Hypothalamic functions in patients with pituitary insufficiency
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Imaging of serotonin transporters with $^{123}$I FP-CIT SPECT in the human hypothalamus

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ABSTRACT

Background: Serotonergic neurons in the rodent hypothalamus are implicated in key neuroendocrine and metabolic functions, including circadian rhythmicity. However, the assessment of the serotonergic system in the human hypothalamus in vivo is difficult, as delineation of the hypothalamus is cumbersome with conventional region-of-interest analysis. In the present study, we aimed to develop a method to visualize serotonin transporters (SERT) specifically in the hypothalamus. Additionally, we tested the hypothesis that hypothalamic SERT binding ratios are different between patients with hypothalamic impairment (HI) or pituitary insufficiency (PI) and control subjects.

Methods: SERT availability was determined in 17 subjects (6 HI, 5 PI and 6 healthy controls), 2 hours after injection of $^{123}$I-$\omega$-fluoropropyl-2$\beta$-carboxymethoxy-3$\beta$-(4-iodophenyl) nortropane ($[^{123}\text{I}]\text{FP-CIT}$), using SPECT (performed on a brain-dedicated system) fused with individual MRIs of the brain. The hypothalamus (representing specific SERT binding) and cerebellum (representing non-specific binding) were manually delineated on each MRI to assess $[^{123}\text{I}]\text{FP-CIT}$ binding and specific to non-specific binding ratios.

Results: In each healthy subject, $[^{123}\text{I}]\text{FP-CIT}$ binding was higher in the hypothalamus than in the cerebellum, and the mean hypothalamic binding ratio of SERT was $0.29 \pm 0.23$. We found no difference in hypothalamic binding ratios between HI, PI and control subjects [HI $0.16 \pm 0.24$, PI $0.45 \pm 0.39$, C $0.29 \pm 0.23$, p-value 0.281].

Conclusions: We were able to demonstrate SERT binding in the human hypothalamus in vivo. However, we did not find altered hypothalamic SERT binding in patients with hypothalamic impairment.
BACKGROUND

The human hypothalamus is a small brain structure of only 4 ml in the diencephalon that directs a multitude of important functions in the body, including pituitary hormone release, diurnal rhythmicity, energy homeostasis and autonomic regulation (1). The serotonergic system is one of the key regulators of these functions (2-6). Animal studies showed that numerous hypothalamic areas receive axon collaterals from serotonergic perikarya located in the midbrain (7;8). Hypothalamic microinjection of serotonergic agents into brain-cannulated rats produces potent and selective effects on feeding patterns and food-choice (9). Moreover, serotonergic stimulation of selected hypothalamic areas in rodents affects energy metabolism (10), circadian rhythmicity (11) and cardiovascular responses (4). By inference, dysfunction of the serotonergic system is likely to be one of the determinants of symptoms in patients with hypothalamic dysfunction such as obesity, disturbed sleep and drowsiness (12-14).

Imaging of serotonin transporters (SERT) with single photon emission computed tomography (SPECT) or positron emission tomography (PET) provides an important opportunity to study the serotonergic system in vivo. SERT are expressed exclusively in the membrane of serotonergic neurons and regulate intrasynaptic neurotransmitter levels. The concentration of transporters is assumed to reflect the homeostatic tone of neurotransmitter systems (15). Several studies have investigated SERT in vivo in the diencephalon in humans (16-19), providing strong evidence for expression of SERT in the human diencephalon. However, the expression of SERT in the hypothalamus was poorly defined, as spatial resolution of nuclear imaging techniques is limited and delineation of a structure as small and heterogeneous as the hypothalamus is cumbersome with conventional region-of-interest (ROI) analysis (20). To our knowledge only one study demonstrated specific hypothalamic SERT binding using PET and [11C]DASB, although the delineation of the hypothalamus was not strictly defined (21).

The aim of this study was to evaluate whether SERT binding can be demonstrated in the human hypothalamus in vivo using SPECT imaging and [123I]-N-ω-fluoropropyl-2β-carboxymethoxy-3β-(4-iodophenyl)nortropane ([123I]FP-CIT). For this purpose we combined conventional magnetic resonance imaging (MRI) for anatomical reference with SPECT imaging of the SERT with [123I]FP-CIT using a brain-dedicated system (22;23). This radiotracer is approved to visualize and quantify dopamine transporters at early as 3 hours after injection (24), but more recent studies showed its capacity to assess binding to extrastriatal SERT as well, optimally between 2 and 3 hours after injection (23;25).

As a next step, we investigated if hypothalamic specific-to-nonspecific [123I]FP-CIT binding ratios are impaired in patients treated for a large sellar tumour giving rise to visual field defects. These tumours are highly suspect for giving rise to hypothalamic impairment by various factors including direct tumour invasion or involvement, trauma related to surgery, and radiation (26). As these patients suffer from pituitary insufficiency, we included a third
subjects with pituitary insufficiency without a history of visual field defects, radiotherapy and surgery to correct for potential confounding by endocrine factors.

METHODS

Subjects
Six healthy control subjects were included in the present study. Exclusion criteria were age below 18 or above 65 years, the use of medication interfering with serotonin or dopamine metabolism (e.g. psychotropic medication like SSRIs or other antidepressants), life-time ecstasy, amphetamine or cocaine use, intravenous drug abuse as measured by self report, participation in another study associated with exposure to ionizing radiation during the last 12 months, pregnancy and the presence of any contra-indication for MRI. All subjects completed the Beck Depression Inventory, the Mini-Mental State Examination (MMSE), the Symptoms Checklist (SCL-90) and Snaith-Hamilton Pleasure Scale (SHAPS) before inclusion to exclude subjects with severe neuropsychiatric problems.

Furthermore, eligible patients with clinical suspicion of hypothalamic impairment and patients with pituitary insufficiency, i.e. at least one impaired anterior pituitary hormonal axis, were recruited from the outpatient clinic of the department of Endocrinology and Metabolism of the Academic Medical Centre and the department of Endocrinology of the VU Medical Centre. All patients were seen on a regular basis by an internist-endocrinologist for clinical and biochemical evaluation. They received conventional hormone replacement therapy consisting of L-thyroxine, hydrocortisone, testosterone, recombinant human growth hormone (rhGH) and/or vasopressin analogues when indicated. Exclusion criteria were identical to those for healthy control subjects.

We selected three groups that were carefully matched for age and gender: 1) control subjects; 2) those with probable hypothalamic impairment (HI), defined as having a history of surgery in the sellar region, cranial radiotherapy as well as compression of the optic chiasm; 3) those with pituitary insufficiency, without a history of cranial surgery, radiotherapy or compression of the optic chiasm (PI). Written informed consent was obtained from all subjects and the study was approved by the Medical Ethical Committee of the Academic Medical Centre from the University of Amsterdam and performed in accordance with the Declaration of Helsinki.

[^123I]FP-CIT Brain SPECT imaging
Subjects were examined using SPECT with the ligand[^123I]FP-CIT, which has a high affinity for the dopamine transporter and somewhat lower affinity for the SERT. Radiosynthesis of[^123I]FP-CIT was performed as described earlier (27). To block uptake of free radioactive iodide in the thyroid, each subject received 300 mg of potassium iodide in the 24 hours
before the SPECT imaging. Acquisition of the SPECT images took place at 2 hours after an intravenous bolus injection of approximately 115 MBq $^{[123]}$FP-CIT (range, 110–120 MBq). They were performed using a 12-detector single-slice brain-dedicated scanner (Neurofocus 810, which is an upgrade of the Strichmann Medical Equipment) with a full-width at half-maximum (FWHM) resolution of approximately 6.5 mm throughout the 20-cm field of view (https://cc3yr1-j8owgedrpj8vz-hh.sec.amc.nl). Subjects were positioned with their head parallel to the orbitomeatal line to acquire axial slices parallel and upward from this line to the vertex in 5 mm steps. The energy window was set at 135–190 keV. Attenuation correction of all images was performed as described earlier (28) and all images were reconstructed in 3-D mode.

**Figure 1.** Schematic illustration of delineation of the hypothalamus in coronal view. (A), (B) and (C) represent different levels of the hypothalamus, from rostral (A), middle (B) to caudal (C). Note that only one side of the hypothalamus is shown.

**Figure 2.** (A) Coronal SPECT image of a healthy subject 2 h after injection of approximately 115 MBq $^{[123]}$FP-CIT at the level of the hypothalamus. (B) Coronal T1 weighted MRI image of the same subject with region of interest (ROI) drawn in the hypothalamus. (C) Coregistered SPECT and T1-weighted MRI image with ROI drawn in the hypothalamus. The SPECT images are colour encoded for low (black) to high activity (yellow).
MRI
For anatomical reference, a T1-weighted 3-D MRI scan was acquired in each individual using a 3-T Philips Intera scanner (Philips Healthcare, Best, The Netherlands) with a standard head coil.

Image Analysis
To analyze the brain SPECT images, we defined ROIs for hypothalamus and cerebellar cortex (excluding vermis) for each participant. These unique ROIs were manually drawn by experienced researchers in the field of the hypothalamus (AA and EF) on each individual T1-weighted 3D MRI scan using in-house developed software (29). AA and EF were blinded to the clinical data. Using the same software, SPECT scans of the subjects were matched with their individual T1-weighted 3D MRI scan and the mean amounts of radioactivity/voxel were determined for each ROI. Activity in the cerebellar cortex (excluding vermis) was assumed to represent non-specific binding. The specific-to-nonspecific binding ratios were calculated as follows: binding in hypothalamus minus non-specific binding in cerebellar cortex / non-specific binding in cerebellar cortex (30;31).

Delineation of the hypothalamus on MRI
We delineated the hypothalamus as visualized schematically in Figure 1, using anatomical landmarks wherever possible. Rostral border: lamina terminalis, where the optic chiasm attaches to the mediobasal hypothalamus. Lateral border: as indicated in Figures 1 and 2. Dorsal border: septum verum. We included the area of the bed nucleus of the stria terminalis and the lateral septum. At a more caudal level (Figure 1B) we used the sulcus hypothalamicus as dorsal border. Caudal border: we included the mammillary bodies as most caudal hypothalamic structures.

Sample size calculation
In a previous study by Booij et al. [123I]FP-CIT binding ratios to SERT in the diencephalon of healthy subjects was 0.51 ± 0.17 (22). Blocking of SERT in the diencephalon by paroxetine (a selective serotonin reuptake inhibitor) decreases this binding ratio to 0.17 ± 0.15. As no preliminary data were available regarding the effect of HI on serotonergic neurotransmission in the hypothalamus, we used these values to calculate our sample size assuming that healthy controls will have a binding ratio of [123I]FP-CIT to SERT in the diencephalon of 0.51 ± 0.17, that HI patients will have a the binding ratio of 0.17 ± 0.15 and that PI patients will have a binding ratio of 0.34 ± 0.16. To detect a difference between the three groups (hypothalamic impairment vs. pituitary insufficiency without hypothalamic impairment vs. healthy controls) with significance level $\alpha = 0.05$, power = 80%, variance of means = 0.019 and a common standard deviation = 0.16, we needed 6 subjects per group (used software: nQuery Advisor 7.0, 1995-2007, Janet D. Elashoff).
Statistics
Statistical analysis was done using PASW Statistics for Windows, version 19.0 (SPSS Inc. Chicago, Illinois, USA). Numerical variables were presented as mean ± SD and categorical variables as counts (percentages). Interobserver variability in the hypothalamic specific-to-nonspecific binding ratios was assessed using intraclass correlation coefficient (ICC). Differences between the three groups were tested with one-way ANOVA, or Chi-square test where appropriate. A two-sided p-value < 0.05 was considered statistically significant.

RESULTS
Subjects
Seventeen subjects were included in this study: 6 healthy control subjects, 6 subjects with HI, and 5 subjects with PI. The HI group consisted of subjects treated for non-functioning macroadenoma (n=3), craniopharyngioma (n=1), GH-producing macroadenoma (n=1) or dysgerminoma (n=1). All subjects with HI were ACTH-, TSH- and LH/FSH deficient, n=5 had GH deficiency and n=2 had ADH deficiency. In the PI group, n=3 subjects had Sheehan syndrome and n=2 subjects had pituitary apoplexy. All subjects with PI were ACTH-, GH-, and LH/FSH deficient and n=4 were TSH deficient.

As expected, the three groups were comparable with respect to age, sex and body mass index (table 1).

SPECT measures of SERT in the hypothalamus in healthy control subjects
In each healthy control subjects, [123I]FP-CIT binding was higher in the hypothalamus than in the cerebellum, and the mean hypothalamic binding ratio of SERT was 0.29 ± 0.23.

Table 1. Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>HI n=6</th>
<th>PI n=5</th>
<th>Controls n=6</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Age - yr</td>
<td>51.0 ± 6.0</td>
<td>53.8 ± 6.1</td>
<td>49.7 ± 7.4</td>
<td>0.590</td>
</tr>
<tr>
<td>Male/Female (n)</td>
<td>2 / 4r</td>
<td>2 / 3</td>
<td>2 / 4r</td>
<td>0.966</td>
</tr>
<tr>
<td>Body mass index - kg/(height) 2</td>
<td>32.7 ± 10.1</td>
<td>29.3 ± 5.5</td>
<td>26.6 ± 2.0</td>
<td>0.333</td>
</tr>
<tr>
<td>ACTH deficiency</td>
<td>6 (100%)</td>
<td>5 (100%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>GH deficiency</td>
<td>5 (83.3%)</td>
<td>5 (100%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>TSH deficiency</td>
<td>6 (100%)</td>
<td>4 (80%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>LH/FSH deficiency</td>
<td>6 (100%)</td>
<td>5 (100%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>ADH deficiency</td>
<td>2 (33.3%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

HI = patients with probable hypothalamic impairment, defined as having a history of surgery in the sellar region, cranial radiotherapy and compression of the optic chiasm; PI = subjects with pituitary insufficiency but without a history of cranial surgery, radiotherapy or compression of the optic chiasm.
We found no difference in hypothalamic binding ratios of SERT between HI, PI and control subjects [HI 0.16 ± 0.24, PI 0.45 ± 0.39, C 0.29 ± 0.23, p-value 0.281]. Of note, there was a very good inter-observer agreement between the two independent observers (ICC 0.951, 95% CI 0.872 – 0.982).

**DISCUSSION**

This study is the first to demonstrate in vivo SERT binding in the human hypothalamus using $^{[123]}$I-FP-CIT SPECT. We were able to demonstrate hypothalamic SERT binding by fusing SPECT scans with individual conventional MRIs. Previous SPECT studies have reported SERT binding in the thalamus/hypothalamus region, hypothalamic/midbrain area or diencephalon (18;22;32) and one study examined hypothalamic SERT binding using PET imaging and $^{[11]}$C-DASB (21). However, the delineation of the hypothalamus in those studies was not as precise as in our present method. We used 3T-MRI for anatomical reference to manually draw unique templates of each hypothalamus. The used technique overcomes the lack of anatomical reference on SPECT images, which has often been a methodological limitation. Moreover, two experts with extensive knowledge on the neuroanatomy of the human hypothalamus delineated the hypothalamus, with a very good inter-observer agreement. This is an important aspect, as the exact borders of the hypothalamus are not a matter of clear-cut certainty (33;34) and the distribution of important cell types is not necessarily limited by classical hypothalamic neuroanatomical landmarks as visualized by Nissl staining.

The mean hypothalamic SERT binding ratio of the present study appeared to be lower than previously reported in the diencephalon (23), possibly related to a more precise delineation of the target area in the present study and to age differences. Central SERT availability declines with physiological aging (35) and our study included older subjects than the study by Koopman et al (23).

We observed a relatively large interindividual variation in SERT binding potential. This is in line with previous observations of SERT in the midbrain and diencephalon areas (21;23;36;37). We cannot rule out to have systematically underestimated the binding ratio as the cerebellum contains small amounts of SERT and due to partial volume effects (38;39). Using the cerebellum to correct for non-specific binding, this underestimation is expected to be 7% at most (40).

Contrary to our hypothesis, we were unable to demonstrate differences in hypothalamic SERT binding ratios between HI, PI and control subjects, suggesting that hypothalamic serotonergic neurotransmission is not severely affected in patients suspect for hypothalamic impairment. This is remarkable, as serotonin plays a very important role in the hypothalamus (2-6), which is supported by immunohistochemical studies in animals (2;41;42) and humans (43) showing strong SERT immunoreactivity in the hypothalamus. Moreover, patients
treated for a sellar tumour giving rise to compression of the optic chiasm often continue to experience some physical and mental impairment despite proper endocrine substitution therapy (44-50). Interestingly, their impairments show many similarities with the diverse functions of the hypothalamus and the serotonergic system. However, several limitations of our study should be mentioned. First, subjects having HI and PI are not readily available as their disease is a relatively rare condition (51). We managed to include 6 patients with HI, in line with our power calculation, and matched each of them with 6 age- and gender matched controls and 5 subjects with PI. Moreover, the power calculation was based on the effect of blocking agents on SERT availability. We cannot exclude that the effect of hypothalamic impairment is more subtle and therefore not detectable with the current design. Furthermore, a straightforward clinical definition for HI is lacking. Subjects in the HI group were selected on the basis of a history of cranial radiation therapy, cranial surgery and expanding tumour of the sellar region. Unfortunately, conclusive proof of hypothalamic impairment is difficult to establish, as the functions of the hypothalamus are highly diverse and validated clinical tests or imaging modalities to assess the integrity of hypothalamic function are lacking. Therefore, it is possible that some of our HI patients have only minor hypothalamic impairment which may mitigate overt serotonergic dysfunction.

**CONCLUSIONS**

We were able to demonstrate SERT-binding in the human hypothalamus *in vivo*. We did not find altered specific-to-nonspecific $[^{123}\text{I}]$FP-CIT binding ratios in patients treated for a large sellar tumour giving rise to visual field defects, although a number of methodological issues preclude a definitive conclusion.

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REFERENCES


42. Legutko R, Gannon RL. 2001 Serotonin transporter localization in the hamster suprachiasmatic nucleus. Brain Res. 893:77-83


