot be ruled out, since all but one of the patients with MDD were on one or more psychotropic drugs.

During the basal state the depressed patients showed a loss of uniform relationships among the adrenal steroids and a loss of the age-related decrease in steroid levels that occurred in the controls. The lost negative correlation of age with basal steroid levels may suggest a genetic predisposition to increased corticosteroid production, which is age-related. While evidence is plentiful suggesting that hypercortisolemia in depression may be caused by abnormalities in the limbic and hypothalamic neuroregulatory systems, abnormalities in peripheral systems also may be involved. This study presents data that warrants further investigation of the role of cortisol precursors and metabolites in depressive illness.

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