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Antidepressant use and salivary cortisol in depressive and anxiety disorders

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Abstract

Antidepressants are an effective treatment for depressive and anxiety disorders. Those disorders are frequently accompanied by heightened cortisol levels. Antidepressants may affect hypothalamic–pituitary–adrenal axis functioning, the alteration of which could be partially responsible for treatment efficacy. The association between antidepressants and cortisol was investigated in 1526 subjects of the Netherlands Study of Depression and Anxiety who were grouped into 'serotonin reuptake inhibitor (SSRI) users' (n=309), 'tricyclic antidepressant (TCA) users' (n=49), 'other antidepressant users' (n=100), and 'non-users' (n=1068). All subjects had a current or past diagnosis of anxiety and/or depression. Subjects provided 7 saliva samples from which 3 cortisol indicators were calculated: cortisol awakening response (CAR), evening cortisol, and cortisol suppression after ingestion of 0.5 mg dexamethasone. As compared to non-users, TCA users had a flattened CAR (effect size: Cohen's d=0.34); SSRI users had higher evening cortisol levels (d=0.04); and SSRI users showed decreased cortisol suppression after dexamethasone ingestion (d=0.03). These findings suggest that antidepressant subtypes are associated with distinct alterations of the HPA axis. TCA users, who showed a flattened CAR, displayed the strongest alterations of salivary cortisol.

1. Introduction

A hyperactive hypothalamic–pituitary–adrenal (HPA) axis, indicated by increased cortisol levels (Bhagwagar et al., 2005), has frequently been found in subjects with depressive and anxiety disorders. Antidepressants used to treat depression

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and anxiety (Rose, 2007) may influence HPA axis activity through changes to the glucocorticoid (GRs) and mineralocorticoid receptors (MRs) (Bjartmar et al., 2000). These alterations could be partially responsible for treatment efficacy (Pariante, 2009). Furthermore, increased cortisol levels have been found to be associated with a number of physical diseases (e.g., diabetes, osteoporosis) (Bruehl et al., 2007; Nieman, 2007) which often co-occur with depression and/or anxiety (Nouwen et al., 2010). It is unclear whether antidepressant treatment affects physical diseases in anxious and depressed subjects through modified cortisol levels. For these reasons, studying the effects of antidepressants on the HPA axis is clinically important. However, research on cortisol levels in antidepressant users has yielded results that differed by antidepressant type and cortisol measure.

Studies were typically limited to a small number of subjects with a diagnosis of depression and the average duration of intervention was five or six weeks (Deuschle et al., 2003). In these studies, TCA treatment usually (Deuschle et al., 1997), but not always (Dam, 1988), resulted in increased cortisol suppression in the dexamethasone suppression test (DST) which measures HPA axis response to the administration of a synthetic analog of cortisol. TCA responders had more strongly suppressed cortisol levels at morning, afternoon, and evening time points (Deuschle et al., 2003) than SSRI responders. Long-term SSRI use decreased cortisol and normalized cortisol suppression in one study (Aihara et al., 2007) but not in another (Vythilingam et al., 2004). In a third study, SSRI treatment did not decrease salivary cortisol regardless of remitted or non-remitted status post-treatment (Weber-Hamann et al., 2007). In contrast to the putative HPA axis dampening effects resulting from longer term use, short-term TCA and SSRI use was found to activate the HPA axis (Holsboer and Barden, 1996). Research on the associations of non-TCA/non-SSRI antidepressant medications and HPA axis function was limited to one study in which tetracyclic antidepressants and selective serotonin reuptake enhancers reduced previously increased corticosterone secretion in stressed rats (Szymanska et al., 2009).

Research on antidepressants and cortisol in anxious subjects was even more scarce. Increased cortisol levels have been associated with some types of anxiety, both in our own work group (Vreeburg et al., 2010) and in other studies (Mantella et al., 2008), though these results have been inconsistent. Antidepressants such as SSRIs and TCAs have been effective treatments for anxiety disorders (Baldwin et al., 2005) and might lower cortisol levels in anxious patients as it has been shown to do in depressed subjects (Deuschle et al., 1997). Though chronic antidepressant use is common, the long-term effects ($\geq$12 months) have not been well studied. More importantly, we were not aware of studies that compared the effects of different groups of antidepressants on cortisol indicators.

A fuller understanding of the biological mechanism underlying the effects of antidepressants could result in improved treatment options for anxious and depressed patients. We present a cross-sectional study which compares salivary cortisol levels in TCA, SSRI, other antidepressant and non-users with a lifetime diagnosis of a depressive and/or an anxiety disorder participating in the Netherlands Study of Depression and Anxiety (NESDA). Based on the literature, we expected to find altered cortisol indicators (decreased CAR, lower basal evening levels, and increased suppression in the DST) in antidepressant users compared to non-users.

2. Experimental procedures

2.1. Subjects

Subjects participated in the Netherlands Study of Depression and Anxiety (NESDA), a longitudinal cohort study consisting of 2981 respondents recruited from the community, general practices, and specialized mental health care institutions. Objectives and methods of the NESDA project have been described in an earlier publication (Penninx et al., 2008). Subjects completed a baseline measurement consisting of a medical exam, an in-person interview, collection of saliva samples, and questionnaires. The study protocol was approved by the Ethical Review Board of each participating center and all subjects signed an informed consent form before participation.

To evaluate the associations between antidepressant use and salivary cortisol, only subjects reporting a current or past diagnosis of a depressive and/or anxiety disorder (referred to as a lifetime disorder) were included. The presence of a depressive disorder (Major Depressive Disorder [MDD] or dysthymia) or anxiety disorder (generalized anxiety disorder, social phobia, or panic disorder) was assessed by the DSM-IV Composite Interview Diagnostic Instrument (WHO version 2.1). As previous research in our study group showed that remitted depressed and anxious subjects had a marginally heightened CAR (Vreeburg et al., 2009a; Vreeburg et al., 2010), indicating an increased biological vulnerability, those with a past diagnosis of either disorder were also included. In addition to subjects without lifetime disorders (n=687), subjects who lacked cortisol data (n=636), used corticosteroids (n=105), were pregnant or breastfeeding (n=10), or reported irregular antidepressant use (n=17) were excluded (Vreeburg et al., 2009a).

The remaining subjects (n=1526) were available for analysis. All subjects who had used any antidepressant medication in the month prior to the baseline interview (n=458) were defined as antidepressant users and further subdivided into SSRI users, TCA users, and other antidepressant users. Subjects who had not used antidepressants in the month prior to baseline interview were defined as non-users (n=1068). Of the non-users, 15% reported some antidepressant use in the past 3 years. The following groups resulted: 309 SSRI users, 49 TCA users, 100 other antidepressant users, and 1068 non-users.

2.2. Measures of antidepressant use

Medication use during the month prior to the baseline assessment was assessed via medication containers brought to the interview (80.7%) and subject self-report. Antidepressant users provided information on the daily dose, frequency of use, duration of treatment, and type of antidepressant used.

Antidepressants were classified as SSRIs (ATC codes N06AB02–N06AB10), TCAs (ATC codes N06AA01–N06AA23), and other antidepressants (ATC codes N06AX05, N06AX11, N06AX16, and N06AX21, including tetracyclic antidepressants [n=25], serotonin–norepinephrine reuptake inhibitors [n=82], and trazodone [n=1]), indicating that 8 subjects took more than one 'other' antidepressant. Due to small group sizes, the other AD user group was not further subdivided. For those reporting the use of multiple antidepressants, we assigned subjects to one of the three user groups based on relative strengths of the prescribed medications. SSRIs are usually prescribed as first-choice treatment for MDD as they have a milder side effect profile than TCAs but similar efficacy in moderately depressed patients. In severely depressed patients, or in those who do not tolerate the adverse effects of SSRIs or do not improve with SSRI treatment, TCAs are recommended (Peretti et al., 2000). Using this information, subjects taking both a TCA and another type of antidepressant (n=5) remained in the TCA user group as TCAs are believed to have stronger effects than other

antidepressant types. Subjects taking an SSRI and another non-TCA antidepressant (n=10) were classified as SSRI users.

In order to compare dosages of different antidepressants, the Mean Daily Dose for each user was calculated by dividing the milligrams of antidepressant used daily by the Defined Daily Dose as defined by the World Health Organization for each antidepressant.

2.3. Cortisol measures

A detailed description of cortisol measures can be found elsewhere (Vreeburg et al., 2009b). To summarize, respondents collected saliva samples at home on a regular, preferably working, day shortly after the baseline interview was conducted. Subjects were instructed to refrain from eating, smoking, drinking tea or coffee, or brushing teeth 15 min prior to sampling and no dental work was allowed in the 24 h preceding sample collection. Seven saliva samples were collected over a two-day period using Salivettes© (Sarstedt, Germany). On day 1, samples were taken at awakening (T1) and at 30 min (T2), 45 min (T3), and 60 min (T4) post-awakening. Two samples were taken in the evening at 22:00 h (T5) and 23:00 h (T6). Subjects ingested 0.5 mg of dexamethasone immediately after T6 and one salivary sample was taken at awakening on day 2 (T7). Samples were refrigerated after collection and returned by mail. In the laboratory, Salivettes were centrifuged at 2000 g for 10 min, aliquoted, and stored at −80 °C and cortisol analysis was carried out using competitive electrochemiluminescence immunoassay (Roche, Switzerland). The functional detection limit was 2.0 nmol/l and the intra- and interassay variability coefficients in the measuring range were less than 10%.

The following three cortisol indicators were calculated from the seven cortisol samples.

Cortisol awakening response (*CAR*): The CAR was calculated by analysis of T1 to T4 with linear mixed models (LMM) and two aggregate indicators: the area under the curve with respect to the ground (AUC_G) and the area under the curve with respect to the increase (AUC_I) (Pruessner et al., 2003). The AUC_G is an estimate of the total cortisol secretion during the first hour after awakening. The AUC_I is a measure of the dynamic of the cortisol awakening response, related to the sensitivity of the system, emphasizing changes over time.

Evening cortisol: The basal cortisol level was defined as the average of the two evening cortisol values (T5 and T6). For subjects with a single missing evening value, the remaining cortisol value was used.

Dexamethasone suppression test (*DST*): In addition to the cortisol level at awakening after dexamethasone ingestion (T7), a cortisol suppression ratio was calculated by dividing the cortisol level at awakening on day 1 (T1) by the post-dexamethasone cortisol level at awakening on day 2 (T7). Higher DST ratios indicated a larger difference between T1 and T7 and, accordingly, a greater cortisol-suppressing effect of dexamethasone.

2.4. Covariates

The four categories of confounding variables were: sociodemographic indicators, psychiatric indicators, health indicators, and sampling factors. These variables altered cortisol levels in previous analyses of the NESDA data (Vreeburg et al., 2009b). A detailed description is located in additional publications (Penninx et al., 2008; Vreeburg et al., 2009a).

Sociodemographic indicators included age, sex, and level of education. Psychiatric indicators consisted of a lifetime diagnosis of comorbid anxiety and depression as subjects with comorbid disorder were found to have a higher CAR in previous research in this study group (Vreeburg et al., 2009a). The Inventory of Depressive Symptomatology Self-Report (IDS-SR), a rating scale for depression severity, was not associated with cortisol levels in NESDA (Vreeburg et al., 2009b). However, as the sample used in our analyses was slightly different to the one defined previously, the IDS-SR was included as a covariate in a sensitivity analysis. In order to account for possible associations between cortisol and anxiety severity, we also included the Beck Anxiety Questionnaire as covariate in a separate sensitivity analysis. Health indicators included tobacco use and the current level of physical activity. Tobacco use was grouped into current smokers, former smokers, and non-smokers. Additionally, the number of smoked cigarettes per day was counted in order to exclude the heavy smokers (defined as smoking >25 cigarettes/day) in a sensitivity analysis. Physical activity was measured using the International Physical Activity Questionnaire and expressed in 1000 MET-minutes per week.

Sampling factors included seasonal light, weekday or weekend status, work status, awakening time, and hours of sleep per night. Seasonal light was evaluated by defining the month of sampling as either a dark month (October through March) or a light month (April through September). Average sleep duration during the four weeks prior to sampling was categorized as ≤6 or >6 h per night.

2.5. Statistical analyses

Characteristics of study groups were expressed by frequencies or means and compared using χ^2 statistics (categorical variables) or analysis of variance (continuous variables). Positively skewed cortisol indicators (T1–T4, AUC_G, evening cortisol, T7 and DST) were naturally log-transformed for subsequent analyses. Back-transformed values are given in Table 2. Post-hoc tests on individual group differences were performed using the Fisher Least Significant Difference test. Differences in AUC_G, AUC_I, evening cortisol, T7, and DST across groups were analyzed using analysis of covariance (ANCOVA), adjusting for covariates. Cohen's *d* (the difference in marginal estimated means, divided by their pooled standard deviation) was calculated as a measure of effect size.

Further analysis of the CAR was carried out with random coefficient analysis of the four morning cortisol data points using Linear Mixed Models (LMM). LMM retains original values on all data points while accommodating for missing data and taking into account correlations between repeated measurements within subjects.

Linear regression analyses on dosage of antidepressant (Mean Daily Dose) and duration of use (months) were conducted on the SSRI and TCA user groups. Other AD users were excluded from these analyses due to the multiple antidepressants types included in this group. Analyses were adjusted for the described covariates. To determine the stability of our results, all analyses were also corrected for severity of depression and anxiety in sensitivity analyses. Additionally, heavy smokers (defined as smoking >25 cigarettes/day), users of multiple antidepressants, and lithium users were excluded in different sensitivity analyses in order to investigate the robustness of our results. Statistical significance was set at $p<0.05$. SPSS 16.0 software was used for all analyses (SPSS Inc, Chicago, Ill.).

3. Results

Characteristics of the study groups are shown in Table 1. TCA users were older than SSRI users, other users, and non-users. Non-users were more likely to have sampled during a light month and on a working day. Antidepressant users more often had comorbid anxiety and depression than non-users. TCA users used smaller antidepressant doses than the other groups. Results of analyses of covariance are shown in Table 2.

Table 1 Characteristics of study groups (n=1526).

	N	SSRI users (n=309)	TCA users (n=49)	Other AD users (n=100)	Non-users (n=1068)	*P*-value
Sociodemographics	1529					
Age	1529	42.8 (41.5–44.1)[a]	48.9 (46.3–51.4)[a,b,c]	43.8 (41.8–45.9)[b]	43.1 (42.3–43.9)[c]	**0.01**
% Male	1529	33.7	26.5	40.0	32.3	0.33
Years of education	1529	11.6 (11.2–12.0)	11.4 (10.5–12.3)	11.9 (11.2–12.6)	11.9 (11.7–12.1)	0.68
Sampling factors	1529					
% Sampled during light months	1529	53.7	49.0	48.0	59.0	0.05
% Sampled on weekday	1529	90.0	89.8	90.0	92.4	0.47
% sampled on work day	1529	52.1	51.0	55.0	63.0	**0.002**
Awakening time	1529	7 h 36 min (7 h 28 min–7 h 45 min)	7 h 30 min (7 h 12 min–7 h 48 min)	7 h 31 min (7 h 17 min–7 h 45 min)	7 h 27 min (7 h 23 min–7 h 32 min)	0.35
% >6 h per night	1529	72.8	69.4	71.0	69.4	0.71
Health indicators	1529					
Tobacco use	1529					0.11
% Former smoker	1529	32.4	38.8	29.0	38.4	
% Current smoker	1529	39.8	44.9	40.0	34.4	
Level of physical activity[1] (in MET-minutes)	1529	22.6 (19.9–25.5)[a]	22.1 (16.7–29.0)	20.0 (16.1–24.8)[b]	27.2 (25.7–28.7)[a,b]	**0.001**
Psychiatric indicators	1529					
Lifetime diagnosis	1529					**<0.001**
% Depressive disorder only	1529	22.3	12.2	22.0	33.2	
% Anxiety disorder only		7.4	12.2	5.0	18.2	
% Comorbid anxiety/depressive disorder	1529	70.2	75.5	73.0	48.6	
Severity of psychopathology						
IDS-SR Mood/Cognition Subscale	1526	8.3 (7.81–8.87)[a]	7.6 (6.21–8.93)[b]	8.6 (7.7–9.5)[c]	6.3 (6.0–6.5)[a,b,c]	**<0.001**
IDS-SR Anxiety/Arousal Subscale	1526	5.8 (5.5–6.1)[a]	6.1 (5.4–6.9)[b]	4.9 (4.7–5.1)[c]	4.9 (4.7–5.1)[a,b,c]	**<0.001**
Beck Anxiety Questionnaire	1520	13.1 (12.0–14.3)[a]	14.0 (11.4–17.1)[b]	12.9 (10.8–15.3)[c]	9.0 (8.6–9.5)[a,b,c]	**<0.001**
Antidepressant use						
Duration of use (mean in months, 95% C.I.)	455	14.4 (12.3–16.9)	19.0 (12.1–29.3)[a]	11.7 (9.2–14.7)[a]	–	0.12
Daily dosage level[2] (mean, 95% C.I.)	455	1.2 (1.2–1.2)[a,b]	1.0 (0.9–1.1)[a,c]	1.1 (1.1–1.2)[b,c]	–	**<0.001**

IDS-SR indicates Inventory of Depressive Symptomatology Self-Report.

P-value was calculated by analysis of variance (ANOVA) or the χ^2 test. Significance is inferred at $P<0.05$. Numbers in bold indicate a significant *P* value. Matching superscript letters are given for values that differ significantly within a row (post-hoc test, $P<0.05$).

CI = confidence interval, SSRI = selective serotonin reuptake inhibitor, TCA = tricyclic antidepressant, and AD = antidepressant.

For duration of use, dosage and physical activity back-transformed means (95%C.I.s) are presented, based on estimated marginal means.

[1]MET-minute = (Metabolic Equivalent minute), multiple of the resting metabolic rate.

[2]A comparative daily dosage level (stated as Mean Daily Dose) was calculated by dividing the milligrams of antidepressant used daily by the defined daily dosage (DDD) for the specific antidepressant.

Table 2 Associations between antidepressant use and salivary cortisol indicators (n = 1526).

Cortisol characteristics	N	SSRI users (n = 309)	SSRI users vs. non-users	TCA users (n = 49)	TCA users vs. non-users	Other AD users (n = 100)	Other AD users vs. non-users	Non-users (n = 1068)
		Mean (C.I. 95%)	*P*-value	Mean (C.I. 95%)	*P*-value	Mean (C.I. 95%)	*P*-value	Mean (C.I. 95%)
Unadjusted values								
Cortisol awakening response (CAR)								
Cortisol T1 (awakening, nmol/l)	1512	15.8 (15.1–16.5)	0.84	18.3 (16.4–20.5)	**0.009**	15.6 (14.4–16.9)	0.92	15.7 (15.3–16.1)
Cortisol T2 (+30 min, nmol/l)	1495	19.8 (18.9–20.9)	0.48	18.1 (15.9–20.4)	0.25	19.0 (17.4–20.7)	0.57	19.5 (19.0–20.0)
Cortisol T3 (+45 min, nmol/l)	1481	18.6 (17.6–19.6)	0.23	18.4 (16.4–20.6)	0.54	18.7 (17.0–20.5)	0.40	17.9 (17.4–18.4)
Cortisol T4 (+60 min, nmol/l)	1491	16.0 (15.1–16.8)	0.56	16.9 (14.7–19.3)	0.31	16.0 (14.5–17.6)	0.67	15.7 (15.2–16.1)
AUC_G (nmol/l/h)	1464	18.5 (17.7–19.3)	0.32	18.9 (17.0–21.0)	0.42	18.0 (16.7–19.4)	0.99	18.0 (17.6–18.5)
AUC_I (nmol/l/h)	1464	2.7 (2.0–3.4)	0.56	0.09 (−1.7–1.9)	**0.01**	2.3 (1.0–3.5)	0.78	2.5 (2.1–2.8)
Evening cortisol (nmol/l/h) [a]	1520	5.3 (5.0–5.6)	**0.007**	5.8 (5.0–6.7)	**0.02**	5.3 (4.8–5.9)	0.09	4.8 (4.7–5.0)
Dexamethasone suppression test (DST)								
Cortisol T7 (awakening day 2, nmol/l)	1470	7.3 (6.9–7.7)	**0.001**	8.5 (7.4–9.7)	<**0.001**	7.3 (6.7–8.1)	**0.03**	6.5 (6.4–6.7)
Cortisol suppression ratio (nmol/l) [b]	1457	2.3 (2.2–2.5)	**0.001**	2.4 (2.0–2.8)	0.29	2.3 (2.0–2.5)	**0.02**	2.6 (2.5–2.7)
Adjusted values [c]								
AUC_G (nmol/l/h)	1464	18.5 (17.8–19.3)	0.33	18.4 (16.5–20.4)	0.72	17.9 (16.6–19.3)	0.81	18.0 (17.6–18.5)
AUC_I (nmol/l/h)	1464	2.8 (2.1–3.5)	0.41	0.2 (−1.6–2.1)	**0.03**	2.4 (1.1–3.7)	0.88	2.4 (2.0–2.8)
Evening cortisol (nmol/l)	1520	5.3 (5.0–5.6)	**0.02**	5.6 (4.9–6.5)	0.05	5.3 (4.8–5.8)	0.18	4.9 (4.7–5.0)
Cortisol T7 (awakening day 2, nmol/l)	1470	7.2 (6.9–7.7)	**0.003**	8.2 (7.1–9.3)	**0.002**	7.2 (6.6–7.9)	0.08	6.6 (6.4–6.8)
Cortisol suppression ratio (nmol/l)	1457	2.3 (2.2–2.5)	**0.002**	2.4 (2.1–2.8)	0.37	2.3 (2.1–2.5)	0.05	2.6 (2.5–2.7)

SSRI = selective serotonin reuptake inhibitor, TCA = tricyclic antidepressant, AD = antidepressant, AUC_G = area under the morning curve with respect to the ground, AUC_I = area under the morning curve with respect to the increase, T = time point, and CI = confidence interval.

For all cortisol indicators except for AUC_I backtransformed means (95%C.I.s) are presented, based on estimated marginal means, calculated by analysis of covariance (ANCOVA). For AUC_I estimated marginal means (95%C.I.s) are presented. P-values are calculated by ANCOVA comparing two groups at a time. Significance is inferred at $P<0.05$. Numbers in bold indicate a significant *P* value.

[a] The evening cortisol level is the average of T5 and T6 (taken at 22:00 h and 23:00 h).

[b] The cortisol suppression ratio is the ratio of salivary cortisol at T1 to salivary cortisol at T7 after 0.5 mg dexamethasone.

[c] Adjusted for sociodemographic variables (sex, age, education), psychiatric indicators (comorbidity), health indicators (smoking and physical activity), and sampling factors (seasonal light, work status, weekday/weekend, awakening time, sleep).

3.1. Cortisol awakening response

A large percentage of the TCA users (42.9%) did not show the characteristic increase in cortisol in the first hour of awakening as compared to only 26.2% of SSRI users, 33.0% other AD users, and 28.1% non-users. Adjusted CAR results showed that TCA, SSRI, and other AD users did not differ from non-users on overall cortisol levels, reflected by analysis of AUC_G and a non significant group effect in LMM analysis ($F(3,1500) = 0.14$, $p = 0.94$).

The time course of the awakening response of TCA users differed from that of non-users as well as of SSRI users and other AD users, reflected by analysis of AUC_I (effect size [Cohen's d] = 0.34, p = 0.03, TCA users vs. non-users, see Table 2) and a significant group * time interaction in the LMM analysis ($F(3,3268) = 4.53$, p = 0.004; TCA users vs. non-users, data not shown). As can be seen in Fig. 1, TCA users had a considerably flattened CAR compared to all other groups. None of the other antidepressant user groups differed from the non-user group on the AUC_I.

3.2. Evening cortisol

Unadjusted and adjusted evening cortisol levels were significantly higher for SSRI users ($d = 0.04$, p = 0.02) and marginally significant for TCA users (effect size = 0.17, p = 0.05) as compared to non-users. Other AD users did not differ from non-users.

3.3. Dexamethasone suppression test

After adjustment for covariates, T7 cortisol levels in SSRI and TCA users were higher (SSRI vs. non-users: $d = 0.19$, p = 0.003; TCA vs. non-users: $d = 0.46$, p = 0.002) but other AD users differed only marginally from non-users at T7.

Unadjusted and adjusted cortisol suppression ratios were lower in SSRI users ($d = 0.03$, p = 0.002) and marginally significant in other AD users ($d = 0.22$, p = 0.05) as compared to non-users, indicating a smaller cortisol suppressing effect of dexamethasone.

Consistent results were found for SSRI users (vs. non-users), who had both higher T7 values and the expected lower cortisol suppression ratio. For the TCA users, results of the DST and T7 were incongruous. TCA users had higher T7 values, but a non-statistically significant lower cortisol suppression ratio. As the number of subjects using TCAs was much smaller than in the SSRI group, type II errors could have occurred for the latter contrasts. Due to these discrepancies between T7 and the DST in the TCA group, no conclusions could be drawn on basis of these findings and only the suppression findings of the SSRI group (which were consistent across cortisol indicators) were considered in the discussion.

Additional adjustment for severity with the IDS-SR and BAI as well as the exclusion of multiple antidepressant users, lithium users, and heavy smokers did not significantly alter the results in any of the conducted analyses (data not shown).

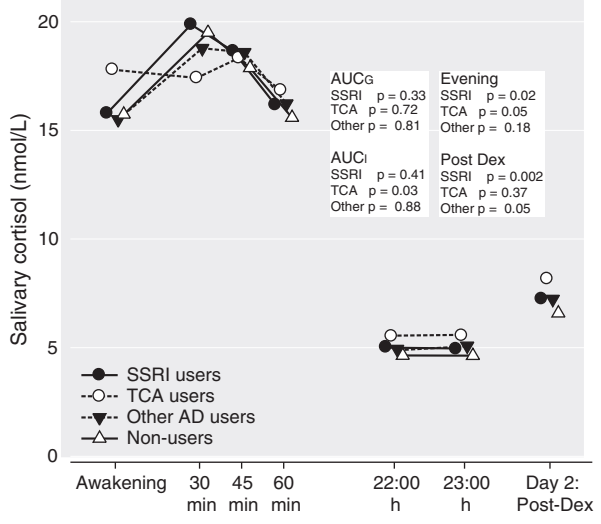

Figure 1 Mean salivary cortisol levels of the CAR, evening cortisol and cortisol after dexamethasone administration adjusted for sociodemographic variables, psychiatric indicators, health indicators, and sampling factors. Numerical values for cortisol indicators are shown.

3.4. Antidepressant dose and duration

In the linear regression analyses, performed in SSRI and TCA users separately (n = 358), dosage level or duration of use was not associated with cortisol indicators (data not shown).

4. Discussion

In this study, the relationship between antidepressant use and multiple salivary cortisol measures was investigated in 1526 NESDA participants with a lifetime diagnosis of depression and/or anxiety. As compared to non-users, TCA users had a flattened CAR, SSRI users had higher evening cortisol levels, and SSRI users displayed a lower suppressing effect of dexamethasone.

Though previous studies have linked lower morning cortisol levels at a single time point with TCA use (Deuschle et al., 2003), the association between TCA use and the CAR has not previously been studied. However, the atypical AUC_I pattern seen in the majority of TCA users in our study is unlikely to indicate that TCAs are clinically effective by producing a flattened CAR. As the CAR is commonly viewed as a healthy reaction to awakening, with awakening representing a natural stressor (Kuehner et al., 2007), the atypical curve most probably reflects an impaired ability to react to the stress of awakening in more severely depressed and difficult-to-treat subjects for whom TCAs are prescribed.

A biological explanation for the atypical curve may lie in the effects of antidepressants on the two main corticosteroid receptors, GR and MR (Bjartmar et al., 2000). GRs are assumed to restore corticosteroid homeostasis after circadian peaks. Consequently, increased expression of GR may increase suppression of cortisol levels (Eiring and Sulser, 1997). Most animal studies (Johansson et al., 1998), but not all of them (Pariante et al., 2003), found that chronic TCA administration resulted in upregulation of GRs, which might contribute to the observed flattened CAR. Additionally, the MR was recently identified as a modulator of the CAR (De Rijk et al., 2006) and found to be upregulated after chronic TCA administration in many (Bjartmar et al., 2000), but not all (Przegalinski and Budziszewska, 1993), animal studies. Thus the MR may also contribute to the flattened CAR. However, the total cortisol

secretion during the first hour after awakening (i.e., AUC_G) was not reduced in TCA users as compared to non-users. Therefore, an increased sensitivity to corticosteroids in response to TCAs is unlikely to be the sole biological explanation for our findings.

We observed higher basal cortisol levels and decreased cortisol suppression in SSRI users. Previous studies reported lower (Vythilingam et al., 2004) or unchanged (Deuschle et al., 2003; Juruena et al., 2010) basal levels and variously altered (decreased, increased and unchanged) cortisol suppression in users (Aihara et al., 2007; Deuschle et al., 2003; Vythilingam et al., 2004; Watson et al., 2006). There are several possible explanations for these discrepancies. Associations of reduced cortisol levels with a positive treatment response (Deuschle et al., 2003) indicated that normalized cortisol levels may not reflect antidepressant use alone. On the other hand, in NESDA (Vreeburg et al., 2009a) and other studies, (Bhagwagar et al., 2003) elevated cortisol levels were found in both current and remitted depressed subjects and an intervention reported no association between suppression after prednisolone administration and treatment response (Juruena et al., 2010). Altered HPA axis activity may reflect a biological vulnerability (Juruena et al., 2010), independent of treatment success (Vreeburg et al., 2009a). However, an intervention study found that impaired response to the prednisolone suppression test was associated with treatment resistance (Juruena et al., 2009). Therefore, additional randomized intervention studies are needed in order to investigate the relationship between the different cortisol indicators and treatment response.

Alternatively, the cortisol-reducing effects of antidepressants in intervention studies may be temporary, implying that conflicting findings were due to inter-study differences in treatment duration. Indeed, an animal study showed the up-regulating effect of antidepressants on MR and GR receptor levels to be transient in nature (Reul et al., 1993). In contrast to the five or six weeks of treatment in intervention studies (Deuschle, 2003), 88.5% of NESDA users reported at least two months of antidepressant use and 47.1% of users reported chronic use ($\geq$12 months). However, as we found an altered CAR in TCA users, the majority of whom were chronic users, altered stress responses might persist in TCA users. Furthermore, dampening of the HPA axis has been hypothesized to be related to therapeutic efficacy of antidepressants (Pariante, 2009) and antidepressants have been shown to be an effective long-term treatment. The long-term effects of antidepressants on cortisol need further investigation in prospective studies before firm conclusions can be drawn.

Alternatively, the discrepancies of our results with previous findings could be due to the inclusion of subjects with lifetime diagnoses, leading to a broader range of illness severity. As subjects in need of antidepressant treatment are usually more severely ill than non-users, this may confound the association between antidepressants and cortisol. However, although antidepressant users scored higher than non-users on the IDS-SR in the present study, severity did not confound the associations found.

Our study had several limitations. We performed a cross-sectional analysis, which precluded causal inferences. Since our study categorized length of use in number of months and the majority of users reported at least two months of use, it was not possible to evaluate cortisol levels after five or six weeks to directly compare our results with those found in short-term intervention studies. In addition, the ambulatory setting and the consequent possibility of non-compliance with instructions on saliva collection may have resulted in measurement error. Despite these limitations, our study had many valuable features. Our large sample size and multiple cortisol measurements allowed us to compare sizable groups of users of different antidepressant types on several cortisol measures indicative of different aspects of HPA axis activity, while adjusting for many potential confounders.

In conclusion, we found an atypical cortisol awakening response in TCA users as well as higher basal cortisol levels and decreased cortisol suppression for SSRI users as compared to non-users within a sample of 1526 NESDA participants with a lifetime disorder of depression and/or anxiety. These findings suggest that antidepressant subtypes may be associated with distinct alterations of the HPA axis. Possible antidepressant-induced alterations of the HPA axis might also affect comorbid physical diseases for which altered cortisol levels are an underlying cause or contributor, such as diabetes, cardiovascular disease and osteoporosis, in subjects with anxiety and depression (Bruehl et al., 2007; Nieman, 2007).

Further research on these effects in randomized trials and prospective cohort studies may lead to a better understanding of the varied efficacies of antidepressants and more effective drug treatments for anxious and depressed patients.

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Contributors

Ms. Manthey and Ms. Leeds searched for literature, analyzed the data, and wrote the article. Ms. Leeds wrote the first draft under the supervision of Ms. Manthey, who later completed the final version. Ms. Manthey was also involved in data gathering (interviews with participants). Dr. Giltay and Ms. van Veen are the supervisors of Ms. Manthey and provided regular feedback on the written material and helped with the statistical analysis. Ms. Vreeburg was responsible for the data cleaning of the salivary cortisol data, contributed to the Experimental procedures section on salivary cortisol, and supplied input on cortisol related content. Prof. Penninx and Prof. Zitman are the promoters of Ms. Manthey, reading the article approximately once every two months and providing feedback.

Conflict of interest

All authors declare that they have no conflicts of interest.

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