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Hypothalamic serotonin-1A receptor binding measured by PET predicts the plasma level of dehydroepiandrosterone sulfate in healthy women

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ABSTRACT

Serotonin modulates the activity of the hypothalamic–pituitary–adrenal (HPA) axis particularly via the serotonin-1A receptor (5-HT_{1A}). Therefore, the rationale of this positron emission tomography (PET) study was to investigate the influence of the 5-HT_{1A} receptor distribution in the human brain on plasma levels of dehydroepiandrosterone sulfate (DHEAS) and cortisol *in vivo*. Eighteen healthy female were measured with PET and the selective 5-HT_{1A} receptor radioligand [carbonyl-^{11}C]WAY-100635. Nine a priori defined brain regions (hypothalamus, orbitofrontal cortex, amygdala, hippocampus, anterior and posterior cingulate cortices, dorsal raphe nucleus, retrosplenial cortex, and insula) and the cerebellum (reference region) were delineated on coregistered MR images. DHEAS and cortisol plasma levels were collected by blood sampling in the morning of the PET day. Linear regression analysis of DHEAS plasma level as dependent variable and hypothalamic 5-HT_{1A} receptor binding potential (BP) as independent variable showed a highly significant association ($r = .691$, $p = .002$). The hypothalamic 5-HT_{1A} BP predicted 47.7% of the variability in DHEAS plasma levels. Regressions were borderline significant ($p < .01$, Bonferroni corrected threshold <.0056) between 5-HT_{1A} BP in the anterior cingulate and orbitofrontal cortices and free cortisol levels. No significant associations between DHEAS or cortisol and the 5-HT_{1A} receptor BP in other investigated brain regions were found. In conclusion, the serotonergic system may influence the DHEAS plasma level by modulating CRH and ACTH release via hypothalamic 5-HT_{1A} receptors as reported for cortisol before. As disturbances of the HPA axis as well as changes of the 5-HT_{1A} receptor distribution have been reported in affective disorders, future studies should focus on these interactions.

Complex interactions between the hypothalamic–pituitary–adrenocortical (HPA) axis and the serotonergic system have been reported [20]. Disturbances in the HPA axis [7,17], as well as changes in the serotonin-1A (5-HT_{1A}) receptor binding potential (BP) in distinct brain regions are strongly associated with affective [25] and anxiety disorders [18,29]. Serotonin modulates the activity of the HPA axis to a large extent through the 5-HT_{1A} receptor [11]. In return, hormones of the HPA axis have regulatory effects on the serotonergic neurotransmission [10]. Cortisol and especially dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS), the hydrophilic storage form in the blood, are major secretory products of the human adrenal glands.

DHEAS levels increase in response to acute stress in parallel with cortisol. However, they decline markedly with chronic stress, even though cortisol levels may remain elevated. In contrast to cortisol, the secretory activity of which appears more sensitive to day-to-day variability, the secretory pattern of DHEA is considerably stable and no distinct circadian rhythm of DHEA plasma level is observed [14]. In contrast to cortisol, DHEAS plasma levels are gradually rising in pre-puberty reaching peak concentrations in the third decade of life before declining again in older age [2].

Serotonergic stimulation of the 5-HT_{1A} receptor in the hypothalamus has been shown to increase the corticotropin-releasing hormone (CRH) and as a consequence to raise the secretion of adrenocorticotrophic hormone (ACTH) [24] by the pituitary gland. CRH has been reported to be a potent stimulator for DHEA secretion in males [15] and for DHEA/DHEAS secretion in females with hyperandrogenism [16]. CRH was shown to be 70% less potent than ACTH at stimulating cortisol production, indicating that its actions were preferentially directed toward increasing DHEAS synthesis [28]. Several challenge studies reported that ACTH stimulates

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dose-dependent DHEA [3] and cortisol secretion of the adrenal glands. Adults secrete both DHEA and DHEAS from the zona reticularis of the adrenal cortex and also DHEA from the ovary and testis [22]. In humans DHEA is converted to DHEAS by the enzyme hydroxysteroid sulfotransferase. Intra- and extra-adrenal regulatory mechanisms have also been reported [23], though ACTH is still the fundamental regulatory factor in the secretion of DHEAS [1]. *In vivo* challenge studies with ACTH revealed that an extremely low dose of ACTH is needed to stimulate the secretion of adrenal steroids, and DHEAS is particularly more sensitive to stimulation by ACTH than cortisol, and therefore DHEAS appears as a highly sensitive indicator of ACTH secretion [3].

In this PET brain study we investigated the relationship between the 5-HT_{1A} receptor BP and the plasma levels of DHEAS and cortisol. The hypothalamus region was of special interest, as this brain region is both an essential part of the HPA axis and a regulatory region of the serotonergic system that is involved in the secretion of CRH, ACTH and, consequently, DHEAS and cortisol. This study was therefore focused on the hypothalamus and eight other selected brain regions. The main aim of this study was to demonstrate a significant association between hypothalamic 5-HT_{1A} receptor BP measured by PET *in vivo* and the adrenal steroid hormones DHEAS and cortisol measured in blood plasma.

18 healthy female volunteers participated in this PET study. Our subjects were in a small age range from 21 to 29 years (24.2 ± 2.6 years, mean age $\pm$ SD) to exclude age effects. One subject was excluded from data analysis since no hormonal data were available. The following exclusion criteria were applied: any psychiatric and any serious medical history including drug abuse, the use of psychotropics or hormonal contraceptives over the past 6 months, including oral contraceptives and anabolic steroids, a positive urine pregnancy test and irregular menstrual cycles. The subjects were physically examined, routine blood tests and an electrocardiogram as well as psychological tests including the MINI-International Neuropsychiatric Interview (M.I.N.I.) were performed. The study was approved by the Ethics Committee at the Medical University of Vienna. All subjects signed written informed consent. The study presented here is an extension of our investigations focused on gender differences in the regional 5-HT_{1A} binding and on the difference in 5-HT_{1A} BP between patients with social phobia and healthy controls [18,30]. In these studies males and females were enrolled, however, DHEAS measurements were just available in females.

For hormone assays, venous blood samples were taken from the cubital vein. Due to circadian variations of cortisol the blood samples were collected in the early morning hours (8.6 ± 1.8 a.m.) depending on the unforced awakening. In order to exclude effects of unusual waking-up time on steroid hormone plasma levels there was no fixed blood sampling time. Furthermore, blood sampling was carried out at minimum intervals of 60 min after awakening in view of data showing increased fluctuations of the cortisol levels within the first hour after awakening. It is presumed that the anticipatory stress towards the blood withdrawal is similar to more stressful than a PET measurement. Blood sampling and PET scans were performed on the same day in the mid-follicular phase of the menstrual cycle, i.e. 7 ± 3 days after the start of menstrual bleeding. Assays were performed using the E170 Module (Roche E170 Modular Analytical System®). For quantification of total DHEAS and cortisol in plasma, we used electrochemoluminescence (ECLIA). Transcortin plasma levels were quantified by using radioimmunoassay (RIA). Free cortisol plasma levels were calculated using the total cortisol and transcortin plasma levels. DHEAS and cortisol levels are given as total plasma levels, except when otherwise indicated in the text. The lower limit of sensitivity was 0.1 μg/dl (0.273 nmol/l) and the interassay coefficient of variation was 5–8% for DHEAS, 0.04 μg/dl (0.11 nmol/l) and 6% for cortisol, respectively (see http://www.kimcl.at). The analyses were carried out by the Clinical Institute for Medical and Chemical Laboratory Diagnostics at the Medical University of Vienna.

In order to investigate the distribution of the 5-HT_{1A} receptor we used [*carbonyl*-^{11}C]WAY-100635 as radioligand. The injected dose was 365.5 ± 13.6 MBq (mean $\pm$ SD). PET measurements were performed on a GE Advance Scanner. To correct tissue attenuation, a transmission scan with duration of 5 min was carried out in 2D mode using a retractable GE ring source. A bolus of [*carbonyl*-^{11}C]WAY-100635 in phosphate-buffered saline was injected. Dynamic scans, in 3D mode, started simultaneously with the injection of the tracer. A series of 30 (15 min × 1 min, 15 min × 5 min) time frames was collected in 90 min, 35 contiguous slices were reconstructed (matrix 128 × 128). The spatial resolution of the final attenuation corrected and reconstructed image (filtered back-projection) had a full-width at half-maximum of 4.36 mm at the centre of the field of view.

The kinetic modelling tool of the biomedical image quantification software PMOD 2.65 (http://www.pmod.com) was used for quantification of the 5-HT_{1A} receptor binding potential (BP). We applied the simplified reference tissue model (SRTM) using the cerebellum as reference region (as it has low 5-HT_{1A} receptor density). Decay corrected time activity curves (TACs) were obtained using the 30 frames of the dynamic PET data and the three-dimensional ROIs. We calculated the regional BP and the regional relative delivery of the radioligand normalised to the cerebellum (R1). The BPs of the right and left ROIs (except for the raphe region, the hypothalamus, and the medial orbitofrontal cortex, which were drawn as a single region each) were combined to improve the signal-to-noise ratio.

To define anatomical areas in the PET images, we acquired structural magnetic resonance images (MRI) using the MPRAGE sequence (256 × 256 matrix, 0.78 mm × 0.86 mm voxel size, slice thickness 1.56 mm, 128 slices) for each subject on a 3 Tesla MR scanner (Bruker BioSpin, Ettlingen, Germany). MR images were coregistered to the summed dynamic PET images (PET integral image, PET_{ADD} images) using SPM2 (http://www.fil.ion.ucl.ac.uk/spm).

Ten a priori defined regions of interest (ROI) were drawn on the coregistered magnetic resonance images (MRI) using a triplanar tracing technique and PMOD 2.65 (http://www.pmod.com). First, individual hypothalamic ROIs were delineated on the coregistered images. These hypothalamic ROIs (0.4 ± 0.2 cm^3, mean $\pm$ SD) were defined on sagittal MR images using the mammillary nuclei, the anterior commissure and the chiasma opticum as inferior–posterior, anterior–superior and anterior–inferior borders, respectively (see Fig. 1). To define the hypothalamic borders for delineation of the ROI we used the criteria given by Talairach and Tournoux stereotaxic atlas. The lateral hypothalamic margin was drawn on axial MR slices using the grey to white matter border. The amygdala (2.1 ± 0.9 cm^3, mean $\pm$ SD), the medial orbitofrontal cortex (3.1 ± 2.8 cm^3), the anterior (3.1 ± 0.7 cm^3) and posterior (1.4 ± 0.1 cm^3) cingulate cortices, the insula (7.4 ± 1.0 cm^3), the hippocampus head (2.1 ± 0.2 cm^3), and the region of reference in the cerebellum (19.4 ± 2.7 cm^3) were traced using the anatomical criteria adopted from Bremner et al. [4]. Based on the central role of the retrosplenial region and ventral posterior cingulate cortex in the processing of self-relevant emotional contents including episodic memory, we traced the retrosplenial region (1.1 ± 0.4 cm^3) using the criteria set out by Vogt et al. The raphe region (fixed ROI, 0.75 cm^3) in the midbrain was directly traced on the PET_{ADD} image (see Fig. 1).

We used SPSS 12.0.1 (©SPSS Inc. 1989–2003) for statistical analysis. In order to check the normal distribution and equality of variance, the Kolmogorov–Smirnov test and Levene's test were carried out. Linear regression analyses with the regional 5-HT_{1A} receptor binding potentials as independent variables (predictors) and the hormone levels of DHEAS or cortisol as dependent