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Exogenous cortisol exerts effects on the startle reflex independent of emotional modulation

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Abstract

Exogenous cortisol's modulation of the acoustic startle reflex (ASR) was tested alone and during exposure to affectively valenced photographs in healthy men and women. During nonmodulated startle, oral hydrocortisone had a biphasic dose effect, with 5 mg increasing and 20 mg decreasing, eyeblink reflex magnitude compared to placebo. During emotion modulation, 20 mg of hydrocortisone reduced reflex magnitude without affecting the usual pattern of modulation across positive, neutral, and negatively affective slides. Gender differences were not found in either relationship. These findings illustrate dose-dependent effects of cortisol on the startle pathway independent of emotional state and consistent across genders. © 2001 Elsevier Science Inc. All rights reserved.

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Cortisol participates in fuel homeostasis and cellular metabolism (Munck et al., 1984). Its diurnal and metabolic secretion is regulated by the hypothalamic–pituitary–adrenocortical axis (HPA) through negative feedback at the hypothalamus and pituitary (McEwen, 1992). In addition, cortisol release is a core component of the stress response, which is regulated above the level of the hypothalamus through a system of neurons expressing corticotropin-releasing hormone (CRH). Feedback of cortisol on these extrahypothalamic systems is poorly understood. In rat models, long-term corticosterone administration appears to increase anxiety behaviors (Corodimas et al., 1994; Korte et al., 1996; Pugh et al., 1997; Shepard et al., 2000), while short-term exposure is anxiolytic (File et al., 1979). Although in humans, affective disorders, including anxiety and depression, are accompanied by altered cortisol regulation (Arborelius et al., 1999; File, 1996), little is known about cortisol's acute effects on affective state and indices of anxiety.

One method to assess anxiety is to employ manipulations that enhance or diminish the acoustic startle reflex (ASR) (Davis, 1979, 1992; Davis et al., 1979; Lang et al., 1998). The ASR is a protective response that involves both skeletal motor and autonomic components (Yeomans and Frankland, 1996). In animal models, the ASR is potentiated by exposure to conditioned fear stimuli or anxiogenic drugs and diminished by reward stimuli or anxiolytic drugs (Davis, 1979, 1992; Davis et al., 1979; Lang et al., 1998). In humans, the ASR is similarly increased by viewing negatively affective photographs, and decreased while viewing positively affective photographs (Cook et al., 1992; Lang et al., 1998; Sutton et al., 1997; Vrana et al., 1988).

Hormones of the HPA axis also affect the ASR. Intracerebroventricular infusions of CRH result in increased ASR magnitude in rats (Swerdlow et al., 1986), an effect that is blocked by the CRH antagonist α-helical CRH (9–41) (Swerdlow et al., 1989). The peptide fragment, adrenocorticotropic hormone$_{4-10}$ (ACTH$_{4-10}$; a structurally related analogue of ACTH), increases the ASR in adult rats after neonatal treatment with high doses of the peptide (McGivern

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et al., 1987). Intraperitoneal injections of corticosterone, on the other hand, decrease the ASR (Sandi et al., 1996). Similarly, antagonism of both the mineralocorticoid (MR) and the glucocorticoid (GR) receptors — and, thus, blockade of negative feedback on the HPA axis — leads to an increase in the ASR (Korte et al., 1996). These studies suggest that corticosteroids may exert a tonic suppression on the ASR, perhaps via negative feedback effects on CRH and ACTH.

The purpose of the present studies was first to evaluate the effects of two doses of cortisol (5 and 20 mg) on the unmodulated ASR and subjective reports of anxiety in healthy men and women (Experiment 1), and then to ascertain whether cortisol-induced changes in the reflex are active via the same mechanisms as emotional modulation of the ASR by affectively valenced photographs (Experiment 2). If cortisol exerts a tonic suppressive effect on the ASR via negative feedback on ACTH and CRH, we would expect a dose-dependent reduction in ASR magnitude in Experiment 1. If cortisol's effects on the ASR act via the same mechanisms of emotional modulation, then cortisol should result in an altered pattern of emotional modulation of the reflex.

1. Experiment 1

1.1. Participants

Participants were 12 healthy volunteers, six men and six women, ranging in age from 20 to 40 years, in good health by history and physical examination, not taking any chronic medication, smoking less than 10 cigarettes per day, consuming less than two alcoholic drinks per day, not allergic to hydrocortisone, with no reported history of alcohol or drug abuse, and having a normal nighttime sleep pattern. All participants signed a consent form approved by the Institutional Review Board of the University of Oklahoma Health Sciences Center and the Veterans Affairs Medical Center and were paid for participation.

1.2. Procedure

In a within-subjects design, participants received either placebo or oral hydrocortisone (5 or 20 mg) single blind in a counterbalanced pseudorandom order on each of three experimental sessions begun between 1300 and 1400 h on separate days. Upon arrival at the laboratory on each test day, an initial saliva cortisol sample was taken and electrodes were attached. Participants were seated in a comfortable chair. At this point, the drug was administered. Following a 30-min adaptation period, participants received sets of startle probes at 30, 60, 90, and 120 min postdrug. At each time point, 12 startle probes were presented over 3 min at pseudorandom intervals ranging from 3 to 45 s. Participants provided self-reports of anxiety and saliva samples at the end of each set of probes.

1.3. Apparatus and materials

Hydrocortisone (5 and 20 mg; Pharmacia and Upjohn, Peapack, NJ) and identical appearing placebo capsules were prepared by a local pharmacy (University Hospital Pharmacy, Oklahoma City, OK).

Startle stimuli were 105 dB, 50 ms white noise bursts with 3–5 ms rise times generated by a Coulbourn V85-05 noise generator with the audio gate set to 0 ms, amplified by a Radio Shack Optimus SA 155 power amplifier, and delivered through matched Telefonics TDH 49 headphones. Sound pressure level was calibrated using an AA-188 Audiometric Analyzer (Quest Electronics, Oconomowoc, WI).

EMG activity from the orbicularis oculi was collected using two In Vivo Metrics (Healdsburg, CA) minielectrodes (3-mm diameter) placed directly below the left eye (as described in Vrana et al., 1988). A third minielectrode was placed behind the right ear over the mastoid region and used as reference. Electrode impedances were less than 10,000 Ω. Raw signals were amplified $\times 10{,}000$ using a Coulbourn V75-05 Bioamplifier with bandpass filters set at 8 and 150 Hz, and then rectified and integrated using a Coulbourn V76-23 contour-following integrator with the time constant set at 10 ms. Analysis of EMG activity $^{2}(\mu v)$ was performed on the integrated signal.

Saliva samples were collected using a commercially available collection device (Salivette, Sarstedt, Germany), centrifuged and stored at $-70°C$ until assayed. Salivary free cortisol concentrations were measured by radioimmunoassay with a commercially available kit (Orion Diagnostica, Espoo, Finland) adapted to measure the low cortisol concentrations observed in saliva. Saliva samples were mixed with a fixed amount of ^{125}I-labelled cortisol derivative and cortisol antiserum. The labelled and unlabelled antigens were then allowed to compete for the high affinity binding sites of the antibody during an incubation period. The separation of bound and unbound antigen was performed with polyethylene glycol. The amount of labelled antigen in the sample is inversely proportional to the concentration of the unlabelled antigen. The actual concentrations in the unknown samples were obtained by means of a standard curve based on known concentrations of unlabelled antigen analyzed in parallel to the unknown. Intra- and inter-assay coefficient of variations were 6% and 12%, respectively. Anxiety was documented using the state version of the State/Trait Anxiety Inventory (STAI; see Spielberger et al., 1970).

1.4. Data acquisition and analyses

Orbicularis oculi EMG activity in response to acoustic startle probes was processed to reflect eyeblink reflex magnitudes. Integrated EMG activity in response to each probe was computer scored and then reviewed. A small percentage of eyeblink reflexes was excluded because of excessive noise. Eyeblink reflex response values were cal-

Table 1
Experiment 1 salivary cortisol levels across days and periods

	20 mg		5 mg		Placebo	
	Men	Women	Men	Women	Men	Women
Bsl	2.3 (0.4)	3.1 (1.5)	2.1 (0.3)	1.4 (0.4)	2.4 (0.4)	1.9 (0.1)
30	7.3 (4.8)	5.7 (2.6)	2.8 (0.9)	2.0 (0.5)	4.3 (2.4)	2.8 (0.3)
60	11.1 (5.3)	27.7 (14.2)	3.2 (0.7)	4.1 (0.8)	2.1 (0.4)	1.9 (0.2)
90	14.2 (2.6)	22.9 (6.2)	3.1 (0.3)	4.2 (0.7)	1.6 (0.2)	1.2 (0.1)
120	18.7 (3.7)	20.4 (2.0)	3.4 (0.6)	5.1 (0.9)	1.3 (0.1)	2.1 (0.7)

Entries show mean (S.E.M.) salivary cortisol levels in nanograms per milliliter at baseline (Bsl) and 30, 60, 90, and 120-min postdrug.

culated by subtracting the integrated EMG signal during the 30 ms before probe onset from the peak magnitude recorded between 40 and 120 ms following probe onset. EMG blink magnitudes are expressed in the standardized *z*-score metric (i.e., mean = 0, S.D. = 1) using the overall mean and standard deviation from all participants across all three sessions.

The primary independent variables were drug dose (5 and 20 mg hydrocortisone or placebo), time of startle measurement (30, 60, 90, and 120-min postdrug), and gender of subject. The dependent variables were the eyeblink EMG magnitude, self-reports of anxiety, and salivary cortisol levels.

The analyses were conducted using a 3 Drug Doses (0, 5, and 20 mg hydrocortisone) × 2 Gender × 4 Time Periods (30, 60, 90, and 120-min postdrug) repeated measures analysis of variance (ANOVA) with gender as a between-subjects variable and drug and time as within-subjects variables for both ASR magnitude and salivary cortisol levels. After finding no gender differences in either startle magnitude or response to cortisol, the data were collapsed over this variable. Predrug differences in anxiety level were countered by using the predrug anxiety level as a covariate in analyses of these data. This resulted in a 3 Drug Doses × 2 Gender × 4 Time Periods repeated measures analysis of covariance (ANCOVA) with averaged predrug anxiety as a covariate. All analyses on within-subjects variables used the mixed-model univariate ANOVA, as opposed to the multivariate approach due to the relatively small sample size, as suggested by Maxwell and Delaney (1990). The Greenhouse–Geisser epsilon correction procedure (Geisser and Greenhouse, 1959) was used in order to control for the inflated Type I error rate associated with the mixed-model univariate ANOVA when the sphericity assumption is not met. When testing pairwise contrasts in repeated measures analyses, the Bonferroni approach for reducing Type I errors was adopted (Maxwell and Delaney, 1990).

2. Results

2.1. Salivary cortisol levels

There were significant differences in cortisol levels measured in saliva across the 3 days, $F(2,20) = 17.5$, $P = .002$, $\varepsilon = 0.517$. As expected, the 20-mg dose resulted in the largest increase in cortisol compared to both the 5-mg, $F(1,10) = 5.6$, $P = .01$, and the placebo days, $F(1,10) = 15.6$, $P = .008$. Women tended to have higher salivary cortisol levels after administration of either dose of cortisol, but this gender difference was not significant, $F(1,10) < 1$ (see Table 1).

2.2. Anxiety reports

Predrug anxiety reports were different across the 3 days [$F(2,10) = 4.3$, $P = .045$]. Analysis of covariance with predrug anxiety level as a covariate revealed no difference in self-reported postdrug anxiety across the three drug doses, $F(2,20) < 1$, $P > .4$, $\varepsilon = 0.718$. Additionally, there were no differences in anxiety reported across periods, $F(2,20) < 1$, $P > .7$, $\varepsilon = 0.569$.

2.3. Startle reflex magnitude

Fig. 1 shows *z*-transformed startle magnitudes across the three doses. There was a marginally significant effect of cortisol on startle magnitude, $F(2,22) = 3.3$, $P = .059$, $\varepsilon = 0.916$. A trend analysis revealed a marginally significant quadratic trend [$F(1,11) = 4.4$, $P = .06$], reflecting a biphasic effect of cortisol on startle. Follow-up contrasts revealed a lower reflex magnitude after the 20-mg dose compared to the 5-mg dose, $F(1,11) = 5.4$, $P = .04$ (means and standard errors of raw EMG data expressed in volts across drug conditions: Placebo: 0.053 ± 0.011; 5 mg: 0.061 ± 0.011; 20 mg: 0.053 ± 0.012). There was no significant difference between either drug dose and placebo. There was a significant reduction in the magnitude of the startle reflex across periods [$F(3,33) = 8.3$, $P = .004$, $\varepsilon = 0.545$], illustrating the well-documented habituation of the reflex over repeated expo-

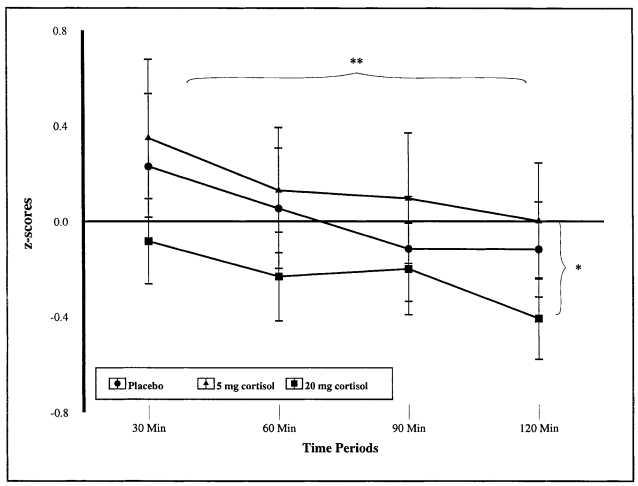

Fig. 1. Experiment 1. Bars show *z*-transformed startle magnitudes for placebo and each cortisol dose across periods. Error bars show ±S.E.M. Time periods reflect time elapsed postdrug. ** Significant reduction in startle magnitude across all three doses ($P = .004$). * Significantly lower startle magnitudes on the 20-mg day compared to the 5-mg day ($P = .04$).

sures. There was no Drug × Period interaction, $F(6,66) < 1$, $P > .4$, $\varepsilon = 0.492$.

An additional 3 Day Order (first, second, and third session) × 5 Time Periods (Baseline, 30, 60, 90, and 120-min postdrug) analysis was conducted to determine whether differences in startle magnitude were due to acclimation to the laboratory across the three sessions irrespective of drug assignment. This analysis revealed no effect of day order [$F(2,22) < 1$, $P > .3$, $\varepsilon = 0.620$], nor was there a Day Order × Period interaction [$F(8,88) = 1.2$, $P > .3$, $\varepsilon = 0.557$].

3. Experiment 2

3.1. Participants

Participants were 48 healthy volunteers (24 men and 24 women). All participants met the same inclusion/exclusion criteria as those in Experiment 1.

3.2. Procedure

In a between-subjects design, participants were randomly assigned to receive either placebo or 20 mg cortisol (in order to approximate the reduction in ASR magnitude from Experiment 1). Capsules were administered orally in a double-blind fashion. Gender was evenly distributed with 12 men and 12 women in each drug group. Upon arrival (between 1300 and 1400 h), participants gave informed consent, gave a saliva sample for measurement of baseline cortisol levels, measures of anxiety were taken, and electrodes were affixed and impedances checked. The participants were seated in a comfortable chair approximately 2 m from a 21-in. color monitor, and the drug was administered. Following drug administration, participants were allowed to relax and watch documentary videos or read during a 50-min drug absorption period.

Before the presentation of the slide sets, participants viewed four neutral slides and received three startle probes to orient them to the procedure. Participants viewed three picture sets each consisting of 20 slides at 60-min postdrug administration. Each picture was presented for 12 s with 12–16 s between pictures as described by Sutton et al. (1997). The order of slides within each set consisted of a random mix of pleasant (e.g., puppies, appetizing food), neutral (e.g., a book, a light bulb) and unpleasant (e.g., aimed gun, mutilated bodies) slides. Each slide set was matched as closely as possible for the variables of arousal and valence. Acoustic startle probes were presented on 75% of the trials pseudorandomly distributed within each picture set and valence. This resulted in blank trials (no noise presented) on five pleasant, five neutral, and five unpleasant slides throughout the total presentation of 60 slides to minimize expectancy effects. The presentation orders of the picture sets were counterbalanced to control for possible order effects.

Saliva samples were taken before drug administration and at 30-, 40-, and 50-min postdrug administration. Before drug administration and at the 50-min time point, a self-report of anxiety was taken. Additional measures of salivary cortisol and anxiety were taken after the three slide sets approximately 90-min postdrug.

3.3. Apparatus and materials

Hydrocortisone (20 mg; Hawkins Chemical, Minneapolis, MN) and placebo capsules were prepared by a local pharmacy (Innovative Pharmacy Solutions, Edmond, OK).

Startle stimuli were 95 dB, 50 ms white noise bursts with immediate rise time generated by a Coulbourn noise generator. The same equipment for measurement of startle, salivary cortisol, and anxiety in Experiment 1 was used in the current experiment (see Experiment 1).

Photographic slides used in the modulation of startle were selected from the International Affective Picture System (IAPS; see Center for the Study of Emotion and Attention, 1995). This selection was based on self-report ratings of valence and arousal from the IAPS technical report (Lang et al., 1995). A list of pictures is available upon request.

3.4. Data acquisition and analyses

Data acquisition and signal processing used the same equipment and methods as Experiment 1. The main analyses focused on differences in startle magnitude between drug groups and gender across slide valence conditions. This resulted in a 2 Drug × 2 Gender × 3 Slide Valence (pleasant, neutral, and unpleasant) repeated measures, within-subjects multivariate analysis of variance (MANOVA) with drug and gender as between-subjects variables and slide valence as a within-subjects variable. Additional analyses focus on differences in saliva levels of cortisol and reported anxiety. This resulted in a 2 Drug × 2 Gender × 5 Period (predrug, 30-, 40-, 50-min postdrug, and postslides) design for cortisol level and a 2 Drug × 2 Gender × 3 Period (predrug, 30-min postdrug, and postslides) design for anxiety. Due to the likely violation of the sphericity assumption in repeated measures designs, MANOVAs were employed, as suggested by Maxwell and Delaney (1990), in order to avoid the inflated Type I error rate associated with the mixed-model univariate ANOVA when the sphericity assumption is not met.

4. Results

4.1. Salivary cortisol levels

As expected, individuals receiving cortisol showed significantly higher levels of salivary cortisol compared to those receiving placebo, $F(1,44) = 40.4$, $P < .0001$. Additionally, women receiving cortisol showed a significantly

Table 2
Experiment 2 salivary cortisol levels across periods

	20 mg		Placebo	
	Men	Women	Men	Women
Bsl	1.7 (0.2)	1.8 (0.2)	2.3 (0.4)	2.2 (0.4)
30	5.3 (1.7)	15.7 (5.2)	2.5 (0.4)	2.4 (0.3)
40	6.7 (2.5)	22.5 (6.8)	1.5 (0.1)	2.1 (0.2)
50	7.5 (2.2)	28.5 (6.6)	2.1 (0.6)	1.9 (0.3)
Postslides	7.9 (2.5)	35.4 (4.3)	2.2 (1.0)	1.5 (0.3)

Entries show mean (S.E.M.) in salivary cortisol levels in nanograms per milliliter at baseline (Bsl), and 30, 40, 50-min postdrug and after viewing the slide sets (postslides; 80-min postdrug).

greater increase in salivary cortisol than men (Gender × Drug interaction: $F(1,44) = 18.1$, $P < .0001$; see Table 2).

4.2. Anxiety reports

There was no effect of gender or cortisol on reported anxiety, F's$(1,44 < 1)$. There was, however, a significant effect of period, $F(2,43) = 19.4$, $P < .0001$. Follow-up pairwise contrasts revealed postslide show anxiety to be greater than both predrug [$F(1,44) = 14.1$, $P = .002$] and preslide show anxiety levels [$F(1,44) = 35.4$, $P < .0001$].

4.3. Startle reflex magnitude

Fig. 2 shows mean blink magnitudes across drug groups and slide valence categories. There was no difference between men and women in either startle magnitude or response to stimuli [F's$(1,43) < 1$], and so the data were collapsed over the variable of gender. There was a significant effect of slide valence, $F(2,42) = 4.3$, $P = .02$. A polynomial contrast revealed a significant linear trend in startle magnitude [$F(1,43) = 7.3$, $P = .01$], illustrating the expected linear increase in startle magnitude from pleasant to neutral to unpleasant slide categories. Follow-up tests illustrated significantly increased startle magnitudes while viewing the unpleasant slides compared to the pleasant slides, $F(1,43) = 7.2$, $P = .01$. There was no difference in startle magnitude between the neutral slides and the unpleasant [$F(1,43) = 2.8$, $P = .29$] or the pleasant slides, $F(1,43) < 1$.

There was a marginally significant effect of cortisol on startle [$F(1,43) = 4.0$, $P = .052$] with those receiving cortisol having smaller blink magnitudes than those receiving placebo (cortisol group mean raw EMG startle response in volts $= 0.016 \pm 0.002$; placebo group mean $= 0.0026 \pm 0.004$). There was, however, no significant interaction between cortisol and slide valence, $F(2,42) < 1$.

5. Discussion

These experiments examined the effects of exogenous cortisol on the ASR, its modulation by emotionally valent pictures and self-reported anxiety in healthy men and women. In Experiment 1, it was predicted that if cortisol exerts a suppressive effect on the ASR via negative feedback on CRH and ACTH, then a dose-dependent reduction of the ASR would be expected. Contrary to expectations, results illustrated a dose-dependent biphasic effect of cortisol on the ASR with 5 mg enhancing and 20 mg reducing the magnitude of the reflex without affecting self-reports of anxiety. In Experiment 2, it was predicted that if the reduction in ASR magnitude by 20 mg of cortisol acts via the same mechanisms as emotional modulation of the reflex, then cortisol should result in an altered pattern of ASR modulation. Both cortisol and placebo groups, however, exhibited the same pattern of emotional modulation, but those receiving cortisol had lower ASR magnitudes across the affective slide sets. In spite of the small magnitude of effects, these results illustrate an influence of cortisol on the ASR, which are independent of both self-reported anxiety

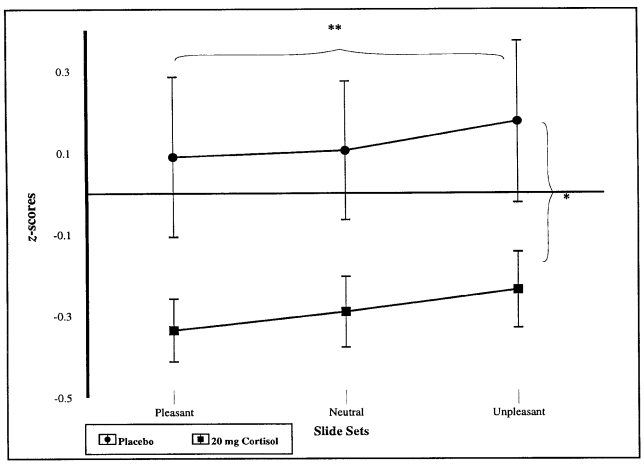

Fig. 2. Experiment 2. Bars show z-transformed startle magnitudes for both groups and slide sets. Error bars show ± S.E.M. Data indicate magnitude of startle reflex during the presentation of pleasant, neutral, and unpleasant emotionally valent photographs. * * Indicates a linear increase in startle magnitude associated with slide valence ($P = .04$). * Indicates a reduction in startle magnitude in the cortisol group compared to the placebo group ($P = .052$).

and modulation of the reflex by emotionally valent pictures. Although women achieved higher cortisol levels in saliva, these relationships were consistent for men and women.

Results from Experiment 1 fit with the often-reported biphasic, or inverted U-shaped pattern of corticosteroid effects on various physiological, behavioral, and cognitive measures — including anxiety (see Lupien and McEwen, 1997; Oitzl et al., 1997). This pattern is thought to be due to different effects of corticosteroids on the two types of corticosteroid receptors, the MR and GR (Oitzl et al., 1997; Plihal et al., 1996). The human GR binds cortisol with one-fifth the affinity of the MR (Damm et al., 1994). Differences in receptor occupation may have resulted in the pattern of results seen here, with the 5-mg dose occupying primarily MR and the 20-mg dose occupying both MR and GR, resulting in different patterns of effects on the central nervous system. This speculation is tentative, however, due to the lack of specific manipulation of each receptor type in the current investigation. Future work should address receptor occupancy, dose effects, and behavior in humans.

The emotion modulated startle paradigm in Experiment 2 resulted in a linear pattern of increasing startle magnitude with the lowest magnitude during pleasant slides, intermediate to neutral slides, and greatest during unpleasant slides in both the placebo and cortisol groups. This pattern of emotional modulation is identical to that reported numerous times across several laboratories (Cook et al., 1992; Lang et al., 1998; Sutton et al., 1997; Vrana et al., 1988). Extensive animal research has illustrated that fear-potentiation of the ASR is dependent on the integrity of connections between the amygdala and the startle reflex circuit (Davis, 1992). This work has been extended to humans by illustrating an overall reduction in ASR magnitude and a lack of ASR potentiation with emotionally valent pictures in a patient with a right amygdala lesion (Angrilli et al., 1996). Those receiving cortisol showed a global reduction in ASR magnitude compared to the placebo group while showing the identical pattern of emotional modulation. The independent effects of cortisol and emotionally valent pictures in Experiment 2 suggest that cortisol's effects on the ASR are independent of emotional modulation of the ASR via amygdaloid activity.

The reduced ASR magnitude to the 20-mg dose in Experiments 1 and 2 is to our knowledge the first such report in humans. The current results replicate previous findings with rats: Sandi et al. (1996) found that a single intraperitoneal injection of corticosterone, 15 min prior to startle testing, resulted in a significant reduction in ASR. In healthy young men, however, Schmidt et al. (1999) found no alteration of ASR magnitude or emotional modulation of the reflex following administration of 160 mg/day of prednisone for 4 days. The high dose of prednisone (a GR-preferring corticosteroid; Reul et al., 1990), and the longer time course of administration make it difficult to compare with the present results. It appears however, that prolonged high occupancy of GR alone by prednisone does not alter the ASR. Both Experiment 2 and the study by Schmidt et al. (1999) illustrate that corticosteroids — within these dose ranges — do not affect the pattern of emotional modulation of the ASR.

Both CRH and ACTH have been associated with enhanced ASR in rats (Corodimas et al., 1994; McGivern et al., 1987; Swerdlow et al., 1986). The reduction in ASR following the 20-mg dose in Experiments 1 and 2 could be an effect of negative feedback of cortisol on both ACTH and CRH. The so-called ''intermediate negative feedback'' begins at about 30 min after exposure to corticosteroids and lasts for a period of hours (Dallman, 2000). Measurements in both Experiments 1 and 2 were well within the time frame of intermediate negative feedback and may reflect reduced secretion of ACTH and CRH. This does not, however, explain the increased ASR following the 5-mg dose. Future research should explicitly measure CRH and ACTH secretion in relation to effects of corticosteroids on ASR in order to clarify these effects. Although chronic elevations of corticosteroids may activate CRH neurons outside the hypothalamus, such as those of the central nucleus of the amygdala and bed nucleus of the stria terminalis, and those of the paraventricular nucleus itself (Schulkin et al., 1994; Shepard et al., 2000; Swanson and Simmons, 1989), these 'positive feedback' effects are not known to occur to the durations and routes of administration used in the present study.

Previous work has suggested an association between cortisol and anxiety (File, 1996; File et al., 1979). One of the many functions of cortisol is the mobilization of energy resources in response to challenges to homeostasis (Munck et al., 1984). The current experiments were designed to examine the feedback effects of the hormone on an index of anxiety in order to better understand the role it plays in acute states of anxiety and fear. Results from both Experiments 1 and 2 showed no effect of either dose of cortisol on reported anxiety, despite differentially affecting the ASR. Self-reported anxiety has also been associated with modulation of the ASR (Grillon et al., 1993). However, anxiety reports from the current studies showed no relationship to changes in ASR magnitude. This lack of association among cortisol, anxiety reports, and ASR modulation suggests that the effects of cortisol on the ASR are independent of reported anxiety. This independence of effects on the ASR serves further to illustrate that the different reactive systems of emotion (e.g., expressive language and physiology) may be differentially affected and expressed by pharmacological manipulations (see Lang et al., 1998 for review).

Numerous studies have shown that corticosteroids have an effect on various forms of sensory functions (see Henkin, 1970 for review; Beckwith et al., 1983; Born et al., 1987). Henkin (1970) observed that sensory detection thresholds were increased with increasing cortisol levels. The reduction of the ASR after administration of 20 mg of cortisol in the current study may reflect a nonspecific effect on auditory perception. The magnitude of the ASR is dependent upon

both the rise time, as well as the intensity of the noise burst (see Hoffman and Ison, 1980), with higher intensity bursts resulting in greater magnitude of the ASR. If the noise bursts were perceived as being less intense following administration of 20 mg of cortisol in the current studies, then a reduction in the magnitude of the reflex would be expected. Auditory perception was not explicitly tested in either of these experiments, however, so this speculation remains tentative.

These studies examined the effect of cortisol on the ASR, emotional modulation of the ASR and self-reports of anxiety. Results showed effects of cortisol on the ASR which are independent of both the emotional modulation of the reflex by affectively valenced pictures and anxiety reports. Findings extend animal research on the relationships among hormones of the HPA axis, the ASR, and anxiety. Future work should address these relationships further in terms of effects of ACTH and CRH on the human ASR and the role of the MR and GR in human anxiety behaviors.

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