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Differential hypothalamic-pituitary-adrenal activation of the neuroactive steroids pregnenolone sulfate and deoxycorticosterone in healthy controls and alcohol-dependent subjects

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Summary

Ethanol and the neuroactive steroids have interactive neuropharmacological effects and chronic ethanol administration blunts the ethanol-induced increase in neuroactive steroid levels in rodent plasma and brain. Few studies have explored neuroactive steroid regulation in alcohol-dependent human subjects. In fact, the regulation of adrenal neuroactive steroids has not been well defined in healthy controls. We thus explored the regulation of two neuroactive steroids, pregnenolone sulfate (PREG-S) and deoxycorticosterone, by pharmacological challenges to the hypothalamic-pituitary-adrenal (HPA) axis in healthy controls and one-month abstinent alcohol-dependent patients with co-occurring nicotine dependence. Plasma levels of PREG-S and deoxycorticosterone were measured by radioimmunoassay in controls and alcohol-dependent patients after challenges of naloxone, ovine corticotrophin releasing hormone (oCRH), dexamethasone, cosyntropin, and cosyntropin following high-dose dexamethasone. In addition, basal diurnal measures of both hormones were obtained. PREG-S plasma levels in healthy controls were increased by cosyntropin challenge (with and without dexamethasone pretreatment) and decreased by dexamethasone challenge. However, PREG-S concentrations were not altered by naloxone or oCRH challenges, suggesting that PREG-S is not solely regulated by hypothalamic or pituitary stimulation. Deoxycorticosterone, in contrast, is regulated by HPA challenge stimulation in a manner similar to cortisol. Alcohol-dependent patients had a blunted PREG-S response to cosyntropin (with and without dexamethasone pretreatment). Furthermore, the time to peak deoxycorticosterone response following oCRH was delayed in alcohol-dependent patients compared to controls. These results indicate that plasma PREG-S and deoxycorticosterone levels are differentially regulated by HPA axis modulation in human plasma. Further, alcohol-dependent patients show a blunted PREG-S response to adrenal stimulation and a delayed deoxycorticosterone response to oCRH challenge.

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Keywords

Pregnenolone Sulfate; Deoxycorticosterone; Hypothalamic-Pituitary-Adrenal Axis; Alcohol Dependence; Neuroactive Steroids

Introduction

Neuroactive steroids are endogenous neuromodulators that rapidly alter neuronal excitability by binding to membrane receptors (Paul and Purdy, 1992). Neuroactive steroids that are synthesized directly in the brain, independent of peripheral sources and still detected in adrenalectomized/orchiectomized animals, have been termed neurosteroids (Baulieu, 1998). Pregnenolone sulfate (PREG-S) and deoxycorticosterone are endogenous neuroactive steroids synthesized from cholesterol. Cholesterol is converted into pregnenolone by the mitochondrial enzyme CYP11A and metabolized to PREG-S via a sulfotransferase enzyme. Alternately, pregnenolone can be converted into progesterone which is further converted into deoxycorticosterone, a precursor of the potent $GABA_A$ receptor agonists 3α21-dihydroxy-5α-pregnan-20-one (3α,5α-THDOC) and 3α,5β-THDOC (Figure 1). Although these conversions can take place in the brain (Stoffel-Wagner, 2001; Davies and MacKenzie, 2003), plasma levels of these steroids measured in healthy human subjects are likely of adrenal origin. Therefore, in this paper we will refer to PREG-S and deoxycorticosterone as *neuroactive steroids* as opposed to *neurosteroids*.

Neuroactive steroids have potent effects on neurotransmission mediated by γ-aminobutyric acid type A ($GABA_A$) and/or glutamate receptors and transporters. PREG-S-is an excitatory neuroactive steroid that inhibits GABA release at nanomolar concentrations (Mtchedlishvili and Kapur, 2003), inhibits $GABA_A$ receptors, and potentiates N-methyl-D-aspartate (NMDA) receptor-mediated excitatory responses at micromolar concentrations (Majewska et al., 1988; Wu et al., 1991). PREG-S modulates learning and memory in rodents (Flood et al., 1992; 1995); its concentrations decrease with age and administration of PREG-S reverses cognitive deficits in aged rats (Vallee et al., 2001). PREG-S also prevents the memory-impairing effects of ethanol (Melchior and Ritzmann, 1996) and plays an important role in fetal alcohol syndrome (Caldeira et al., 2004; Mameli and Valenzuela, 2006). PREG-S concentrations are increased by corticotrophin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) challenges in rat brain and plasma (Torres and Ortega, 2003) and by ACTH challenge in humans (De Peretti and Mappus, 1983; Boizel et al., 1986).

In contrast, deoxycorticosterone is an inhibitory neuroactive steroid with anticonvulsant properties (Selye, 1942; Craig, 1966) that have been attributed to its conversion to 3α,5α-THDOC, a potent GABAergic modulator (Reddy and Rogawski, 2002). Deoxycorticosterone and 3α,5α-THDOC are increased following acute ethanol administration in rats (Barbaccia et al., 1999; Khisti et al., 2005). In humans, deoxycorticosterone plasma levels are increased following ACTH administration and decreased after dexamethasone administration (Brown and Strott, 1971; Tan and Mulrow, 1975; Kater et al., 1990), suggesting potential regulation by the hypothalamic-pituitary-adrenal (HPA) axis. In agreement, (Porcu et al. 2006a) have shown that plasma deoxycorticosterone levels in cynomolgus monkeys are increased by naloxone and CRH challenges and decreased by dexamethasone challenge.

Animal models of ethanol dependence show adaptations in stimulated neuroactive steroid concentrations. Chronic ethanol consumption in rodents decreases corticosterone responses to stress (Spencer and McEwen, 1990) and blunts ethanol challenge-induced elevation of plasma and brain levels of the neuroactive steroids 3α-hydroxy,5-pregnan-20-one (3α,5α-THP) and deoxycorticosterone (Morrow et al., 2001; Khisti et al., 2005). Following long-term ethanol

self-administration in cynomolgus monkeys, basal deoxycorticosterone levels are elevated and deoxycorticosterone responses to HPA axis modulation by CRH and dexamethasone are blunted (Porcu et al., 2006b). In humans, Adinoff et al. (2005a,b) have shown that one-month abstinent alcohol-dependent patients have decreased cortisol concentrations compared to healthy controls. The cortisol responses to ovine CRH (oCRH) and dexamethasone plus cosyntropin challenges were blunted in alcohol-dependent patients. In addition, both ACTH and cortisol were suppressed to a greater extent in the alcohol-dependent patients after dexamethasone challenge. Several other investigators (Adinoff et al., 1990; Wand and Dobs, 1991; Inder et al, 1995; Costa et al., 1996; Ehrenreich et al., 1997) have demonstrated an attenuated glucocorticoid response in alcohol-dependent subjects following both psychosocial and pharmacological challenges.

Despite both a sizable literature confirming a disruption in HPA axis regulation in alcohol-dependent subjects and a link between HPA axis activation and neuroactive steroid release, no studies of basal or stimulated levels of deoxycorticosterone or PREG-S in alcohol dependence have been conducted to date. We therefore assessed PREG-S and deoxycorticosterone responses to pharmacological challenges of the HPA axis in the healthy control and alcohol-dependent subjects previously described by Adinoff et al. (2005a,b). The aims of the present work were: a) to determine how the neuroactive steroids PREG-S and deoxycorticosterone respond to hypothalamic, pituitary, and adrenal stimulation by pharmacological challenges with naloxone, oCRH, cosyntropin, dexamethasone plus cosyntropin and dexamethasone alone in healthy human subjects and, b) to determine if the HPA regulation of these neuroactive steroids is altered in abstinent alcohol-dependent subjects.

A number of pharmacological challenges were used to selectively explore HPA axis functioning in these studies. As background, activation of the HPA axis stimulates the release of CRH from the hypothalamus. CRH induces the synthesis and release of ACTH from the pituitary, which stimulates the adrenal gland to release glucocorticoids and neuroactive steroids. Glucocorticoids, primarily cortisol in humans and non-human primates and corticosterone in rodents, elicit negative feedback upon the hypothalamus and pituitary. Likewise, the GABAergic neuroactive steroids inhibit CRH production, ACTH release and corticosterone increase in rodents (Owens et al., 1992; Patchev et al., 1994; Patchev et al., 1996). Thus, administration of naloxone was used to assess HPA axis activity by blocking the inhibitory opioid input to CRH neurons in the hypothalamus. The pituitary ACTH response was tested by administering oCRH, while the adrenal secretion of cortisol and neuroactive steroids was assessed by administration of cosyntropin (synthetic ACTH, corticotrophin α_{1-24}). Administration of cosyntropin following high dose dexamethasone pretreatment also tested the adrenal secretion after near complete suppression of endogenous pituitary ACTH release. The dexamethasone suppression test further assessed the sensitivity of negative feedback on pituitary corticotrophs.

Methods

Subjects

Twenty-one male alcohol-dependent participants were recruited from patients requesting treatment for alcohol dependence at the Dallas VA Medical Center and Homeward Bound, Inc. as previously reported (Adinoff et al., 2005a,b). Due to the volume of blood required, two different populations of alcohol-dependent subjects were studied (Group 1: oCRH and naloxone challenges, Group 2: cosyntropin and dexamethasone plus cosyntropin challenges). Eleven patients (aged 43.5 ± 4.8 years [mean ± SD]; range, 36 – 49 years) participated in group 1. Ten patients (aged 42.8 ± 4.0 years [mean ± SD]; range, 38 – 49 years) participated in group 2. Patients reported an alcohol intake of at least 80 g of absolute alcohol on a daily basis for at least two weeks before the cessation of drinking and had at least a 10-year history of

problematic drinking. 90% of the patients were also nicotine-dependent. Patients with a lifetime history of other DSM-IV (American Psychiatric Association, 1994) Axis I psychiatric disorders (such as anxiety, post-traumatic stress, or mood disorders) not associated with alcohol use, other substance use disorders (or weekly drug use) within the previous 12 months (excluding caffeine or nicotine use disorders), active medical disorders (i.e., hypertension, diabetes, chronic pain, or cardiac or pulmonary disorders), or a history of major head trauma were excluded from the study. Furthermore, patients taking medications that may interfere with HPA axis functioning (i.e. psychotropic, antihypertensive or hypoglycemic agents), patients with Beck Depression Inventory (Beck et al., 1979) scores above 15 the week before testing, and patients with alanine aminotransferase (ALT) or aspartate aminotransferase (AST) three times greater than the clinical laboratory's upper limit of normal, were also excluded.

Fourteen healthy men were individually age-matched within a five-year period with alcohol-dependent patients. Ten subjects (aged 38.2 ± 5.4 years [mean ± SD];range, 30 – 47 years) underwent the naloxone and oCRH tests and eleven subjects (aged 39.6 ± 5.7 years [mean ± SD]; range, 31 – 47 years) underwent the cosyntropin and dexamethasone plus cosyntropin tests. Six subjects underwent all tests, with at least six weeks separating the two sets of endocrine challenges. Controls reported no lifetime history of any *DSM-IV* axis I disorder (except caffeine or nicotine use disorders), reported no medical disorders, and were taking no medications. To avoid possible effects of family history of substance abuse or other psychiatric disorders on HPA axis functioning, controls with a single first-degree relative or two second-degree relatives with an axis I disorder were excluded.

All participants underwent a history and physical examination, clinical laboratory testing (including complete blood count, liver function tests, routine blood chemistries, thyroid-stimulating hormone test, and HIV test), electrocardiography, and urine drug screening. The study was conducted in accordance with the Declaration of Helsinki (http://www.wma.net/e/ethicsunit/helsinki.htm) and was approved by the Institutional Review Boards at both the University of Texas Southwestern Medical Center and the Dallas VA Medical Center. Informed consent was obtained after the study was fully explained, and participants were financially compensated for their participation.

Neuroendocrine Challenge Tests

All neuroendocrine studies were performed at the General Clinical Research Center (GCRC) at the University of Texas Southwestern Medical Center as previously described (Adinoff et al., 2005a,b). Both healthy controls and alcohol-dependent participants arrived at the GCRC at least two hours before the studies. Alcohol-dependent patients were accompanied to the GCRC from their treatment site. The studies were performed in separate sessions and session order was balanced. Nicotine-dependent subjects were placed on nicotine patches appropriate to their daily cigarette intake upon admission to the GCRC so that nicotine withdrawal symptoms were avoided.

Blood samples were obtained every ten min for 30 min prior to pharmacological challenge. Blood samples were then obtained every five min during the first hour and every 10 min during the second hour following challenge infusion. PREG-S and deoxycorticosterone were post-hoc assays run on those time points where sufficient plasma was available. Samples from two to three different time points were combined to obtain sufficient plasma for the analysis [samples from 2035h, 2040h and 2045h or from 2100h and 2110h were combined and the mean interval (i.e. 2040h or 2105h) is shown in the figures].

Group 1—These tests have previously been described in Adinoff et al. (2005b).

oCRH challenge: Participants were admitted to the GCRC at 1800h on day one. An intravenous catheter was inserted in each arm at 0700h on day two. Blood sampling was initiated for pulsatile measures of ACTH and cortisol one hour later (at 0800h) and continued through 2000h. Blood sampling at 1915h was used as baseline for PREG-S and deoxycorticosterone determinations. After the collection of the final basal measure at 2000h, 0.4 μg/kg oCRH was administered IV over one min. oCRH was administered at 2000h during the glucocorticoid diurnal troughs.

Naloxone challenge: Participants returned to the GCRC by 1700h, two days after the completion of the oCRH challenge. An intravenous catheter was placed in both arms at approximately 1800h on the day of the study. Basal measures of ACTH and cortisol were obtained from 1900h to 2000h, and the 1940h was used for baseline PREG-S and deoxycorticosterone determinations. Naloxone 125 μg/kg was administered IV over one min immediately after the 2000h blood withdrawal. One patient did not return to the GCRC for the naloxone challenge after completion of the oCRH challenge.

Group 2—All subjects were administered both the Cosyntropin challenge and the Dexamethasone plus Cosyntropin challenge. Challenges were administered seven days apart. Test order was randomly determined and balanced between groups (described in Adinoff et al., 2005a).

Cosyntropin challenge: Participants were brought to the GCRC at 1800h on day one. A 24-h urine collection was initiated at 2000h on day one for urinary free cortisol and continued until 2000h on day two. An intravenous catheter was inserted in each arm at 1900h on day two. One hour later (at 2000h) blood sampling was initiated for 12-h basal measures of ACTH and cortisol and continued through 0800h on day three. Blood sampling at 0715h was used as baseline for PREG-S and deoxycorticosterone determinations. After the 0800h blood withdrawal on day three, 0.01 μg/kg cosyntropin was administered IV over one min.

Dexamethasone plus Cosyntropin challenge: This study included the pituitary and adrenocortical response to dexamethasone and the cosyntropin challenge after dexamethasone. Participants arrived at the GCRC at 2000h and intravenous lines were placed into both arms at 2100h. Blood samples for the measurement of ACTH and cortisol were obtained every 10 min from 2200h through 2300h. Dexamethasone 8 mg in 50 ml of dextrose 5% water (D5W) was administered IV from 2300h to 2330h. Blood sampling was continued from 2300h to 0500h and then restarted at 0700h to 0800h every 10 min (blood sampling was not obtained from 0500h to 0700h because of limitations on blood volume). The cosyntropin challenge was performed as described above.

Assays

PREG-S assay—PREG-S levels were determined by radioimmunoassay (RIA) as follows. Plasma samples (250 μl) were extracted three times with 2 ml diethyl ether to remove free pregnenolone and 3α,5α-THP from the sample. [^{3}H]pregnenolone (1000 cpm; SpA = 14 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA) and [^{3}H]PREG-S (1000 cpm; SpA = 20 Ci/mmol; Custom synthesis from American Radiolabeled Chemicals, Inc., Saint Louis, MO, USA) were added to each sample for recovery estimation and to confirm that diethyl ether selectively extracted free pregnenolone, while the PREG-S was left in the aqueous phase. However, a percentage of [^{3}H]pregnenolone was found in the aqueous phase (1.0 ± 0.6%). These values were incorporated in the recovery estimates. The aqueous phase was then purified with OASIS HLB cartridges (60 mg; Waters Chromatography, Milford, MA, USA), as described by Higashi et al. (2003). Prior to use, cartridges were conditioned with 1 ml ethyl acetate, 2 ml ethyl alcohol (100%) and 2 ml double-distilled water. The aqueous phase was

then applied to the cartridge and washed with 2 ml double-distilled water followed by 1 ml ethyl acetate. PREG-S was eluted with 1.5 ml ethyl alcohol and samples were dried in a speed vacuum concentrator. The dried extracts were resuspended in 2 ml RIA buffer of which 0.5 ml was used for the assay (run in duplicate) and for recovery determination. Average final recovery of PREG-S was 82 ± 0.9%. The antiserum for PREG-S was purchased from MP Biomedicals (Orangeburg, NY, USA) and diluted according to manufacturer's instructions. This antiserum cross-reacts 100% with pregnenolone and PREG-S; it also cross reacts with 3α,5α-THP 16%, 3α,5β-THP 5.9%, progesterone 3.1%, 3α,5α-THDOC 1.1%. Less than 1% cross-reactivity was observed for 5α-dihydroprogesterone, 17α-hydroxyprogesterone, 20α-dihydroprogesterone, 17α-hydroxypregnenolone, deoxycorticosterone, cortisol, 11-deoxycortisol, corticosterone, androsterone, 5α-dihydrotestosterone, cholesterol, 17β-estradiol, estrone and estriol. Unknown samples are compared to concurrently run standards for PREG-S using a one-site competition model and adjusted for extraction efficiency. Values are expressed as ng/ml plasma. The sensitivity of the assay is 50 pg/ml. Intra-assay and inter-assay coefficients of variation were 12.1% and 14.3%, respectively.

Deoxycorticosterone assay—Deoxycorticosterone levels were determined by RIAas previously described (Porcu et al., 2006a). Briefly, plasma samples (200 μl) were extracted twice with 2 ml ethyl acetate/hexane (3:2) 1000 cpm aliquot of [^{3}H]deoxycorticosterone (SpA = 50 Ci/mmol; American Radiolabeled Chemicals, Inc. Saint Louis, MO, USA) was added to each sample for recovery estimation. The dried extracts were resuspended in 1.5 ml RIA buffer of which 0.5 ml was used for the assay (run in duplicate) and 0.3 ml was used for recovery determination. The antiserum for deoxycorticosterone was purchased from MP Biomedicals (Orangeburg, NY, USA) and diluted according to manufacturer's instructions. This antiserum was highly specific for deoxycorticosterone as shown by the following cross-reactivity tests: deoxycorticosterone 100%, 3α,5α-THDOC 4.7%, progesterone 2.5%, corticosterone 1.7%. Less than 1% cross-reactivity was observed for 3α,5α-THP, 3α-hydroxy-pregn-4-en-20-one, pregnenolone, 20-hydroxy-pregnen-4,3-one, testosterone, androstenedione, 17α-hydroxyprogesterone, 11-deoxycortisol, 5α-dihydrotestosterone, cortisol, cholesterol, 17β-estradiol, estrone and estriol. Unknown samples were compared to concurrently run standards using a one-site competition model and adjusted for extraction efficiency. Deoxycorticosterone values are expressed as ng/ml plasma. The sensitivity of the assay is 10 pg/ml. Intra-assay and inter-assay coefficients of variation were 4.86% and 4.51%, respectively.

Data Analysis

Steroid levels were analyzed using a commercially available statistical program (GraphPad Prism 4.0, GraphPad Software, San Diego, CA, USA). ANOVAs were performed on log-transformed values to control for potential heterogeneity of variance. Two-way repeated measures ANOVAs considering the factors group (control vs. patient) and time, as the repeated factor, were performed. Two-way ANOVAs were performed when missing values did not allow the repeated measures test. Post-hoc comparisons were performed by the Bonferroni test. Values are expressed as mean ± SEM of the raw data and p values less than 0.05 were considered statistically significant. Net area under the curve (AUC) was calculated using Sigma Plot 8.0 (Systat Software, Inc., Point Richmond, CA, USA). PREG-S, deoxycorticosterone, and cortisol peak responses (% increase vs. basal) to each challenge test were correlated using the Pearson's coefficient.

Results

Baseline and Diurnal Cycle of PREG-S and deoxycorticosterone

Demographic characteristics of participants are shown in Table 1 and were previously described in Adinoff et al. (2005a,b).

Differences in the diurnal secretion of PREG-S and deoxycorticosterone plasma levels were determined from morning (0715h) and evening (1915h) basal values prior to the cosyntropin and the oCRH and naloxone challenges, respectively. Two-way ANOVA for PREG-S showed no significant effect of time (0715h vs. 1915h) [$F(1,18)=0.13$; $p=0.73$], no significant effect of group (controls vs. alcohol-dependent patients) [$F(1,18)=0.36$; $p= 0.56$] and no significant interaction [$F(1,18)=0.02$; $p=0.89$]. PREG-S levels did not show a diurnal variation in either healthy controls or alcohol-dependent patients. Furthermore, no differences were observed in PREG-S basal values between abstinent alcohol-dependent subjects vs healthy controls (Figure 2A).

In contrast, two-way ANOVA for deoxycorticosterone revealed a significant effect of time (0715h vs. 1915h) [$F(1,18)=31.17$; $p<0.0001$] but no effect of group (controls vs. alcohol-dependent patients) [$F(1,18)=0.90$; $p=0.35$] or significant interaction [$F(1,18)=2.49$; $p=0.12$]. Thus, deoxycorticosterone levels were lower in the evening relative to the morning [50% lower in controls and 30% lower in alcohol-dependent patients, respectively]. However, no differences were observed between controls and alcohol-dependent patients (Figure 2B).

HPA Axis Regulation of PREG-S and Deoxycorticosterone Levels

Naloxone challenge—PREG-S and deoxycorticosterone measures were determined 20 min before and 40, 55, 65, 85, 105 and 120 min following naloxone infusion (125 μg/kg, IV).

Two-way repeated measures ANOVA for PREG-S found a significant effect of time [$F(6,18)=4.34$; $p=0.0005$], but no effect of group (controls vs. alcohol-dependent patients) [$F(1,18)=0.17$; $p=0.68$] or significant interaction [$F(6,18)=0.24$; $p=0.96$]. However, PREG-S levels did not significantly differ from basal measures in either healthy controls or alcohol-dependent patients (Figure 3A).

Two-way repeated measures ANOVA for deoxycorticosterone also revealed a significant effect of time [$F(6,18)=7.25$; $p<0.0001$], but no effect of group (controls vs. alcohol-dependent patients) [$F(1,18)=0.87$; $p=0.36$] or significant interaction [$F(6,18)=0.51$; $p=0.80$]. Naloxone increased deoxycorticosterone levels in both groups compared to basal measurements (Figure 3B). In healthy controls, deoxycorticosterone levels peaked 55 min after naloxone administration (+81%) and remained significantly elevated at 65 and 85 min (+47 and +55%, respectively). In alcohol-dependent patients, deoxycorticosterone levels were increased by 62% at 55 min, peaked at 65 min (+72%) and were still elevated at 85 min (+56%).

oCRH challenge—PREG-S and deoxycorticosterone measures were determined 45 min before and 55, 65, 85, 105 and 120 min following oCRH infusion (0.4 μg/kg, IV). Only deoxycorticosterone levels were measured at 40 min due to insufficient plasma availability for the PREG-S assay.

Two-way ANOVA for PREG-S showed a significant effect of time [$F(5,18)=2.61$; $p=0.03$], but no effect of group (controls vs. alcohol-dependent patients) [$F(1,18)=2.79$; $p=0.10$] or significant interaction [$F(5,18)=0.43$; $p=0.82$]. Indeed, PREG-S levels following oCRH did not change in healthy controls or alcohol-dependent patients relative to the basal measure (Figure 4A).

Two-way ANOVA for deoxycorticosterone revealed a significant effect of time [$F(6,18)=19.23$; $p<0.0001$], no effect of group (controls vs. alcohol-dependent patients) [$F(1,18)=0.30$; $p=0.58$], but a significant interaction between group and time variables [$F(6,18)=3.42$; $p=0.004$]. oCRH increased deoxycorticosterone levels in both controls and alcohol-dependent patients compared to the 45 min pre-drug basal values (Figure 4B). In the control group, deoxycorticosterone levels were significantly increased at all time points following oCRH

infusion with the peak occurring at 40 min (+224%). In alcohol-dependent patients, deoxycorticosterone levels were elevated at 40 min (+44%), peaked at 85 min (+161%) and remained increased at 105 and 120 min (+136 and 156%, respectively). Furthermore, deoxycorticosterone levels were 52% lower in alcohol-dependent patients 40 min following oCRH administration compared to the respective time point in the control group. Further analysis revealed that the time to peak response following oCRH administration was shifted in alcohol-dependent patients compared to controls; the mean time for peak response in controls was 50 min, compared to 87 min in alcohol-dependent patients (Figure 4C).

Cosyntropin challenge—Blood samples for PREG-S and deoxycorticosterone measures were taken 45 minutes before and 50 to 55 min following cosyntropin (0.01 μg/kg, IV) administration.

Two-way repeated measures ANOVA for PREG-S revealed a significant effect of time [$F(1,18)=19.42$; $p=0.0003$], no effect of group (controls vs. alcohol-dependent patients) [$F(1,18)=1.90$; $p=0.19$] and a significant interaction [$F(1,18)=9.14$; $p=0.007$]. PREG-S levels increased by 49% in healthy controls 55 min after infusion, but did not change in alcohol-dependent patients (Figure 5A).

Two-way repeated measures ANOVA for deoxycorticosterone revealed a significant effect of time [$F(1,18)=26.86$; $p<0.0001$], but no effect of group (controls vs. alcohol-dependent patients) [$F(1,18)=4.23$; $p=0.055$] and no interaction [$F(1,18)=0.08$; $p=0.78$]. Cosyntropin administration increased deoxycorticosterone levels in both healthy controls (+66%) and alcohol-dependent patients (+37%) 55 min after infusion compared to the respective pre-drug basal values (Figure 5B).

Dexamethasone plus Cosyntropin challenge—In this test, cosyntropin was administered nine hours following dexamethasone (8 mg, IV) and blood samples were obtained 45 minutes before and 30 and 55 minutes after cosyntropin.

Two-way ANOVA for PREG-S revealed a significant effect of time [$F(2,18)=15.90$; $p<0.0001$], a significant effect of group (controls vs. alcohol-dependent patients) [$F(1,18)=14.24$; $p=0.0004$] but no significant interaction [$F(2,18)=0.09$; $p=0.92$]. PREG-S levels were increased in both groups 30 and 55 min following cosyntropin administration compared to their respective basal values prior to cosyntropin (and after dexamethasone). In healthy subjects, PREG-S levels were increased by 141% and by 68% at 30 and 55 min, respectively, following cosyntropin. In alcohol-dependent patients, PREG-S increased by 113% and by 105% at 30 and 55 min, respectively, following cosyntropin (Figure 6A). Despite the ANOVA showing a significant effect of group, the post-hoc analysis failed to reveal any significance between controls and alcohol-dependent subjects. However, further analysis using the net area under the curve (AUC) revealed that PREG-S AUC in alcohol-dependent patients was significantly lower than controls (−38%, Figure 6B).

For deoxycorticosterone, two-way ANOVA revealed a significant effect of time [$F(2,18)=1.68$; $p<0.0001$], but no effect of group (controls vs. alcohol-dependent patients) [$F(1,18)=0.09$; $p=0.11$] or significant interaction [$F(2,18)=0.01$; $p=0.78$]. Cosyntropin administration nine hours after dexamethasone increased deoxycorticosterone levels in both controls and alcohol-dependent patients compared to their respective pre-cosyntropin basal values. In healthy subjects, deoxycorticosterone levels were increased by 281% and by 100% 30 and 55 min, respectively, after cosyntropin infusion; in alcohol-dependent patients, deoxycorticosterone was increased by 335% and by 151%, respectively (Figure 6C).

Dexamethasone Challenge—The effect of dexamethasone alone on PREG-S and deoxycorticosterone levels was evaluated by comparing the 0715h baseline from the cosyntropin stimulation test alone and the one obtained nine hours after dexamethasone but before cosyntropin administration.

Two-way repeated measures ANOVA for PREG-S revealed a significant effect of time [$F(1,18)=70.03$; $p<0.0001$], no effect of group (controls vs. alcohol-dependent patients) [$F(1,18)=2.43$; $p=0.14$] and no interaction [$F(1,18)=2.99$; $p=0.10$]. Dexamethasone decreased PREG-S levels by 47% in control subjects and by 64% in alcohol-dependent patients (Figure 7A).

Two-way repeated measures ANOVA for deoxycorticosterone revealed a significant effect of time [$F(1,18)=36.70$; $p<0.0001$], a significant effect of group (controls vs. alcohol-dependent patients) [$F(1,18)=6.94$; $p=0.012$] but no interaction [$F(1,18)=0.03$; $p=0.86$]. Deoxycorticosterone levels were decreased by 43% in control subjects and by 44% in alcohol-dependent patients compared to their respective basal values (Figure 7B). Again, despite the ANOVA finding a significant effect of group, the post-hoc test failed to reveal any significant differences between controls and alcohol-dependent subjects.

Correlations among Neuroendocrine Measures

We analyzed potential correlations between the PREG-S and deoxycorticosterone peak responses obtained in these subjects following each pharmacological challenge and the cortisol peak responses reported by Adinoff et al. (2005a,b). Data from healthy controls and alcohol-dependent subjects were combined, given the lack of significant differences in the correlations between the two groups. Time points of peak responses were variable for each steroid. The peak percent increase in PREG-S was correlated with the peak percent increase in deoxycorticosterone following the naloxone, cosyntropin and dexamethasone plus cosyntropin challenges ($r=0.48$, $p=0.034$; $r=0.46$, $p=0.049$; $r=0.64$, $p=0.001$, respectively). The peak percent increase in PREG-S was correlated with the peak percent increase in cortisol following the cosyntropin challenge only ($r=0.47$, $p=0.037$). The peak percent increase in deoxycorticosterone was correlated with the peak percent increase in cortisol following the naloxone, oCRH and cosyntropin challenges ($r=0.50$, $p=0.026$; $r=0.68$, $p=0.001$; $r=0.65$, $p=0.003$, respectively).

Discussion

Little is known about HPA axis regulation of neuroactive steroid synthesis in humans. In this study, we show that PREG-S levels are regulated by adrenal activation of the HPA axis and are sensitive to negative feedback mechanisms; however, they do not exhibit diurnal variation or significantly change after naloxone and oCRH infusions. In contrast, deoxycorticosterone levels exhibit diurnal variations and are regulated by the HPA axis at all levels examined. Abstinent alcohol-dependent patients, primarily nicotine dependent, have blunted PREG-S response to adrenal stimulation with cosyntropin, butno other alterations in PREG-S and deoxycorticosterone responses to HPA axis challenges were found (see Table 2 for recapitulative results). To the best of our knowledge, this is the first comprehensive study of the selective modulation of the HPA axis with naloxone, oCRH, cosyntropin and dexamethasone on PREG-S and deoxycorticosterone levels in men.

The finding that naloxone or oCRH challenges did not alter PREG-S levels could indicate that PREG-S levels are independent of hypothalamic or pituitary activation of the HPA axis. This would explain the lack of diurnal changes in circulating PREG-S levels that we and others (Laatikainen and Vihko, 1968) have observed in humans. The lack of change in PREG-S levels following naloxone or oCRH stimulation in humans could be due to the preferential metabolism of pregnenolone to progesterone (ratherthan PREG-S) mediated by an effect of naloxone or

oCRH on the steroidogenic enzymes sulfotransferase and sulfatase that interconvert pregnenolone and PREG-S. Indeed, naloxone, but not CRH, increases plasma pregnenolone levels in cynomolgus monkeys (Porcu et al., 2006c). However, CRH does increase progesterone and its neuroactive metabolite 3α,5α-THP in humans (Genazzani et al., 1998), suggesting that CRH may promote pregnenolone metabolism to progesterone rather than PREG-S. Alternatively, it is possible that activation of the HPA axis via hypothalamic or pituitary stimulation induces the release of other hormones, in addition to ACTH, that could interfere with PREG-S biosynthesis. For instance, it is known that ACTH derives from a large precursor molecule, pro-opiomelanocortin, that gives rise to numerous hormones, including opioid peptides and melanocyte stimulating hormone (Raffin-Sanson et al., 2003).

In contrast to PREG-S, deoxycorticosterone had a circadian variation (Tan and Mulrow, 1975) which is similar to that observed for ACTH and cortisol in the same subjects (Adinoff et al., 2005a). Furthermore, plasma deoxycorticosterone and cortisol appear to be similarly regulated by challenges to the HPA axis, as shown by the correlations in deoxycorticosterone and cortisol responses following naloxone, oCRH, and cosyntropin challenges. In addition, naloxone, oCRH, cosyntropin and cosyntropin following dexamethasone all significantly increase deoxycorticosterone levels and dexamethasone decreases deoxycorticosterone levels. These findings are consistent with other studies assessing deoxycorticosterone response to pharmacological challenges (Brown and Strott, 1971; Tan and Mulrow, 1975; Kater et al., 1990).

The second aim of this study was to explore putative differences in basal and stimulated PREG-S and deoxycorticosterone levels between control subjects and abstinent alcohol-dependent patients. The finding that basal PREG-S did not differ between healthy controls and abstinent alcohol-dependent patients contrasts with a previous study showing higher plasma PREG-S levels in premenopausal women undergoing alcohol detoxification treatment compared to controls (Hill et al., 2005). Either sex differences or length of abstinence could be responsible for this discrepancy. The blunted PREG-S response in alcohol-dependent patients following cosyntropin is similar to the blunted cortisol response observed in the same subjects by Adinoff et al. (2005a) (following dexamethasone pretreatment) and Wand and Dobs (1991) (without dexamethasone pretreatment). Other experimental evidence suggests that suppressed PREG-S levels might be relevant in alcoholism. Administration of PREG-S to rodents improves cognition (Vallee et al., 2001) and cognitive impairment has been observed inalcoholics (Bates et al., 2002). In agreement, PREG-S prevents the memory-impairing effects of ethanol in mice (Melchior and Ritzmann, 1996). Therefore, diminished PREG-S responses to adrenal activation may contribute to cognitive impairment in alcoholism.

Overall, we did not observe significant differences in basal or stimulated deoxycorticosterone levels between controls and alcohol-dependent subjects. Basal deoxycorticosterone levels have never previously been measured in alcohol-dependent subjects. In cynomolgus monkeys basal deoxycorticosterone levels were dramatically increased after long-term alcohol exposure (Porcu et al., 2006b). Furthermore, cynomolgus monkeys show a blunted deoxycorticosterone response to CRH and dexamethasone challenges (Porcu et al., 2006b). In addition, rats after chronic ethanol exposure had blunted deoxycorticosterone responses to an acute ethanol challenge (Khisti et al., 2005). These discrepancies might be the result of species differences or they might be due to different experimental conditions: assessment immediately following chronic alcohol exposure in rats and cynomolgus monkeys versus one-month abstinence in humans. In addition, more subtle findings of deoxycorticosterone alterations in the one-month abstinent alcohol-dependent patients, including a delayed response to oCRH and a non-statistically significant ($p=0.055$) suppressed response to cosyntropin, may yield significant differences in future studies with a more thorough time course and increased group size.

A methodological strength of this study is the population. Patients and controls were similar in age and race. Although there was a statistically significant difference in age between the two groups, the HPA axis remains functioning stable over this age range. Neither group had significant medical disorders nor was taking any psychotropic drugs or other medications. Period of abstinence in alcohol-dependent patients was controlled such that the study occurred within four-six weeks after the last drink. However, alcohol-dependent patients were almost all nicotine-dependent while controls were not. Although a previous study reported that cigarette smoking in abstinent alcohol-dependent subjects did not alter the pituitary-adrenal response to a pharmacological challenge (Anthenelli et al., 2001), a more recent study found that 3α,5α-THP levels may be up-regulated in smokers and dehydroepiandrosterone sulfate levels may predict nicotine dependence (Marx et al., 2006). Indeed, acute administration of nicotine alters pregnenolone, progesterone, 3α,5α-THP and 3α,5α-THDOC levels in rat brain and plasma (Porcu et al., 2003).

The major limitation of the study is that we had only few time points available; thus, our basal measures preceded the challenge infusions by 20 to 45 min and time points after the infusion were analyzed based on plasma availability. Therefore, it is possible that we may have missed the actual peak level of PREG-S and deoxycorticosterone. Other limitations include the high prevalence of psychologicalstress (divorced, unemployed, homeless) in the alcohol-dependent group, although these subjects did not meet criterion for depression or anxiety disorders. Stress is known to interfere with HPA axis functioning and we cannot rule out the possibility that the alterations in PREG-S and deoxycorticosterone responses to HPA axis challenge seen in these patients are strictly related to the alcohol-dependence. Also, recent findings using highly sensitive mass spectrometry techniques suggest that PREG-S is not present in rat brain and plasma (Liu et al., 2003; Liere et al., 2004). However, these authors were able to measure PREG-S levels in human plasma and values are comparable to the ones we obtained using a radioimmunoassay with a commercially available antibody.

In conclusion, we have shown that HPA axis modulation by pharmacological challenges differentially regulates the neuroactive steroids PREG-S and deoxycorticosterone in humans. The small number of subjects is a limitation that precludes definitive conclusions and argues for further studies in alcohol-dependent subjects, examining other stages of alcohol dependence (we only looked at one-month abstinent alcohol-dependent patients) and women. Moreover, since deoxycorticosterone is the precursor of other GABAergic neuroactive steroids, it is possible that its metabolites, or other pregnenolone metabolites, may be altered in alcohol dependence. Nonetheless, these results add new information on neuroactive steroids in alcohol-dependent subjects; since neuroactive steroids influence ethanol sensitivity (Morrow et al., 2006), they might contribute to the underlying pathology.

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References

Adinoff B, Krebaum SR, Chandler PA, Ye W, Brown MB, Williams MJ. Dissection of hypothalamic-pituitary-adrenal axis pathology in 1-month-abstinent alcohol-dependent men, part 1: adrenocortical and pituitary glucocorticoid responsiveness. Alcohol Clin Exp Res 2005a;29:517–527. [PubMed: 15834216]

Adinoff B, Krebaum SR, Chandler PA, Ye W, Brown MB, Williams MJ. Dissection of hypothalamic-pituitary-adrenal axis pathology in 1-month-abstinent alcohol-dependent men, part 2: response to ovine corticotropin-releasing factor and naloxone. Alcohol Clin Exp Res 2005b;29:528–537. [PubMed: 15834217]

Adinoff B, Martin PR, Bone GH, Eckardt MJ, Roehrich L, George DT, Moss HB, Eskay R, Linnoila M, Gold PW. Hypothalamic-pituitary-adrenal axis functioning and cerebrospinal fluid corticotropin releasing hormone and corticotropin levels in alcoholics after recent and long-term abstinence. Arch Gen Psychiatry 1990;47:325–330. [PubMed: 2157379]

Anthenelli RM, Maxwell RA, Geracioti TDJ, Hauger R. Stress hormone dysregulation at rest and after serotonergic stimulation among alcohol-dependent men with extended abstinence and controls. Alcohol Clin Exp Res 2001;25:692–703. [PubMed: 11411461]

Barbaccia ML, Affricano D, Trabucchi M, Purdy RH, Colombo G, Agabio R, Gessa GL. Ethanol markedly increases "GABAergic" neurosteroids in alcohol-preferring rats. Eur J Pharmacol 1999;384:R1–R2. [PubMed: 10611449]

Bates ME, Bowden SC, Barry D. Neurocognitive impairment associated with alcohol use disorders: implications for treatment. Exp Clin Psychopharmacol 2002;10:193–212. [PubMed: 12233981]

Baulieu EE. Neurosteroids: a novel function of the brain. Psychoneuroendocrinology 1998;23:963–987. [PubMed: 9924747]

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1979;4:561–571. [PubMed: 13688369]

Boizel R, De Peretti E, Cathiard A, Halimi S, Bost M, Berthezene F, Saez J. Pattern of plasma levels of cortisol, dehydroepiandrosterone and pregnenolone sulphate in normal subjects and in patients with homozygous familial hypercholesterolaemia during ACTH infusion. Clin Endocrinol (Oxf) 1986;25:363–371. [PubMed: 3040299]

Brown RD, Strott CA. Plasma deoxycorticosterone in man. J Clin Endocr 1971;32:744–750. [PubMed: 4325217]

Caldeira JC, Wu Y, Mameli M, Purdy RH, Li P-K, Akwa Y, Savage DD, Engen JR, Valenzuela CF. Fetal alcohol exposure alters neurosteroid levels in the developing rat brain. J Neurochem 2004;90:1530–1539. [PubMed: 15341536]

Costa A, Bono G, Martignoni E, Merlo P, Sances G, Nappi G. An assessment of hypothalamo-pituitary-adrenal axis functioning in nondepressed early abstinent alcoholics. Psychoneuroendocrinology 1996;21:263–275. [PubMed: 8817725]

Craig CR. Anticonvulsant activity of steroids: separability of anticonvulsant from hormonal effects. J Pharmacol Exp Ther 1966;153:337–343.

Davies E, MacKenzie SM. Extra-adrenal production of corticosteroids. Clin Exp Pharmacol Physiol 2003;30:437–445. [PubMed: 12823256]

De Peretti E, Mappus E. Pattern of plasma pregnenolone sulfate levels in humans from birth to adulthood. J Clin Endocrinol Metab 1983;57:550–556. [PubMed: 6308031]

Ehrenreich H, Schuck J, Stender N, Pilz J, Gefeller O, Schilling L, Poser W, Kaw S. Endocrine and hemodynamic effects of stress versus systemic CRF in alcoholics during early and medium term abstinence. Alcohol Clin Exp Res 1997;21:1285–1293. [PubMed: 9347091]

Flood JF, Morley JE, Roberts E. Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it. Proc Natl Acad Sci U S A 1992;89:1567–1571. [PubMed: 1531874]

Flood JF, Morley JE, Roberts E. Pregnenolone sulfate enhances post-training memory processes when injected in very low doses into limbic system structures: The amygdala is by far the most sensitive. Proc Natl Acad Sci U S A 1995;92:10806–10810. [PubMed: 7479888]

Genazzani AR, Petraglia F, Bernardi F, Casarosa E, Salvestroni C, Tonetti A, Nappi RE, Luisi S, Palumbo M, Purdy RH, Luisi M. Circulating levels of allopregnanolone in humans: gender, age, and endocrine influences. J Clin Endocrinol Metab 1998;83(6):2099–2013. [PubMed: 9626145]

Higashi T, Sugitani H, Yagi T, Shimada K. Studies on neurosteroids XVI. Levels of pregnenolone sulfate in rat brains determined by enzyme-linked immunosorbent assay not requiring solvolysis. Biol Pharm Bull 2003;26:709–711. [PubMed: 12736517]

Hill M, Popov P, Havlikova H, Kancheva L, Vrbikova J, Kancheva R, Pouzar V, Cerny I, Starka L. Altered profiles of serum neuroactive steroids in premenopausal women treated for alcohol addiction. Steroids 2005;70:515–524. [PubMed: 15894035]

Inder WJ, Joyce PR, Ellis MJ, Evans MJ, Livesey JH, Donald RA. The effects of alcoholism on the hypothalamic-pituitary-adrenal axis: interaction with endogenous opioid peptides. Clin Endocrinol (Oxf) 1995;43:283–290. [PubMed: 7586596]

Kater CE, Irony I, Biglieri EG, Faiçal S. Continuous adrenocorticotropin administration in hypopituitarism produces asynchronous increases of deoxycorticosterone and 11-deoxycortisol relative to other reduced zona fasciculata steroids. J Clin Endocrinol Metab 1990;71:305–310. [PubMed: 2166067]

Khisti RT, Boyd KN, Kumar S, Morrow AL. Systemic ethanol administration elevates deoxycorticosterone levels and chronic ethanol exposure attenuates this response. Brain Res 2005;1049:104–111. [PubMed: 15936001]

Laatikainen T, Vihko R. Diurnal variation in the concentrations of solvolyzable steroids in human plasma. J Clin Endocr 1968;28:1356–1360. [PubMed: 4300561]

Liere P, Pianos A, Eychenne B, Cambourg A, Liu S, Griffiths W, Schumacher M, Sjovall J, Baulieu EE. Novel lipoidal derivatives of pregnenolone and dehydroepiandrosterone and absence of their sulfated counterparts in rodent brain. J Lipid Res 2004;45:2287–2302. [PubMed: 15342680]

Liu S, Griffiths WJ, Sjovall J. Capillary liquid chromatography/electrospray mass spectrometry for analysis of steroid sulfates in biological samples. Anal Chem 2003;75:791–797. [PubMed: 12622368]

Majewska MD, Mienville JM, Vicini S. Neurosteroid pregnenolone sulfate antagonizes electrophysiological responses to GABA in neurons. Neurosci Lett 1988;90:279–284. [PubMed: 3138576]

Mameli M, Valenzuela CF. Alcohol increases efficacy of immature synapses in a neurosteroid-dependent manner. Eur J Neurosci 2006;23:835–839. [PubMed: 16487164]

Marx CE, Trost WT, Shampine L, Behm FM, Giordano LA, Massing MW, Rose JE. Neuroactive steroids, negative affect, and nicotine dependence severity in male smokers. Psychopharmacology 2006;186:462–472. [PubMed: 16402195]

Melchior CL, Ritzmann RF. Neurosteroids block the memory-impairing effects of ethanol in mice. Pharmacol Biochem Behav 1996;53:51–56. [PubMed: 8848460]

Morrow AL, Porcu P, Boyd KN, Grant KA. Hypothalamic-pituitary-adrenal axis modulation of GABAergic neuroactive steroids influences ethanol sensitivity and drinking behavior. Dialogues Clin Neurosci 2006;8:463–477. [PubMed: 17290803]

Morrow AL, VanDoren MJ, Penland SN, Matthews DB. The role of GABAergic neuroactive steroids in ethanol action, tolerance and dependence. Brain Res Brain Res Rev 2001;37:98–109. [PubMed: 11744078]

Mtchedlishvili Z, Kapur J. A presynaptic action of the neurosteroid pregnenolone sulfate on GABAergic synaptic transmission. Mol Pharmacol 2003;64:857–864. [PubMed: 14500742]

Owens MJ, Ritchie JC, Nemeroff CB. 5α-Pregnane-3α,21-diol-20-one (THDOC) attenuates mild stress-induced increases in plasma corticosterone via a non-glucocorticoid mechanism: comparison with alprazolam. Brain Res 1992;573:353–355. [PubMed: 1504771]

Patchev VK, Hassan AHS, Holsboer F, Almeida OFX. The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. Neuropsychopharmacology 1996;15:533–540. [PubMed: 8946427]

Patchev VK, Shoaib M, Holsboer F, Almeida OFX. The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. Neuroscience 1994;62:265–271. [PubMed: 7816204]

Paul SM, Purdy RH. Neuroactive steroids. FASEB Journal 1992;6:2311–2322. [PubMed: 1347506]

Porcu P, Grant KA, Green HL, Rogers LS, Morrow AL. Hypothalamic-pituitary-adrenal axis and ethanol modulation of deoxycorticosterone levels in cynomolgus monkeys. Psychopharmacology 2006a; 186:293–301. [PubMed: 16133132]

Porcu, P.; Grant, KA.; Morrow, AL. 2006 Neuroscience Meeting Planner. Atlanta, GA: Society for Neuroscience; 2006b. Long-term voluntary alcohol consumption increases plasma deoxycorticosterone levels and alters hypothalamic-pituitary-adrenal axis modulation in cynomolgus monkeys. Online Program No. 192.3

Porcu P, Rogers LSM, Morrow AL, Grant KA. Plasma pregnenolone levels in cynomolgus monkeys following pharmacological challenges of the hypothalamic-pituitary-adrenal axis. Pharmacol Biochem Behav 2006c;84:618–627. [PubMed: 16790266]

Porcu P, Sogliano C, Cinus M, Purdy RH, Biggio G, Concas A. Nicotine-induced changes in cerebrocortical neuroactive steroids and plasma corticosterone concentrations in the rat. Pharmacol Biochem Behav 2003;74:683–690. [PubMed: 12543235]

Raffin-Sanson ML, de Keyzer Y, Bertagna X. Proopiomelanocortin, a polypeptide precursor with multiple functions: from physiology to pathological conditions. Eur J Endocrinol 2003;149:79–90. [PubMed: 12887283]

Reddy DS, Rogawski MA. Stress-induced deoxycorticosterone-derived neurosteroids modulate GABAA receptor function and seizure susceptibility. J Neurosci 2002;22:3795–3805. [PubMed: 11978855]

Selye H. The antagonism between anesthetic steroid hormones and pentamethylenetetrazol. J Lab Clin Med 1942;27:1051–1053.

Spencer RL, McEwen BS. Adaptation of the hypothalamic-pituitary-adrenal axis to chronic ethanol stress. Neuroendocrinology 1990;52:481–489. [PubMed: 2126355]

Stoffel-Wagner B. Neurosteroid metabolism in the human brain. Eur J Endocrinol 2001;145:669–679. [PubMed: 11720889]

Tan SY, Mulrow PJ. The contribution of the zona fasciculata and glomerulosa to plasma 11-deoxycorticosterone levels in man. J Clin Endocrinol Metab 1975;41:126–130. [PubMed: 168227]

Torres JM, Ortega E. DHEA, PREG and their sulphate derivatives on plasma and brain after CRH and ACTH administration. Nurochem Res 2003;28:1187–1191.

Vallee M, Mayo W, Le Moal M. Role of pregnenolone, dehydroepiandrosterone and their sulfate esters on learning and memory in cognitive aging. Brain Res Brain Res Rev 2001;37:301–312. [PubMed: 11744095]

Wand GS, Dobs AS. Alterations in the hypothalamic-pituitary-adrenal axis in actively drinking alcoholics. J Clin Endocrinol Metab 1991;72:1290–1295. [PubMed: 2026749]

Wu F-S, Gibbs TT, Farb DH. Pregnenolone sulfate: A positive allosteric modulator at the *N*-methyl-D-aspartate receptor. Mol Pharmacol 1991;40:333–336. [PubMed: 1654510]

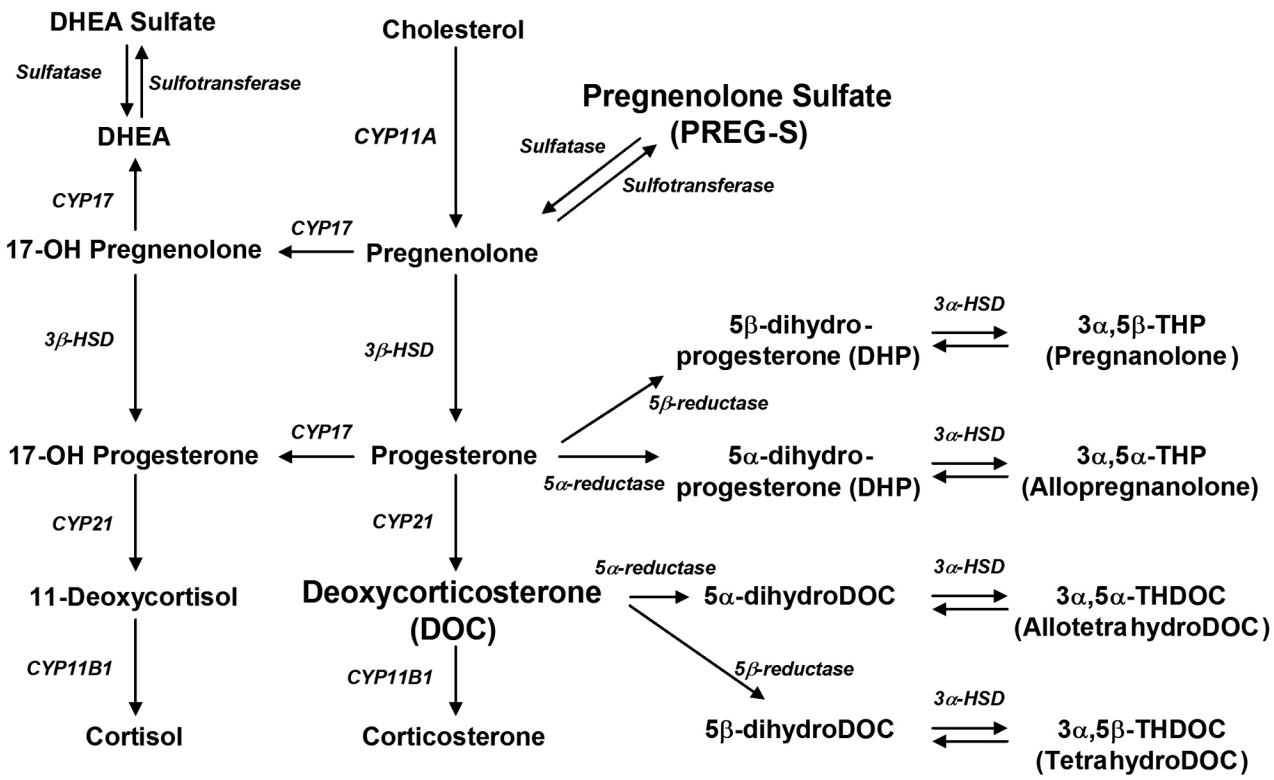

Figure 1.
Schematic representation of the neuroactive steroids biosynthetic pathway from human adrenal glands.

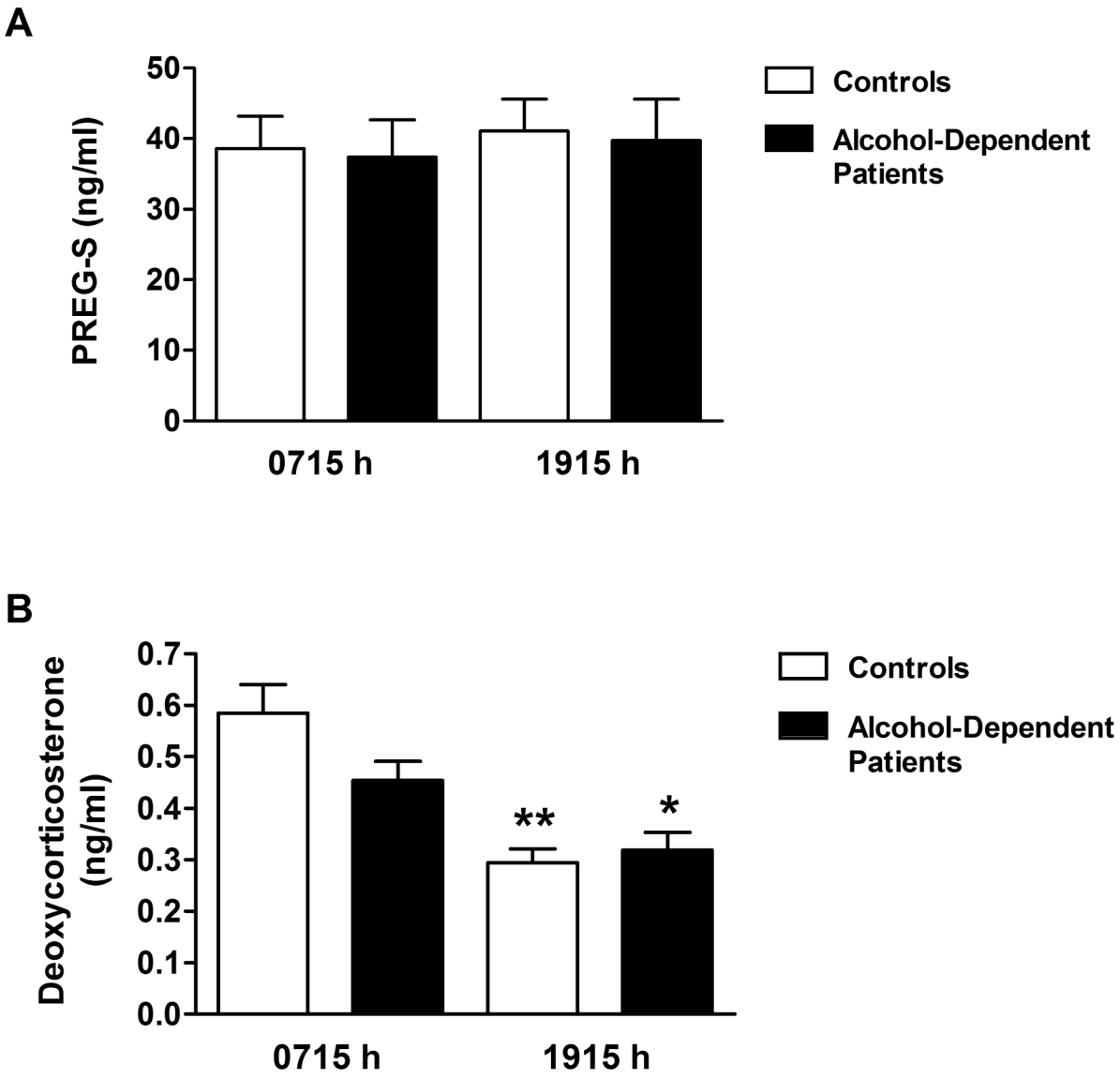

Figure 2.

Diurnal baseline plasma PREG-S (A) and deoxycorticosterone (B) levels in control subjects (n=10) and alcohol-dependent patients (n=11). Data (mean ± SEM) are expressed as ng/ml. Asterisks indicate significant difference between the time points: *p<0.05 and **p<0.001 (two-way ANOVA).

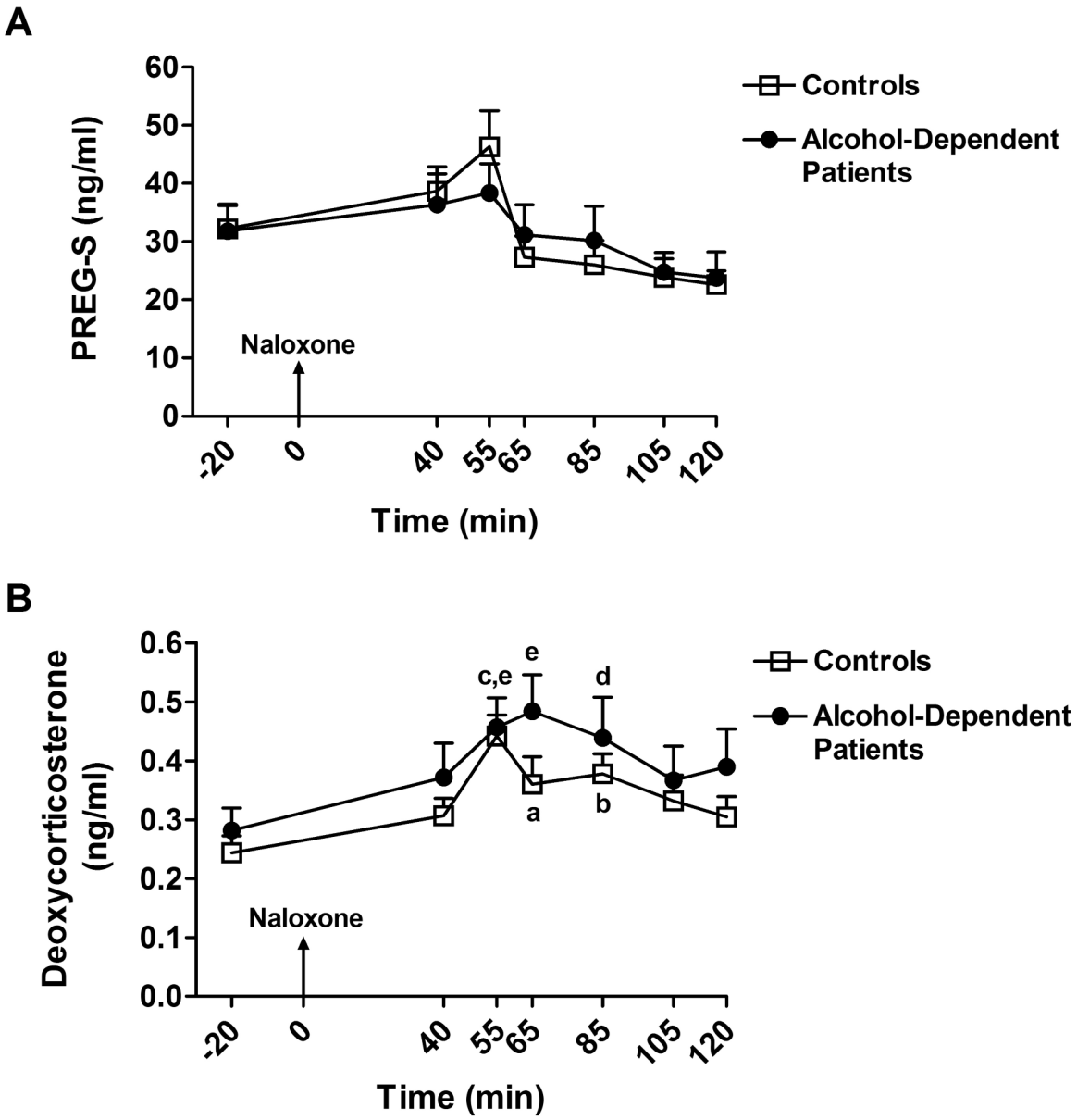

Figure 3.

Time course of plasma PREG-S (A) and deoxycorticosterone (B) levels in control subjects (n=10) and alcohol-dependent patients (n=10) after administration of naloxone (125 μg/kg, IV) at 2000h. Data (mean ± SEM) are expressed as ng/ml. Letters indicate significant difference from basal measures: [a]$p<0.05$, [b]$p<0.01$ and [c]$p<0.001$ vs. −20 min for control subjects; [d]$p<0.01$ and [e]$p<0.001$ vs. −20 min for alcohol-dependent patients (two-way repeated measures ANOVA).

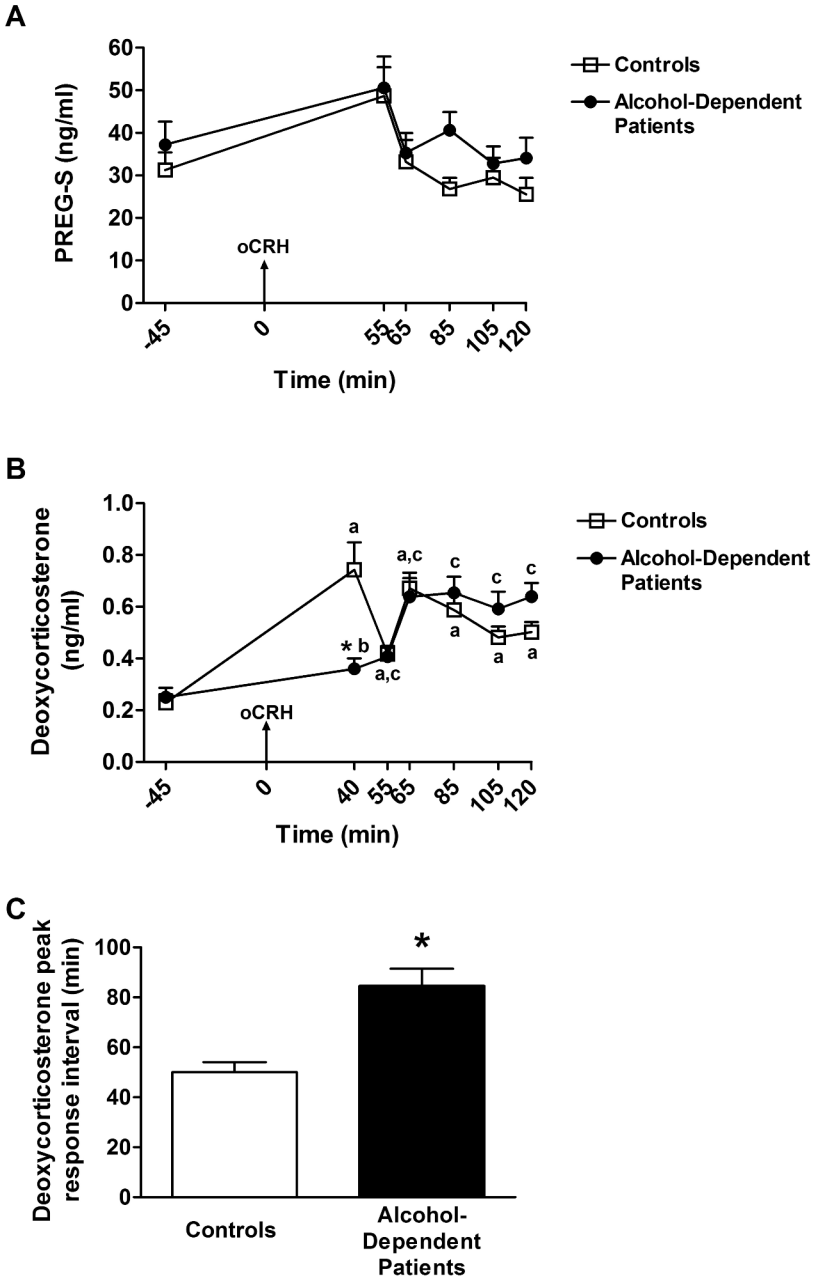

Figure 4.

Time course of plasma PREG-S (A) and deoxycorticosterone (B) levels in control subjects (n=10) and alcohol-dependent patients (n=11) after administration of oCRH (0.4 μg/kg, IV) at 2000h. Data (mean ± SEM) are expressed as ng/ml. Asterisk indicates significant difference between controls and alcohol-dependent patients: *p<0.001; letters indicate significant difference from basal measures: [a]p<0.001 vs. −45 min for control subjects; [b]p<0.05 and [c]p<0.001 vs. −45 min for alcohol-dependent patients (two-way ANOVA). (C) Data (mean ± SEM) represent the mean time interval (minutes) necessary to reach the deoxycorticosterone peak response after administration of oCRH in control subjects and alcohol-dependent patients (*p=0.0023, paired t test).

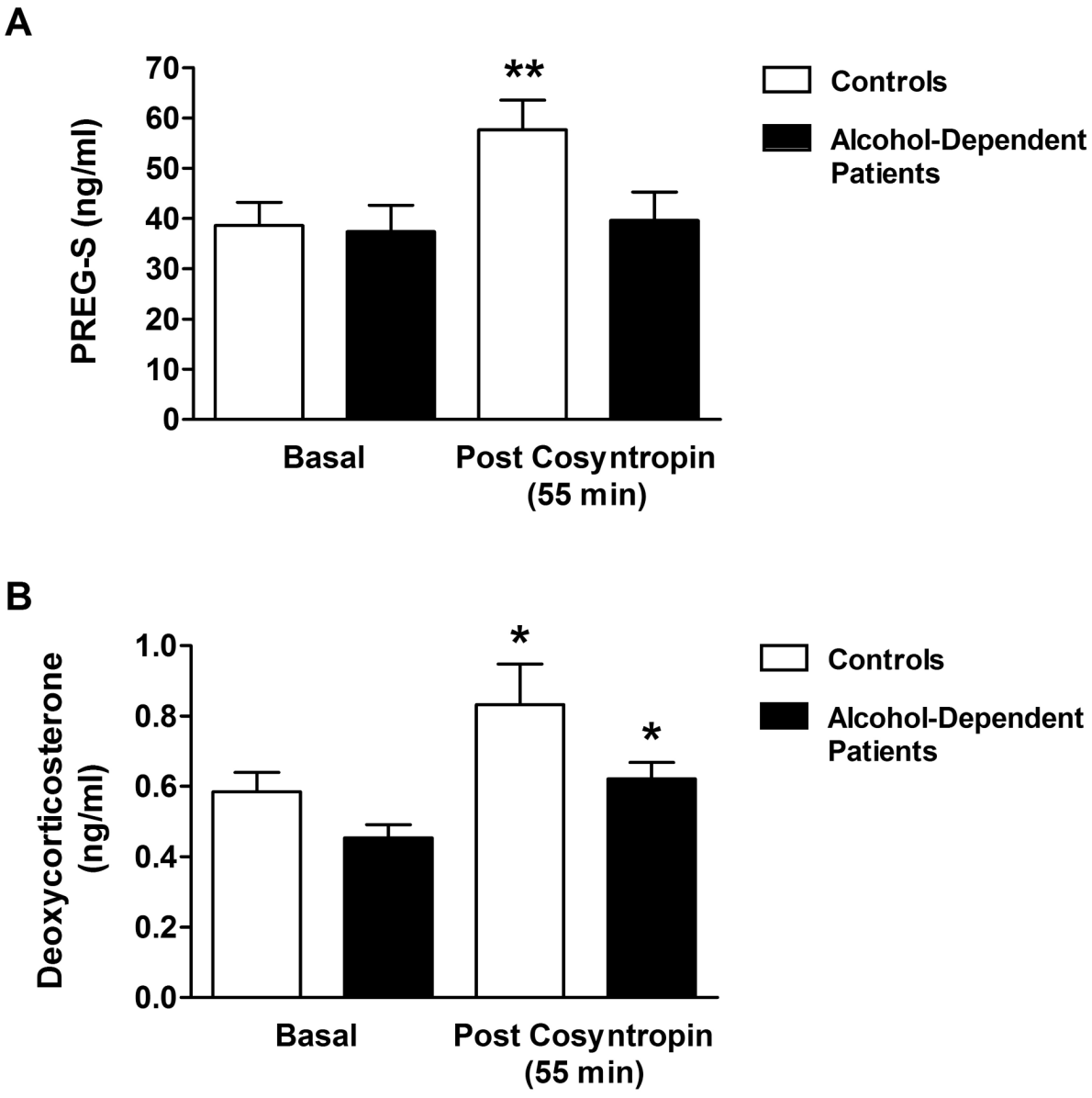

Figure 5.

Effect of cosyntropin on plasma PREG-S (A) and deoxycorticosterone (B) levels incontrol subjects (n=10) and alcohol-dependent patients (n=11). Cosyntropin (0.01 μg/kg, IV) was administered at 0800h. Data (mean ± SEM) are expressed as ng/ml. Asterisks indicate significant differences between basal and cosyntropin groups: *p<0.01 and**p<0.001 vs. the respective basal group (two-way repeated measures ANOVA).

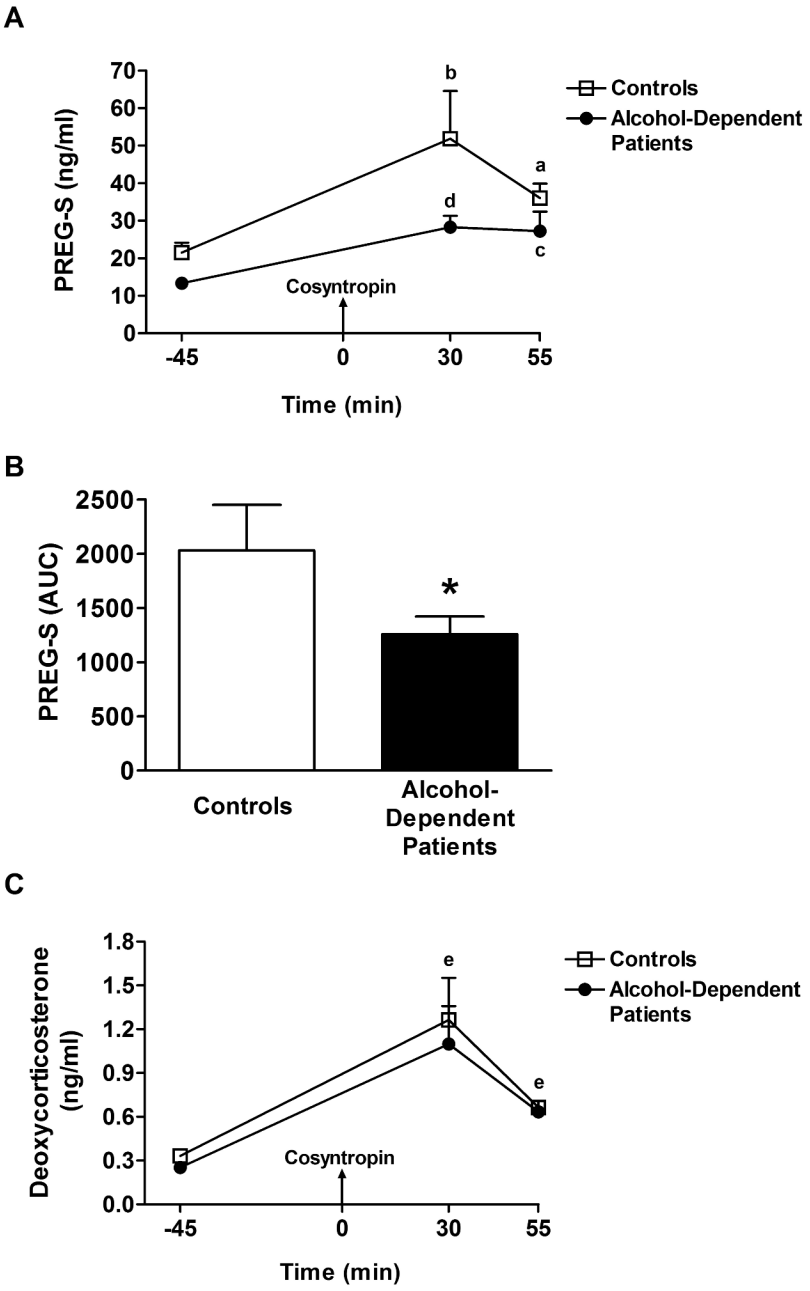

Figure 6.

Time course of plasma PREG-S (A) and deoxycorticosterone (C) levels in control subjects (n=10) and alcohol-dependent patients (n=11) after administration of dexamethasone plus cosyntropin. Dexamethasone (8 mg, IV) was administered at 2300h; cosyntropin (0.01 μg/kg, IV) was administered at 0800h the following day. Data (mean ± SEM) are expressed as ng/ml. Letters indicate significant difference from basal measures: $^{a}p<0.05$ and $^{b}p<0.01$ for control subjects; $^{c}p<0.01$ and $^{d}p<0.001$ for alcohol-dependent patients; $^{e}p<0.001$ for both groups (two-way ANOVA). Section B shows the PREG-S area under the curve (AUC) for controls and alcohol-dependent patients following dexamethasone plus cosyntropin administration.

Asterisk indicates significant difference between controls and alcohol-dependent patients: *p=0.037 (Unpaired t test).

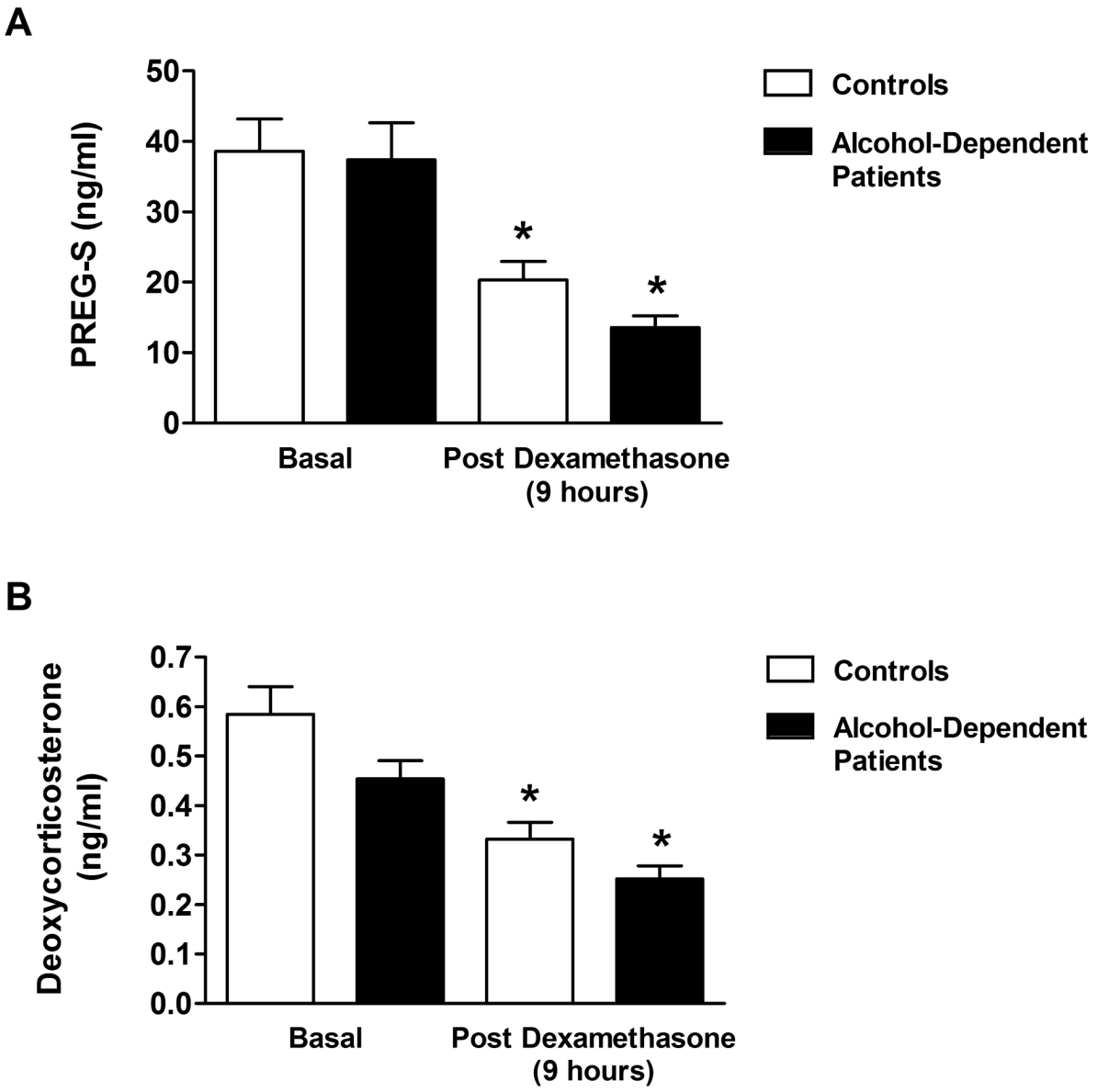

Figure 7.

Effect of dexamethasone on plasma PREG-S (A) and deoxycorticosterone (B) levels incontrol subjects (n=10) and alcohol-dependent patients (n=11). Dexamethasone (8 mg, IV) was administered at 2300h and PREG-S and deoxycorticosterone levels were measured nine hours later. Basal levels were obtained from a 0715h sample. Data (mean ± SEM) are expressed as ng/ml. Asterisks indicate significant differences between basal and dexamethasone groups: *p<0.001 vs. the respective basal group (two-way repeated measures ANOVA).

Table 1

Demographic and clinical characteristics of alcohol-dependent participants and healthy controls.

	Group 1		Group 2	
	Patients (n = 11)	**Controls (n = 10)**	**Patients (n = 11)**	**Controls (n = 10)**
Age (years)	43.5 ± 4.8	38.2 ± 5.4	42.8 ± 4.0	39.6 ± 5.7
Race				
Asian	0	1	0	1
White	8	8	6	7
African-American	3	0	3	1
Hispanic	0	1	2	1
Marital status				
Single	1	0	0	2
Married	0	5	1	7
Separated	1	0	4	0
Divorced	9	1	6	1
Employed	6	10	3	10
Homeless	3	0	8	0
Education (years)	12.3 ± 1.7	16.9 ± 2.8	13.0 ± 1.3	16.4 ± 2.9
Nicotine dependence	10	0	9	1
Beck Depression Score	10.3 ± 7.8	0.7 ± 1.6	8.0 ± 4.2	0.9 ± 1.7
ALT (units/liter)	24.8 ± 18.2	27.7 ± 12.0	59.0 ± 60.5	31.1 ± 10.4
AST (units/liter)	21.6 ± 8.5	24.1 ± 6.0	57.1 ± 47.6	33.8 ± 27.0
DrInC*	34.1 ± 10.7	3.1 ± 2.8	37.6 ± 6.3	3.7 ± 3.5
Years problem drinking	23.9 ± 5.4		20.8 ± 5.8	
Drinks in past 30 days	637 ± 331		853 ± 577	
Lifetime drinks	146193 ± 82485		131129 ± 106528	

Table is adapted from Adinoff et al., 2005a,b.

Data are mean ± Standard Deviation.

*Drinker Inventory of Consequences

Table 2

Recapitulative table of results.

	PREG-S		Deoxycorticosterone	
	Controls	**Patients**	**Controls**	**Patients**
Basal measures	No change	No change	Evening decrease	Evening decrease
Naloxone challenge	No change	No change	Increase	Increase
oCRH challenge	No change	No change	Increase	Delayed increase*
Cosyntropin challenge	Increase	No change*	Increase	Increase
Dexamethasone plus cosyntropin challenge	Increase	Blunted increase*	Increase	Increase
Dexamethasone challenge	Decrease	Decrease	Decrease	Decrease

Summary of the pharmacological effects of each challenge on PREG-S and deoxycorticosterone plasma levels in healthy subjects (Controls) and one month abstinent alcohol-dependent subjects (Patients). Asterisks indicate significant differences between controls and patients.