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The modulatory effects of corticosteroids on cognition: studies in young human populations

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Abstract

In the present article, we report on two studies performed in young human populations which tested the cognitive impact of glucocorticoids (GC) in situations of decreased or increased ratio of mineralocorticoid (MR) and glucocorticoid (GR) receptor occupation. In the first study, we used a hormone replacement protocol in which we pharmacologically decreased cortisol levels by administration of metyrapone and then restored baseline cortisol levels by a subsequent hydrocortisone replacement treatment. Memory function was tested after each pharmacological manipulation. We observed that metyrapone treatment significantly impaired delayed recall, while hydrocortisone replacement restored performance at placebo level. In the second study, we took advantage of the circadian variation of circulating levels in cortisol and tested the impact of a bolus injection of 35 mg of hydrocortisone in the late afternoon, at a time of very low cortisol concentrations. In a previous study with young normal controls, we injected a similar dose of hydrocortisone in the morning, at the time of the circadian peak, and reported detrimental effects of GC on cognitive function. Here, when we injected a similar dose of hydrocortisone in the afternoon, at the time of the circadian trough, we observed positive effects of GC on memory function. The results of these two studies provide evidence that GC are necessary for learning and memory in human populations. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Corticosteroids; Memory; Mineralocorticoids; Glucocorticoids; Humans

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1. Introduction

For the last two decades, glucocorticoid (GC; corticosterone in rat, cortisol in human) actions on the brain have been envisioned as being mainly negative. This view emerged principally from animal (Landfield et al. 1978, 1981; Sapolsky et al., 1986) and human (Lupien et al. 1994, 1995) studies showing that cumulative exposure to high levels of GC has a detrimental impact on hippocampal function and leads to both memory impairments, and hippocampal atrophy.

However, studies measuring the acute impact of GC on the brain have reported mixed results. In humans, most studies report negative effects of GC on cognitive function after acute (Beckwith et al., 1986; Fehm-Wolfsdorf et al., 1993; Kirschbaum et al., 1996; Lupien et al., 1999), or after four (Wolkowitz et al., 1990; Newcomer et al. 1994, 1999; Schmidt et al., 1999) to ten days (Young et al., 1999) administration of synthetic glucocorticoids. In contrast, various studies performed in rodents report a positive impact of GC on learning and memory (Micco et al., 1979; Micco and McEwen, 1980; Bohus et al., 1983; Veldhuis et al., 1985; De Kloet et al., 1988; Mitchell and Meaney, 1991). Two possible reasons for the discrepancy between rodent and human studies was recently discussed by De Kloet et al. (1999). The first one relates to the fact that, in contrast to animal studies, human studies administer synthetic cortisol out of the context of the learning, or stressful experience (De Kloet et al., 1999). The second one relates to the fact that most human studies performed to this day measured the impact of physiological increases of GC on learning and memory, while rodent studies have used hormone replacement protocols and central administration of selective antagonists for the mineralocorticoid (MR) and glucocorticoid (GR) receptors. These rodent studies have shown that a physiological decrease of circulating glucocorticoids, or blockade of their action is as detrimental to memory function as is a physiological increase.

The reason why hormone replacement protocols might permit assessing the positive impact of GC on cognitive function relates directly to the differential involvement of MRs and GRs in cognitive function. Circulating GC bind with high affinity to two GC receptor subtypes; the MRs and GRs. Although both receptor types have been implicated in mediating GC feedback effects (see Reul and De Kloet, 1985), there is a major difference between them. MRs bind GC with an affinity that is about 6- to 10 times higher than that of GRs. This differential affinity results in a striking difference in occupation of the two receptor types under different conditions and time of day. Under basal conditions, cortisol secretion exhibits a 24-h circadian profile in which cortisol concentrations present a morning maximum in humans (the circadian peak), and slowly declining levels during the late afternoon, evening and nocturnal period (the circadian trough), and an abrupt elevation after the first few hours of sleep. Thus, during the circadian trough (the PM phase in humans and the AM phase in rats), the endogenous hormone occupies more than 90% of Type I receptors, but only 10% of Type II receptors. However, during stress and/or the circadian peak of GC secretion (the AM phase in humans and the PM phase in rats), Type I receptors are saturated, and there is occupation of approximately 67–74% of Type II receptors (Reul and De Kloet, 1985).

Many studies performed in rodents have reported that the ratio of MR/GR occupation (or MR/GR actuation given that steroid receptor activity largely depends on co-activators and co-repressors) is a major determinant of the direction of GC-induced cognitive changes (for a review, see De Kloet et al., 1999). For example, long-term potentiation (LTP), a proposed neurobiological substrate of memory formation, has been shown to be optimal when GC levels are mildly elevated, ie. when the ratio of MR/GR occupation is high (see Diamond et al., 1992). In contrast, significant decreases in LTP are observed after adrenalectomy, when MR occupancy is very low (Dubrovsky et al., 1987; Filipini et al., 1991), or after exogenous administration of synthetic GC (Pavlides et al., 1993; Bennett et al., 1991), which activate GRs and deplete cortisol, again resulting in low occupancy of MRs. In their recent paper, De Kloet et al. (1999) have re-interpreted the well-known inverted-U shape function between circulating levels of GC and cognitive performance in line with the MR/GR ratio hypothesis. In this view, cognitive function can be enhanced when most of the MRs and only part of the GRs are activated (top of the interted-U shape function; increased MR/GR ratio). However, when circulating levels of corticosteroids are significantly decreased or increased (extremes of the inverted-U shape function; low MR/GR ratio), cognitive impairments will result. The authors suggested that the negative view of GC actions on human cognitive function could be partly explained by limitations in previous human experimental designs, which did not allow differential manipulation of MR and GR levels. In order to do this, such studies should measure cognitive function when GC occupancy is decreased (rather than increased), thus allowing functional measures of MR/GR occupancy on learning and memory.

In the present article, we report on two studies performed in young human populations which tested the cognitive impact of GC in situations of increased MR/GR ratio. In the first study, we used a hormone replacement protocol in which we pharmacologically decreased cortisol levels by administration of metyrapone (a potent inhibitor of cortisol synthesis) and then restored baseline cortisol levels by a subsequent hydrocortisone replacement treatment. Memory function was tested after each pharmacological manipulation and compared to performance under appropriate placebo conditions. We observed that metyrapone treatment significantly impaired delayed recall, while hydrocortisone replacement restored performance at placebo level. In the second study, we took advantage of the circadian variation in circulating levels of cortisol and tested the impact of a bolus injection of 35 mg of hydrocortisone in the late afternoon, at a time of very low cortisol concentrations. In a previous study with young normal controls, we injected a similar dose of hydrocortisone in the morning, at the time of the circadian peak, and reported detrimental effects of GC on cognitive function (Lupien et al., 1999). Here, when we injected a similar dose of hydrocortisone in the afternoon, at the time of the circadian trough, we observed positive effects of GC on memory function.

2. Study 1: hormone replacement protocol

2.1. Population

Fourteen young right handed normal student males aged between 20 and 30 years old (mean age=23.1±2.9) and presenting no psychiatric nor neurological history participated in our study. Informed consent was obtained from all participants. To avoid fluctuation in the cognitive performance due to the effects of the different phases in the menstrual cycle on memory (Hampson, 1990), females were excluded from the study. Each participant was seen on two occasions, for a within-subject hormone replacement protocol (see below). At the end of the experimentation, participants received $150.00 as a compensation for their participation in the study.

Subjects were selected based on the absence of past or present Axis 1 psychiatric disorders (DSM-III-R criteria) ascertained by a clinical interview and modified DIS. All subjects gave informed consent. In all subjects, a complete medical history was obtained followed by a physical and neurological examination, and routine laboratory screening (Chem 20 and CBC). Subjects were required to have no lifetime history of psychiatric disorders, no history of Axis I disorders in first-degree relatives, no active medical problems, no current medication use and no significant abnormalities on laboratory tests.

2.2. Neuroendocrine protocol

Each subject was studied in one active drug condition and a placebo condition administered in a counterbalanced order within subjects. Subjects and experimenter were blinded with respect to study condition. In the active drug condition, oral metyrapone was followed by a cortisol infusion of 0.06 mg/kg/h following a protocol designed by Wilkinson et al. (1997, 2001) and tested without any side effects in young and aged human populations. In the placebo condition, placebo tablets were followed by a normal saline infusion. Studies were separated by at least two weeks for all subjects. Details of the protocol were as follows: Subjects fasted from midnight prior to study and were maintained at bed rest throughout the study. In the active drug condition, metyrapone 750 mg p.o. was administered at 06:00 h. At 06:30 h, an intravenous catheter was inserted in the subject's arm and was used for blood sampling and cortisol infusion. Blood samples were taken at 07:30 and 08:00 h. At 08:05 h, a small snack (yogurt and fruits) was given to participants and a third blood sample was taken at 08:30 h, followed by a second dose of 750 mg p.o.metyrapone at 09:00 h. After a fourth blood sample obtained at 09:15 h, the neuropsychological testing was performed at 09:20 h and lasted for 30 min. Further blood samples were obtained after the first neuropsychological testing at 09:50, 10:30 and 10:40 h, and a light breakfast was given at 09:55 h. At 10:45 h, cortisol infusion began and was maintained at a rate of 0.06 mg/kg/h for 60 min. The second neuropsychological testing begun 30 min after the start of the cortisol infusion (11:15 h), and lasted 30 min. An additional blood sample was obtained at 12:00 h, at which time subjects were served with a lunch and were free to go home after being checked by the

medical supervisor of the study (Dr N.P.V. Nair). Blood samples were not obtained during the time of neuropsychological testing in order not to disturb participants in their performance. The placebo condition protocol was identical to the active drug condition except that subjects received placebo tablets and a placebo normal saline infusion equivalent in volume to the cortisol infusion.

Blood samples (10 ml) were collected in Vacutainer tubes containing the anticoagulant EDTA, and immediately centrifuged at 900 ***g*** at 4°. The isolated plasma samples were divided into two equal aliquots, one for cortisol, 11-desoxycortisol and glucose measurements, and the other for ACTH determination. In the latter, 50 ml of the protease inhibitor, N-ethylmaleimide (0.1 M solution) was added. The plasma samples were stored in polypropylene tubes at −80°. All hormone assays were carried out within six months.

2.3. Measures of memory

At each neuropsychological testing period, declarative memory was measured using a free recall test of a 12-word list presented over three trials. Each subject was presented with a list of 12 imageable and concrete words. The subject had to read each word aloud and try to memorize them for subsequent declarative free recall. Each list was presented three times and free recall was assessed after each trial. Free recall performance over the three trials constituted the measure of learning capacity for each subject. Delayed (20 min) memory performance was also tested on a fourth recall, during which subjects were asked to recall the previously presented words. Delayed memory performance served as a measure of the rate of forgetting in each subject. Four lists of words were created in order to measure learning and memory capacity after each experimental condition. Preliminary pilot studies with normal young subjects (n=10) were performed in order to ensure that each list was equivalent in terms of recall performance. List order was counterbalanced within and across subjects.

2.4. Endocrine and glucose assays

Cortisol levels were determined using validated radioimmunoassay (RIA) kits, purchased from Johnson and Johnson (Mississauga, Ont., Canada). In this assay, cortisol is released from corticosteroid binding globulin by a chemical blocking agent contained in the kit, and the total cortisol determined with the 125I-labeled cortisol and polymer-bound sheep anti-cortisol antibody provided. The intra- and inter-assay coefficients of variation were 4.3% and 7.7% for mean concentrations of 8.15 μg/dl and 8.28 μg/dl, respectively. The sensitivity of the assay was 0.1 μg/dl. For the determination of 11-desoxycortisol levels, validated RIA kits were purchased from ICN Pharmaceuticals, Inc (Costa Mesa, California, USA). This is a double antibody assay using 125I-labeled 11-desoxycortisol. The intra- and inter-assay coefficients of variation were 2.1% and 13.7% for mean concentrations of 2.55 ng/ml and 2.63 ng/ml respectively. The sensitivity of the assay was 0.02 ng/ml. The ACTH levels were determined using the validated immunoradiometric assay (IRMA) kit of Nichols

Institute Diagnostics (San Juan Capistrano, California, USA). This assay uses a 125I-labeled monoclonal antibody binding the N-terminal regions of ACTH, and a polyclonal antibody binding the C-terminal region. It measures the amount of intact ACTH in plasma samples. The intra- and inter-assay coefficients of variation were 3.0% and 7.8% for mean concentrations of 35 pg/ml and 36 pg/ml respectively. The sensitivity of the assay was 1 pg/ml. Plasma glucose levels were determined on an automated chemistry analyser, Beckmann LX20, at St Mary's Hospital (McGill University). The assay is based on the glucose oxidase method.

2.5. Statistical analyses

Treatment effects on baseline nontransformed endocrine measures (cortisol, 11-desoxycortisol, ACTH and glucose) were assessed using an Analysis of Variance with Treatment (Placebo vs Treatment) and Samples (1 through 8) as the within-subjects factors. Significant interactions were decomposed using Tukey Honest Significant Different (HSD) Test. Data were checked for assumption of sphericity and Greenhouse and Geisser (1959) correction was applied if sphericity was not met. For cognitive data, we performed planned orthogonal comparisons instead of an omnibus *F* test because the study was confirmatory rather than exploratory, which then cuts down the probability of a Type I error (see Stevens, 1995; Hays, 1981). Also, the use of a within-subject design with a tight control of placebo conditions permitted us to measure the impact of metyrapone and hydrocortisone when compared to their appropriate placebo condition, taking into account fatigue effects that might be induced by testing at two timepoints of the same experimental day. The two contrasts performed answered the following questions: 1) Does metyrapone change cognitive performance when compared to its controlled placebo condition (first placebo condition); 2) Does hydrocortisone change cognitive performance when compared to its controlled placebo condition (second placebo condition). We did not compare metyrapone to hydrocortisone condition (which would add a contrast) because hydrocortisone condition was always given after the metyrapone condition, leading to possible fatigue effects difficult to interpret in terms of drug treatment effects. Performance on Trial 4 (delayed recall) was not included in the same contrasts as performance on Trials 1 to 3 given that performance on this trial was different from that on trials 1 to 3 due to the fact that words were not presented in the delayed recall condition.

3. Results

3.1. Endocrine data

Cortisol levels were significantly decreased below 6 μg/dl after metyrapone treatment, and they significantly increased above placebo levels after hydrocortisone replacement (significant interaction between Treatment×Sample; $F(7,91)=17.91$;

$P<0.000005$; see Fig. 1A). Also, in the placebo group, cortisol increased from 08:05 h to 08:30 h sample (see Fig. 1A), possibly due to the administration of a light snack at 08:10 h (see Follenius et al., 1982; Ishizuka et al., 1983). ACTH levels significantly increased after the second dose of metyrapone, and stayed elevated until hydrocortisone replacement, at which time they started to decline (significant interaction between Treatment×Sample; $F(7,91)=20.87$; $P<0.0004$; see Fig. 1B). 11-Desoxycortisol levels were significantly increased compared to placebo condition at each sampling time, although the largest increase was observed after the second metyrapone dose (significant interaction between Treatment×Sample; $F(7,91)=2.19$; $P<0.04$; see Fig. 1C). Contrary to cortisol, ACTH, and 11-desoxycortisol, glucose levels were not significantly changed in response to treatment (see Fig. 1D). The ANOVA performed on glucose levels only revealed a main effect of sample ($F(7,91)=10.03$; $P<0.0002$], which showed increases in glucose levels after the snacks offered to subjects.

3.2. Cognitive data

Fig. 2 presents memory performance after metyrapone and hydrocortisone replacement, when compared to their respective placebo conditions. The contrasts performed on learning performance (trials 1 to 3) did not reveal any significant impact of either metyrapone ($P>0.1$) or hydrocortisone replacement ($P>0.1$), although a significant main effect of List was observed on each treatment condition (metyrapone/first pla-

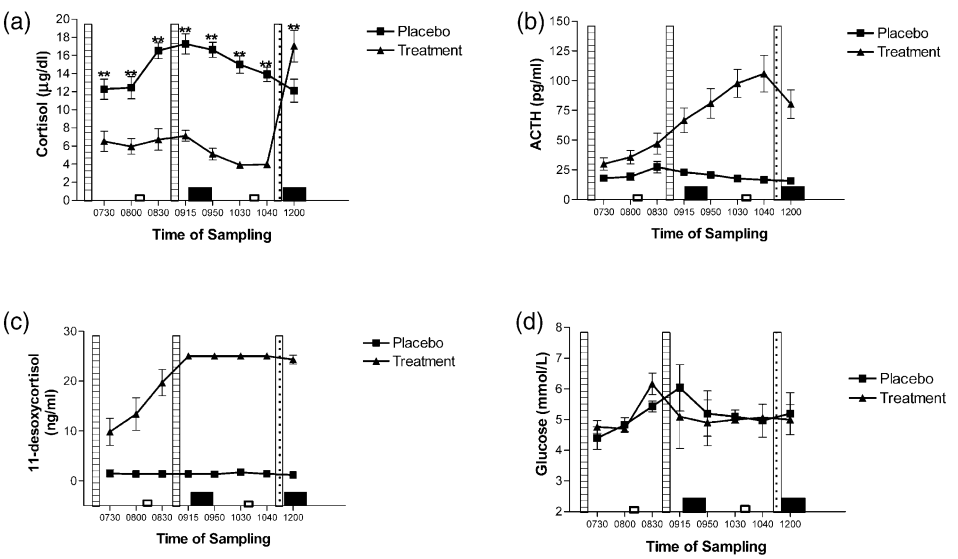

Fig. 1. Cortisol (panel a), ACTH (panel b), 11-desoxycortisol (panel c) and glucose (panel d) levels after placebo and metyrapone (line pattern) and hydrocortisone (dotted pattern) conditions in young normal controls. Open white squares represent time of administration of snacks, while black squares represent time of neuropsychological testing.

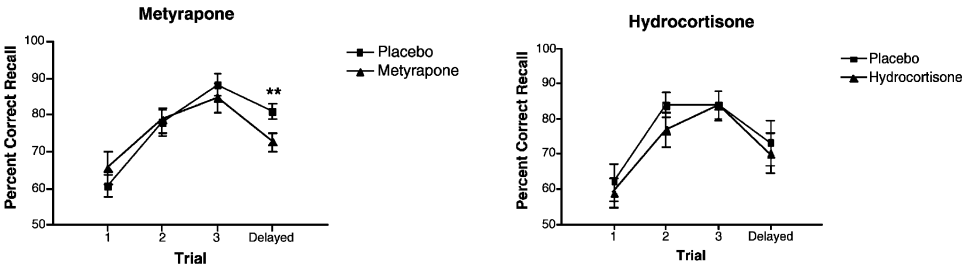

Fig. 2. Memory performance over three immediate recall trials (1 through 3) and one delayed (20 min) recall trial after conditions of metyrapone treatment and hydrocortisone replacement when compared to their appropriate placebo conditions.

cebo; $F(2,26)=65.44$; $P<0.00001$ and hydrocortisone/second placebo; $F(2,26)=35.14$; $P<0.0001$), revealing that performance of participants significantly increased over repeated exposure to the list. Contrasts on the delayed recall condition revealed a significant impairing effect of metyrapone on delayed recall when compared to placebo condition ($F(1,13)=4.55$; $P<0.05$; 8% decrease in memory performance when compared to treatment-controlled placebo condition). No changes in delayed recall performance was observed after hydrocortisone replacement ($F(1,13)=0.39$; $P=0.54$) showing that hydrocortisone replacement was able to restore delayed recall performance to the level observed on the placebo condition.

In summary, the results of this study revealed that administration of metyrapone, which acutely inhibits cortisol secretion, increased the rate of forgetting, while hydrocortisone replacement restored memory performance to the level it was on a placebo condition.

4. Study 2: afternoon hydrocortisone administration

4.1. Population

Eighteen young right handed normal student males aged between 20 and 30 years old (mean age=24.2±4.3) and presenting no psychiatric nor neurological history participated in our study. Informed consent was obtained from all participants. Again, in order to avoid fluctuation in the cognitive performance due to the effects of the different phases in the menstrual cycle on memory (Hampson, 1990), females were excluded from the study. Each participant was seen on one occasion, for a between-subject protocol (see below). At the end of the experimentation, participants received $50.00 as a compensation for their participation in the study. Subjects were selected based on the same criteria as those described in Study 1.

4.2. Cognitive task

Many of the studies performed in human populations and which have reported a negative impact of GC on cognitive function used incidental encoding of information

(for a review, see Lupien and McEwen, 1997). Incidental encoding differs from intentional encoding in that in the former, the participant is not aware that he/she is encoding information that will later be used for recall, while in the latter, the participants are aware that the information presented will later be used for recall. Intentional encoding usually leads to better recall performance than incidental encoding, because during intentional encoding, the information is encoded in a deeper manner (using conscious semantic strategies by the participant) than during incidental encoding. Also, recent results reported in humans showed that GC can have a negative impact when given before retrieval of the information (De Quervain et al., 2000). In order to assess whether GC administration in the afternoon could lead to positive effects on these particular processes, we measured the impact of hydrocortisone administration on an incidental encoding task using two levels of difficulties, and we administered the drug before retrieval of the information.

The cognitive task we used was a modification of Heckers task (1998) that used two levels of depth of processing (deep vs shallow) on an incidental encoding task. A series of 20 words were used at the time of incidental encoding. Half of the words was used for deep encoding while the other half was used for shallow encoding. Shallow encoding was induced by asking the subjects to answer if the word presented to them comprised the letter "T". These words were presented once and subjects had to answer "yes" or "no" using a response pad. Deep encoding was induced by asking the subjects if the word presented to them had more then one signification (ie., homonym). These words were presented randomly four times and subjects had to answer with the response pad using the same keys as for shallow encoding.

During the recognition task, 30 word stems were presented randomly to the subject. Each word stem comprised the first three letters of a word and the task of the participant was to answer whether the presented word stem was part of a word previously presented or not. Twenty of these word stems were the first three letters of the words encoded earlier (10 shallow encoded words and 10 deep encoded words). The other 10 word stems were the first three letters of new words. The subject had to answer using the response pad which recorded errors and reaction times.

4.3. Psychoneuroendocrine protocol

The psychoneuroendocrine protocol took place between 3:00 and 6:00 pm for each participant. Participants were asked to refrain from smoking, eating, or drinking coffee at least three hours before their participation in the study. A bolus dose of 35 mg of hydrocortisone or placebo was administrated intravenously to the subjects five minutes before the recognition task (word-stem completion). Cortisol is highly lipid-soluble and freely and rapidly crosses the blood–brain barrier. In a study in which a bolus of cortisol was injected intravenously in anesthetized monkeys, CSF cortisol reached maximal levels by 10 minutes (the first sampling time of the study) and remained constant at that level throughout the following 60 minutes (Martensz et al., 1983).

For each subject, one saliva sample was collected before administration of hydro-

cortisone or saline solution and a second sample was collected at the end of the recognition task, i.e., 20 minutes after the first sample. The percent change in cortisol levels was used to assess drug-induced elevations in circulating levels of cortisol. The saliva was collected by the insertion of a 3.5×5 cm sterile paper filter (Whatman no. 42 filter paper, Clifton, NJ) in the mouth of the subject until the paper was completely wet with saliva. Only 4 cm of the paper filter had to be wet, leaving about 1 cm for manipulation. The paper at this area was delimited by a tiny cut in the paper. The cortisol was extracted from the saliva with ethanol and quantified by the mean of radioimmunoassay (RIA), in which we use a radioactive marker for cortisol ([^{3}H]cortisol) and a specific antibody for cortisol (B-63, originating from Endocrine Sciences, Tarzana, CA). B-63 reacts less than 4% with deshydrocorticosterone or desoxycortisol and less than 0,5% with the other adrenal steroids. Between and within variability are respectively 5% and 3,5%.

5. Results

Cortisol levels for the two groups were the same before the injection of either hydrocortisone or saline solution while there was a significant cortisol increase after injection of hydrocortisone in the experimental group (significant interaction between Group×Sample; $F(1,15)=40.24$; $P<0.0001$; see Fig. 3). Performance on the word-stem recognition task was not different for the hydrocortisone and placebo groups, although there was a significant main effect of depth of processing ($F(1,16)=20.79$; $P<.0003$), with shallowly encoded words leading to a lower recognition performance, when compared to deeply encoded words. However, we found a significant main effect of Group ($F(1,16)=5.27$; $P>0.05$) for reaction times data calculated on correct answers. This effect revealed that the hydrocortisone group responded significantly faster for correct trials when compared to the placebo group. This facilitatory effect in the hydrocortisone group was present for both deep and shallow recog-

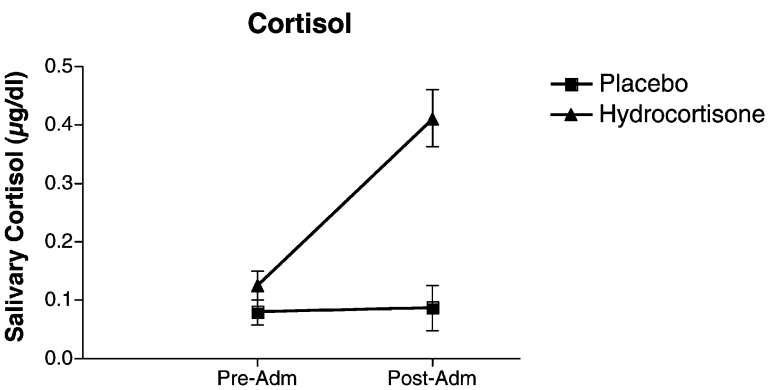

Fig. 3. Salivary cortisol before (Pre-Adm) and 20 minutes after (Post-Adm) administration of placebo or 35 mg dose hydrocortisone in young normal controls.

nition conditions (see Fig. 4). Finally, we also found a main effect of depth of processing on reaction time data ($F(1,16)=17.81$; $P<0.0006$) showing that deeply encoded words were recognized significantly faster than shallow encoded words.

In summary, the results of this study revealed that the bolus injection of 35 mg of hydrocortisone in late afternoon, at the time of the circadian trough, leads to faster reaction times on a recognition memory test.

6. Discussion

The results of these two very different studies provide new evidence in a young human population that GC can have modulatory effects on learning and memory. This suggestion is based on data from our first study showing that a marked reduction in circulating levels of GC decreases performance on a 20 min. delayed memory task when compared to a placebo condition. Also, data from our second study show that administration of a high dose of GC at the time of the circadian trough has a positive impact on cognitive efficiency by significantly decreasing reaction times on a recognition memory task when compared to a placebo condition.

The metyrapone-induced memory impairment observed in study No. 1 was specific to delayed memory, showing that reduction in circulating cortisol, and inability to mount a cortisol response had a negative impact on retrieval of previously learned information. This latter result could be interpreted as showing that retrieval is more sensitive then encoding to the impact of metyrapone. However, in a recent study performed with human elderly individuals and using the same protocol, we reported metyrapone-induced memory impairments for both learning capacity, and forgetting rate (Lupien et al., 2001). This latter result in aged humans, in line with the data in young individuals reported here, suggests that sensitivity, rather than specificity of

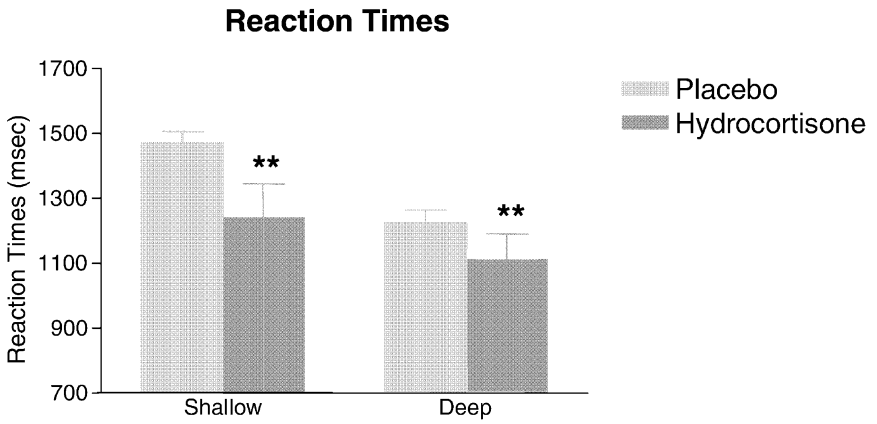

Fig. 4. Reaction time performance (in milliseconds) during recall of deeply and shallowly encoded words after administration of placebo or 35 mg hydrocortisone.

the learning and memory process is the important variable to take into account in explaining the impact of metyrapone on human cognitive function.

The results of our metyrapone-hydrocortisone study provide the first evidence in humans that GC can modulate memory, instead of only having an unidirectional impact on human learning and memory. We showed that metyrapone-induced memory impairments can be acutely restored to placebo baseline levels following hydrocortisone replacement in humans. The GC-induced memory modulation reported here can not be explained by GC-induced changes in glucose levels, since glucose levels were not influenced by our pharmacological manipulation. The results of our metyrapone–hydrocortisone study are similar to those reported in rodents' hormone replacement protocols. These studies showed that pre-training (Micco et al., 1979; Micco and McEwen, 1980; Mitchell and Meaney, 1991) as well as post-training (Bohus et al., 1983; Veldhuis et al., 1985; De Kloet et al., 1988; Mitchell and Meaney, 1991) administration of corticosterone restores an impaired learned behavior or extinction pattern induced by adrenalectomy. In the present study, because modulation of circulating levels of GC gave rise to a concomitant modulation of memory performance, a direct implication of GC in memory can be demonstrated.

The results obtained in study No. 2 showed that a significant increase in circulating levels of GC at the time of the circadian trough has a positive impact on cognitive efficiency, although this effect is nonspecific, affecting mainly response times instead of performance. These results are in accordance with previous data obtained by Fehm-Wolfsdorf et al. (1993) showing that 1) recall performance on a memory test is better in the AM phase, compared to the PM phase in young normal controls, and 2) hydrocortisone administration (50 mg) suppresses this circadian variation in cognitive performance. These results, along with others by Newcomer et al. (1999) showing that 4-days administration of cortisol in the PM phase impairs performance on the fourth day, show that the impact of GC on cognitive are relative rather than absolute, i.e., that the direction and magnitude of the effects of GCs on cognition depend both on the time of day, as well as on the timecourse of the administration.

Altogether, the results of these two studies suggest that GCs can modulate human memory function through a differential activation of MRs and GRs. Indeed, in the metyrapone condition of study No. 1, MR occupancy was low, given the significant decrease of cortisol secretion induced by metyrapone. At this point, impairment in delayed memory was observed. In contrast, administration of a 35 mg dose of hydrocortisone at the time of circadian trough in study No. 2 might have led to partial activation of GRs, thus increasing cognitive efficiency in the group of participants who received hydrocortisone, when compared to placebo. This later finding is interesting in line with data obtained by Oitzl and De Kloet (1992) and recently reviewed by De Kloet et al. (1999), suggesting that MRs and GRs mediate different effects of cortisol in different time domains. According to this view, MR activation is involved in behavioral reactivity in response to environmental cues, while GR-mediated effects promote consolidation of acquired information. The significant decrease in reaction times observed after GC administration in the PM phase in our study No. 2 are in line with a MR-mediated effect of behavioral reactivity (what we call here "cognitive efficiency"; a nonspecific process), while the delayed memory

impairment observed after metyrapone administration is in line with a GR-mediated effect of memory consolidation. However, it is clear that only a careful study manipulating activation of MRs and GRs in humans could permit confirmation of the above suggestion.

Although the MR/GR balance theory of GC action on the brain (Oitzl et al., 1995; De Kloet et al., 1999) could help explain the positive and negative effects of GCs under various conditions, it is important to note that the acute effects of GCs we observed in both studies are too rapid to be explained by a genomic action. Also, it is difficult at this point to explain the results of study No. 2 in terms of MR/GR mediated effects, given that the timecourse of the experiment left very little time for the hormone to activate the cellular and network machinery underlying complex information processing. In this case, acute effects of GCs other than via reduced MR/GR activation have to be considered. For example, GCs increase $Ca2^{+}$ influx and the $Ca2^{+}$-dependent afterhyperpolarization, thereby substantially altering neuronal function (for a recent review, see Joels, 2000). MR activation in the hippocampus is associated with reduced calcium currents, and stable synaptic responses to excitatory or inhibitory amino acid neurotransmitters (Hesen and Joels 1995, 1996). In contrast, higher levels of GCs activate GRs which result in increased calcium currents and exaggerated afterhyperpolarization, dampening synaptic responses to excitatory inputs (Joels and De Kloet, 1994). These results, in line with others showing a differential (cortical vs subcortical) distribution of MRs and GRs in the primate brain (Sanchez et al., 2000; Patel et al., 2000) could help shed a new light on the acute impact of GCs on human cognitive function.

As a last note, it is important to remind the reader that the results of these two studies were obtained in a population of male participants, so that the results obtained are not generalizable to women. The complex interaction occurring between female gonadal hormones and steroid hormones might lead to very different results than those obtained in the present study. It is clear at this point that further studies measuring the acute, short-term, and long-term effects of GCs on cognitive function in female participants will be of great help in disentangling the exact impact (positive, negative, or modulatory) of GCs on human cognitive function.

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