Universiteit Leiden
The Netherlands

Pharmacological aspects of corticotrophinergic and vasopressinergic function tests for HPA axis activation

Jacobs, G.E.

CHAPTER 7

Hypothalamic glutamate levels following serotonergic stimulation: a pilot study using 7-Tesla magnetic resonance spectroscopy in healthy volunteers

Prog Neuropsychopharmacol Biol Psychiatry. 2010 Apr 16; 34(3): 486-91. Epub 2010 Feb 4.

G.E. Jacobs MD[1,3], J. van der Grond PhD[2], W.M. Teeuwisse[2,5] MSc, T.J.C. Langeveld[1], J. van Pelt[4] PhD, J.C.M. Verhagen MSc[4], M.L. de Kam MSc[1], A.F. Cohen[1] MD PhD, F.G. Zitman[1,3] MD PhD, J.M.A. van Gerven MD PhD[1,3]

1 Centre for Human Drug Research, Leiden, The Netherlands
2 Department of Radiology, Leiden University Medical Centre, Leiden, The Netherlands
3 Department of Psychiatry, Leiden University Medical Centre, Leiden, The Netherlands
4 Department of Clinical Chemistry, Leiden University Medical Centre, Leiden, The Netherlands
5 C.J. Gorter Center for High-Field MRI, Leiden University Medical Center, Leiden, The Netherlands

Abstract

INTRODUCTION AND PURPOSE Functional proton magnetic resonance spectroscopy (MRS) can be applied to measure pharmacodynamic effects of central nervous system (CNS)-active drugs. The serotonin precursor 5-hydroxytryptophan (5-HTP), administered together with carbidopa and granisetron to improve kinetics and reduce adverse effects, acutely enhances central serotonergic neurotransmission and induces hypothalamus-pituitary-adrenal-(HPA) axis activation. We studied the hypothalamic levels of glutamate/glutamine (Glx), choline (Chol), N-acetyl-aspartate (NAA) and creatine using 7-Tesla (7T) MRS, and adrenocorticotrophic hormone (ACTH) and cortisol in peripheral blood, after the administration of the 5-HTP function test in healthy volunteers.

METHODS A randomized, double blind, placebo-controlled, two-way cross-over study was performed in 12 healthy males with a 7 day wash-out period. After administration of the oral 5-HTP function test, ACTH and cortisol were measured over four hours and MRS scans at 7T were performed every 30 minutes over three hours measuring Glx: Creatine, Chol: Creatine and NAA: Creatine ratios.

RESULTS In the hypothalamus, the administration of 5-HTP had no effect on the average Glx, Chol or NAA levels over 180 minutes but induced a significant decrease of Glx at 60 minutes on post-hoc analysis. 5-HTP induced significant ACTH release reaching an E_{MAX} of 60.2 ng/l at 80 minutes followed by cortisol with an E_{MAX} of 246.4 ng/ml at 110 minutes.

CONCLUSIONS The reduction in hypothalamic Glx-levels after serotonergic stimulation is compatible with activation of excitatory neurons in this region, which is expected to cause depletion of local glutamate stores. The hypothalamic MRS-response reached its maximum prior to subsequent increases of ACTH and cortisol, which supports the functional relevance of hypothalamic Glx-depletion for activation of the HPA-axis. This exploratory study shows that MRS is capable of detecting neuronal activation following functional stimulation of a targeted brain area.

Introduction

Pharmacological function tests are used to quantify the functionality of central neurotransmitter and neuropeptide systems in health and disease, and following treatment. The serotonergic system can be pharmacologically stimulated with serotonin precursors, selective agonists or re-uptake inhibitors. A test consisting of orally administered (p.o.) L-5-hydroxytryptophan (5-HTP), carbidopa and granisetron acutely increases synaptic availability of serotonin (or 5-hydroxytrytamine, 5-HT) (Gijsman *et al.*, 2002; Smarius *et al.*, 2008). Carbidopa prevents peripheral conversion of 5-HTP to 5-HT (which would preclude brain penetration), while granisetron limits serotonergic side-effects (such as gastro-intestinal stimulation and vomiting) without influencing the neuroendocrine response or 5-HTP pharmacokinetics (PK) (Jacobs *et al.*, 2008). Thus, in the presence of carbidopa and granisetron peripherally, the direct serotonin precursor 5-HTP is decarboxylized centrally into 5-HT. The conversion of 5-HTP to 5-HT is believed to occur in the dorsal raphé nuclei of the brainstem and its associated cortical, subcortical and limbic projections (Dinan, 1996; Leonard, 2005; Sotty *et al.*, 2009). We have previously demonstrated reproducible, concentration-dependent pharmacodynamic effects with acceptable variability associated with this serotonergic function test in healthy volunteers. Also, 5-HTP plasma concentrations demonstrated dose-dependence with a limited PK variability (Smarius *et al.*, 2008). Taken together, these features argue for a reliable pharmacological research tool in examining the central 5-HT system.

Enhanced serotonergic neurotransmission ensuing from the decarboxylation of 5-HTP into 5-HT potently activates the hypothalamus-pituitary-adrenal axis (HPA). The stimulation of $5HT_{2A}$ or $5HT_{2C}$ receptors in the paraventricular nucleus (PVN) of the hypothalamus is believed to release corticotrophin releasing hormone (CRH) (Gartside and Cowen, 1990). Subsequently, CRH stimulates adrenocorticotrophic hormone (ACTH) release from the anterior pituitary which in turn induces cortisol release from the adrenal cortices. Ultimately, cortisol exerts negative feedback on CRH and ACTH release via the mineralocorticoid (MR) and glucocorticoid (GR) receptors (de Kloet *et al.*, 2005), leading to a suppression and ultimate cessation of the 5-HTP induced neuroendocrine response.

ACTH and cortisol therefore are the peripheral neuroendocrine reflections of increased central 5-HT neurotransmission.

Neuroendocrine hormone responses in plasma are merely indirect measures of increased neurotransmission, influenced by both central and peripheral amplification, the negative feedback mechanism and the peripheral clearance of the hormones. More direct measurements of drug-induced neuronal activity in the brain could lead to a better understanding of the CNS processes induced by the stimulation of neurotransmitter systems. Neuroimaging techniques of pharmacological CNS-processes such as functional magnetic resonance imaging (fMRI) is often used to demonstrate regional shifts in blood oxygen level dependent (BOLD) signals. For instance, the specific 5-HT_{2C} agonist meta-chlorophenylpiperazine (mCPP) increased the BOLD signal in human brain areas that are particularly rich in 5-HT_{2C} receptors, such as the PVN, hippocampus, caudate, pallidum, amygdala and anterior cingulate (Anderson *et al.*, 2002) which was prevented by the 5-HT_{2C} receptor antagonist SB242084 in rats (Stark *et al.*, 2008). However, BOLD-MRI is not quantitative and the signal tends to drift with time. Consequently, the BOLD-effects of a drug can only be reliably pursued for no more than 30 or 60 minutes, which for most oral drugs is unsuitable. Also, the BOLD-MRI signal is sensitive to nonspecific confounding factors such as (drug-induced) vascular reactivity and shows substantial intersession variance in cross-over designs (Anderson *et al.*, 2008; Stark *et al.*, 2006). These factors limit the application of fMRI in the quantification of drug-induced neuronal activity. Alternatively, in vivo proton magnetic resonance spectroscopy (MRS) could be a candidate neuroimaging technique to directly quantify drug-induced neurotransmission. MRS of the brain determines concentrations of protons from tissue chemicals other than water, such as glutamate-glutamine (which cannot be distinguished reliably, hence combined as (Glx)), N-acetyl-aspartate (NAA), myo-inositol, lactate, choline (Chol) and creatine (Creat). In this context, NAA and GLx are generally regarded as surrogate MRS markers for neuronal activity, whereas Chol is rather considered a metabolic marker of membrane density and integrity (Soares and Law, 2009; Wise and Tracey, 2006). To semiquantify changes in the levels of these metabolites, creatine is frequently used as an internal reference, since this is related to neuronal membrane turnover

and is also expected to remain stable after acute administration of (non-proapoptotic) CNS active drugs (Mason and Krystal, 2006). MRS is typically applied in neurodegenerative disease and malignant brain tumours, exhibiting relatively specific abnormalities in NAA (Soares and Law, 2009; De Stefano *et al.*, 2007). Recently, a study using MRS found Glx-increases in the occipital lobe after 7 to 10 days of treatment with the specific serotonin reuptake inhibitor (SSRI) citalopram, while this was not the case for the noradrenaline reuptake inhibitor (NRI) (Taylor *et al.*, 2008). These findings indicate that MRS is not only able to demonstrate and quantify changes in neuronal biochemistry in structural brain disorders but potentially also during (prolonged) pharmacological treatment.

We studied whether neuronal changes can also be measured after acute pharmacological stimulation with a CNS-active drug using MRS. For this, a 5-HTP function test was used that causes a robust serotonergic stimulation of the hypothalamus of healthy male volunteers. This was an exploratory study, since neither the extent and variability nor the time course of hypothalamic MRS-changes could be predicted in advance.

Methods

Study design

A randomized, double blind, placebo controlled, two-way crossover trial with administration of 5-HTP combined with carbidopa and granisetron was performed as described previously. The treatments were administered with a wash-out period of at least 7 days. The study protocol was approved by the Medical Ethics Committee of Leiden University Medical Centre (LUMC) and performed according to legal and regulatory requirements.

Drug administration

The function test consisted of, the administration of carbidopa 100 mg (at t=-180 minutes) and granisetron 2 mg (at t=-60 minutes), followed by a single oral dose of 5-HTP 200mg (at t=0 minutes) and carbidopa 50mg (at t=180 minutes). For all three active drugs, matching double-dummies were administered as placebo (Jacobs *et al.*, 2008; Gijsman *et al.*, 2002; Smarius *et al.*, 2008).

Subjects

Twelve healthy, male volunteers participated in the study. Volunteers without a personal or a first-degree family history of a significant psychiatric disorder according to DSM-IV and who have never used MDMA, methamphetamines or ecstasy were included in the study. Volunteers who used more than 4 units alcohol and/or xanthines on average per day; smoked more than 5 cigarettes per day; used any drugs of abuse or substance within two weeks before the first dosing; had a cardiac pacemaker, piercings or other metal objects attached to the body and suffering from claustrophobia were excluded from study participation. No xanthine or tryptophan containing foods or beverages, tobacco or alcohol were allowed during study days. Concomitant medication other than paracetamol was not permitted during the study period.

5-HTP and carbidopa

5-HTP and carbidopa were obtained from BUFA b.v. (Uitgeest, The Netherlands). Granisetron was obtained from the Department of Clinical Pharmacy of the LUMC. All medication and matching placebo were prepared by the Department of Clinical Pharmacy of the LUMC.

Study days

Volunteers arrived at the Centre for Human Drug Research (CHDR) at 22h00 on the evening preceding each study day. After waking up the next morning, a low tryptophane breakfast was supplied. One intravenous cannula for blood sampling was inserted into the antecubital vein of one arm for blood sampling. At t=-180 minutes volunteers received carbidopa 100 mg or placebo at CHDR after which volunteers were transported to the 7T-MRI-unit by taxi, accompanied by a research physician. Granisetron or placebo was administered at t=-60 minutes and 5-HTP or placebo subsequently at t=0 minutes. Six MRS scans were performed at 30 min before and 30, 60, 90, 120 and 180 minutes following 5-HTP administration. A research physician was present throughout the entire study day. Carbidopa 50 mg was given at t=180 minutes. Volunteers were

supplied with a standard meal at t=420 minutes. Volunteers were transported back to CHDR after all scanning sessions were performed, and subsequently went home after dinner.

Biochemical measurements

1.2 ml venous blood was collected in prechilled EDTA collection tubes for the determination of plasma ACTH on -10 and 1, 10, 25, 45, 55, 75, 80, 85, 105, 110, 150, 170, 240 and 300 minutes relative to 5-HTP administration. Samples were immediately placed on ice, processed within 30 minutes and stored at -80°C. ACTH was analyzed within six weeks using the Immulite 2500 Analyzer Assay (EURO/DCP, United Kingdom) at the Laboratory for Clinical Chemistry, LUMC, Leiden, the Netherlands. 3 ml venous blood was collected in non-additive tubes for the determination of serum cortisol on -10 and 1, 10, 25, 45, 75, 110, 170, 240 and 300 minutes relative to 5-HTP administration. Samples were allowed to coagulate for 30 minutes at room temperature, centrifuged within 1 hour of collection and serum stored at -20°C until analysis. Serum cortisol was analyzed using the ECLIA assay method on Modular Analytics E170 (Roche, Switzerland) at the Laboratory for Clinical Chemistry, LUMC, Leiden, the Netherlands. The 5-HTP assay was performed at Laboratory for Clinical Chemistry, LUMC, Leiden, the Netherlands according to methods described previously (Gijsman *et al*., 2002).

MRS acquisition

MRS scans were performed in a Philips Achieva 7.0 Tesla whole body MRI scanner (Philips Healthcare, Best, The Netherlands) installed at the LUMC based C.J. Gorter Center for High-Field MRI. Each individual scanning session had a duration of approximately 10 minutes: first, a T1-weighted MRI scan was performed (with a duration of approximately 3 minutes) followed by MRS (with a duration of approximately 7 minutes). MRS of the hypothalamus was performed using single voxel stimulated echo acquisition mode (STEAM) with the following parameters; repetition time (TR): 2000ms, echo time (TE): 19ms, mixing time (TM): 20ms, 128 averages, 2048 time domain points, and 4000Hz bandwith. Typical voxel size dimensions were 13 x 12 x 10 mm, in left-right (LR),

anterior-posterior (AP) and feet-head (FH) direction respectively. A typical example of MRS voxel positioning is shown in *figure 1*. For optimization of B0 field homogeneity, 2nd order pencil beam shimming was applied and 6 saturation slabs were positioned on either side of the voxel to suppress signal from surrounding tissue. After zero-filling to 4,096 data points, exponential multiplication of 2 Hz, Fourier transformation, and linear baseline correction was performed. The integrated area under the curve of N-acetylaspartate (NAA) (referenced at 2.0 parts per million), Glx, Chol and Creat were determined using integration software routines, which were provided by the manufacturer. Creat was used as internal reference for Chol, Glx and NAA. A typical example of the MRS spectra is shown in *figure 2*.

Side-effects

Adverse events were registered from spontaneous reports and hourly inquiries.

Statistical analysis

Since the responses and variability of the MRS measurements were unknown, power estimates could not be based on the expected MRS responses. Based on previous experiments with the 5-HTP-function test (Gijsman *et al*., 2002; Jacobs *et al*., 2008; Smarius *et al*., 2008), a sample size of six was considered large enough to show statistically significant ACTH responses. To confirm that the power calculation was adequate, a blinded interim analysis was executed after dosing of the first six subjects. For the final analysis pharmacodynamic data were logarithmically (LOG) transformed except for the MRS parameters. Within the repeated measures mixed model ANCOVA design, estimated means (least squared means; LSMs) of the neuroendocrine parameters (ACTH, cortisol) were calculated over four hours and LSMs of the MRS ratios (Chol: Creat, Glx: Creat, NAA: Creat) were calculated over 180 minutes using treatment, time, study day and treatment by time as fixed factors, subject, subject by treatment and subject by time as random factors and average prevalue as covariate. The difference from placebo was estimated percentually for ACTH and cortisol (due to

LOG-transformation) and that for the MRS ratios in terms of absolute changes. All estimated differences were presented with 95% confidence intervals.

Pharmacokinetic (PK) analysis

The mean C_{MAX}, T_{MAX}, terminal half life and $AUC_{0-\infty}$ for 5-HTP were calculated with noncompartmental analysis using WinNonLin Professional for windows V5.0 (Pharsight Corporation, 800 West El Camino Real, Suite 200, Mountain View, CA 94040).

Results

Demographic data and subject disposition

The study recruited 12 volunteers who were included after having provided informed consent (mean age 27 years; range 20 - 38 years). Incomplete data sets had occurred during 5-HTP study days due to vomiting (2 study occasions), flawed MRS baseline scans (2 study occasions), technical MRI failure (1 study occasion) and withdrawal of informed consent (1 study occasion). Consequently, neuroendocrine data for 10 subjects and MRS data for 8 subjects were available for 5-HTP analysis, while the data of 12 subjects were available for placebo analysis.

Adverse events

The most common side-effect during placebo treatment was headache (2/12 occasions). Side-effects associated with 5-HTP treatment were 5-HT related and included mild nausea (10/11 occasions), vomiting (5/11 occasions), abdominal discomfort (3/11 occasions) dizziness (3/11 occasions) and headache (3/11 occasions).

Neuroendocrine effects

The mean ACTH and cortisol responses differed significantly from placebo for 5-HTP (Table 1). ACTH reached a maximum mean concentration (E_{MAX}) of 60.2 ng/l at 80 minutes (Figure 4) and cortisol an E_{MAX} of 246.4 ng/ml at 110 minutes (Figure 5) after administering 5-HTP.

MRS effects

Overall, the mean NAA: creat, Chol: creat and Glx: creat ratios did not differ significantly from placebo over the period of 0 to 180 minutes (Table 2). Based on the time profiles, a post hoc statistical analysis was performed of the effects at the average peak time. This secondary analysis showed a significant decline of Glx: creat, one hour after administration of 5-HTP (Table 2 and Figure 3). This Glx: creat trough at 60 minutes preceded the E_{MAX} of ACTH at 80 minutes and E_{MAX} of cortisol at 110 minutes (Figure 6). The other MRS ratios either did not show a clear peak, or this did not differ significantly from the placebo effect at the same time.

5-HTP PK

PK results are presented as means with standard deviations between brackets: oral 200 mg 5-HTP led to a C_{MAX} of 1867 (392) ng/ml, at 176 (82) minutes after its administration (Figure 6). Terminal half life ($T_{1/2}$) was 265.2 (91.1) minutes and $AUC_{0-\infty}$ was 848135 (313093) min*ng/ml. Since 5-HTP was not sampled sufficiently until the end of its concentration-time profile curve, the terminal half-life was not accurately determinable.

Discussion

5-HTP induced a significant hypothalamic glutamate-glutamine (Glx) trough 60 minutes after its administration, while its effects on Glx, Chol and N-acetyl-aspartate (NAA) levels over the entire 180 minutes observation period were probably too short lasted or too small or variable to be detectable in a group of 12 subjects. ACTH reached its E_{MAX} of 60.2 ng/l at 80 minutes followed by cortisol, with its E_{MAX} of 246.4 ng/ml, at 110 minutes after the administration of 5-HTP. The MRS Glx spectrum is constituted by the combined extracellular peaks of glutamate and glutamine since these spectra are inseparable. Glutamate is the most abundant excitatory neurotransmitter in the brain and one of the most important components of cellular energy metabolism (Mason and Krystal, 2006). This would arguably make it the most informative MRS-measurable metabolite associated with (drug-induced excitatory) neurotransmission (Soares and Law, 2009). However,

little research has been done on the effects of serotonergic agents on cerebral Glx levels and it is unclear how 5-HT and Glx are linked mechanistically. In this context, other areas not directly involved in the HPA-axis may be stimulated by the 5-HTP function test, influencing the hypothalamus indirectly. Spectroscopy of a (limbic) control region with lower serotonergic receptor density than the hypothalamus would also have strengthened the evidence for a specific 5-HT mediated hypothalamic effect. However, the time course of the different effects following oral 5-HTP-administration provides support for a functional connection between Glx and the neuroendocrine responses. The timing of the Glx trough, which preceded the E_{MAX} of ACTH by 20 minutes and that of cortisol by 50 minutes, can be reconciled with a serotonin–induced (and probably $5HT_{2A}$ or $5HT_{2C}$ mediated) release of CRH from the hypothalamic PVN.
Glx showed a significant trough at 60 minutes, ACTH peaked at 80 minutes followed by cortisol at 110 minutes and 5-HTP reached its maximal concentration around 170 minutes (*figure 7*). At the same time, ACTH and cortisol had started to decline from their peaks at 80 minutes and 110 minutes onwards (*figure 7)*. Therefore, we can assume that the major part of the 5-HTP-induced hypothalamic activation must have taken place during the 50 to 70 minute period after 5-HTP-administration. From the moment of its release cortisol exerts negative feedback via the mineralocorticoid receptor (MR) and at higher concentrations also via the glucocorticoid receptor (GR) (de Kloet *et al*., 2005). The decline of ACTH and cortisol in blood is thus a net consequence of both its clearance from the body and negative feedback on the production. In this study, negative feedback would have inhibited hypothalamic 5-HT neurotransmission and consequently also the release of Glx. Thus, the short-lasting effects on Glx levels in the hypothalamus were, at least in part, most probably due to the inhibiting effects of cortisol feedback.

Taylor also looked at the effects of serotonergic treatment on MRS parameters in the brain (Taylor *et al*., 2008). In this trial, Glx increased in the occipital lobes of healthy subjects treated for 7 to 10 days with 20mg citalopram. These findings are difficult to compare to ours, since there are obvious functional and metabolic differences between the occipital lobe and the hypothalamus. However, there is also a pharmacological difference between acute

administration of a precursor and prolonged treatment with a reuptake inhibitor of serotonin. Serotonergic systems are activated by acute administration of 5-HTP, whereas they are desensitized during chronic treatment with a selective serotonin reuptake inhibitor (SSRI). In this respect, a divergent Glx-response is perhaps not surprising. It is not easy to explain elevations of occipital Glx-levels following medium-term SSRI administration. However, acute Glx-depletion following synaptic stimulation is perhaps more in line with predictions and is also supported by a reduction in Glx after acute cortical excitation with transcranial magnetic stimulation (TMS) (Singh *et al.*, 2009). Following neuronal excitation (in this case by serotonergic stimulation of the hypothalamus), glutamate is released into the synaptic cleft by exocytosis. To interrupt post-synaptic activation and prevent accumulation of toxic glutamate levels, the neurotransmitter is rapidly cleared from the synapse by neuronal and astrocytic reuptake via excitatory amino acid Na^+-coupled glutamate transporters (EAATs). Astrocytes convert glutamate back into glutamine and release it via the system-N-transporter (SN) into the synaptic cleft for reuptake by neurons (Danbolt, 2001; Iversen *et al.*, 2009). The net effect of this process on local Glx-levels is therefore expected amount to zero, because MRS cannot distinguish glutamate from glutamine, nor intracellular (astrocytic or neuronal) from extracellular (synaptic or interstitial) sources of these metabolites. However, secondary to increased release of extracellular glutamate and glutamine, these compounds are also cleared from the synaptic cleft by diffusion and blood flow (Danbolt, 2001), which could lead to the Glx-reductions that were observed with acute administration of the 5-HTP function test.

It is uncertain whether MRS would be feasible as a neuroimaging technique in the detection of other functional drug-induced changes. 7-Tesla MRS is reliable for volumes of at least 1 to 1.5cm^3. Measurements in a structure with a smaller volume of interest (VOI) can be disturbed by a low signal to noise ratio. Also, imaging multiple brain regions at the same time is associated with its own complexities. We performed spectroscopy of a hypothalamic VOI of ca. 1.5cm^3 surrounding the PVN, while the PVN itself typically comprises a volume of only 15 to 30 mm^3. This is only a very small fraction of the scanned VOI, which has undoubtedly limited the sensitivity of the technique to detect PVN-activation. Furthermore,

since we exclusively performed spectroscopy on the hypothalamus, non-specific effects (such as nausea or vomiting) or a more generalized serotonergic effect of 5-HTP on the observed reduction in Glx cannot be excluded. The fact that changes were found nonetheless may indicate that the MRS-changes in the PVN must have been substantial. Detection of this small activation area was also due to the relatively low intersession variability of the much larger surrounding 'dead' volume. The variability of the predose (baseline) Glx: creat ratios was around 20% and the intrasubject variability was 15%. The low baseline variability and the high sensitivity of 7-Tesla MRS despite a fairly large VOI suggest that this technique can be particularly useful for drugs that can be predicted to act on a specific brain region. Such functional anatomical predictions can be made using preclinical *in vivo* techniques like brain tissue microdialysis and registration of local neurophysiological activity.

Creat was used as internal reference for NAA, Chol and Glx. It is possible that the Creat concentrations may have changed during the course of each experimental session. The Glx: creat trough might therefore reflect an increase in Creat concentrations rather than a decrease in Glx concentrations. However, it is impossible to reliably estimate changes in absolute quantitative Creat concentrations because hypothalamic 7T metabolite and water T1 and T2 relaxation values are unknown. Moreover, absolute Creat concentrations are usually calculated based on the unsuppressed water signal as internal reference which may change during the course of each experiment. Metabolite referencing to Creat is therefore not optimal and limits the interpretation of our results. This is however a general problem when using MRS and is not specific for the present trial.

A weakness of this study is that the power for the detection of changes by MRS may be relatively low. Measuring 5-HTP-induced activation of the hypothalamus using MRS at 7T is a novel technique. We based our initial power calculation on 5-HTP's effects on ACTH, and performed a blinded interim assessment to determine if MRS would be just as robust. As expected, the 5-HTP neuroendocrine responses were reliable and comparable to our previous experiences with 5-HTP (Danbolt, 2001; Gijsman *et al.*, 2002; Jacobs *et al.*, 2008; Smarius *et al.*, 2008). However, the MRS-analyses were complicated by technical issues and unforeseen dropouts. Statistical

power was also limited by the short duration of the hypothalamic activation as detected by MRS, which was may have been due to the inhibitory effects of cortisol feedback on the hypothalamus.

The potential applications of MRS as novel technique in CNS pharmacology need to be investigated further in well-designed experiments. In this context, careful consideration should be given to the expected CNS effects of the drug under investigation, including the size of the activated area relative to the sampling volume, and the timing and duration of the physiological responses.

THE AUTHORS DECLARE NO CONFLICT OF INTEREST.

Table 1 Neuroendocrine parameters for the period 0 minutes to 240 minutes for the 200mg 5-HTP function test: Estimated means (back transformed Least Square Means) for plasma ACTH (ng/l) and serum cortisol (ng/ml) and estimated difference (%) with 95% confidence interval from placebo for the 200 mg 5-HTP function test.

	Least Square Means		Estimated difference (%)	
Neuroendocrine parameter	placebo n=12	5-HTP n=10	5-HTP vs placebo	p-value
ACTH (ng/L)	16.9	33.4	+97.8 (56.7, 149.6)	p<0.0001
Serum cortisol (ng/mL)	95.6	159.8	+67.2 (44.2;93.9)	P<0.0001

Table 2 MRS ratios for the period 0 minutes to 180 minutes and peak effects at 60 minutes for the 200mg 5-HTP function test: Estimated means (untransformed Least Square Means) from placebo for Chol: Creat , Glx: Creat and NAA: Creat and estimated difference with 95% confidence interval from placebo for the 200mg 5-HTP function test.

MRS ratio		Least Square Means		Estimated difference	p-value
		Placebo	5-HTP	5-HTP vs placebo	
		n=12	n=8		
Chol:Creat ratio	0 to 180 minutes	1.071	1.117	0.046 (-0.017,0.109)	0.128
	Peak effect at 60 minutes	1.159	1.064	0.096 (0.004, 0.195)	0.059
Glx:Creat ratio	0 to 180 minutes	0.229	0.259	-0.030 (-0.066, 0.006)	0.090
	Peak effect at 60 minutes	0.196	0.252	-0.055 (-0.110, -0.001)	0.045
NAA:Creat ratio	0 to 180 minutes	1.041	1.057	0.016 (-0.049, 0.080)	0.597
	Peak effect at 60 minutes	1.036	1.057	0.021 (-0.076, 0.118)	0.670

Figure 1 Typical example of the MRS planning. The white rectangles represent the borders of the MRS voxel that contains the hypothalamus.

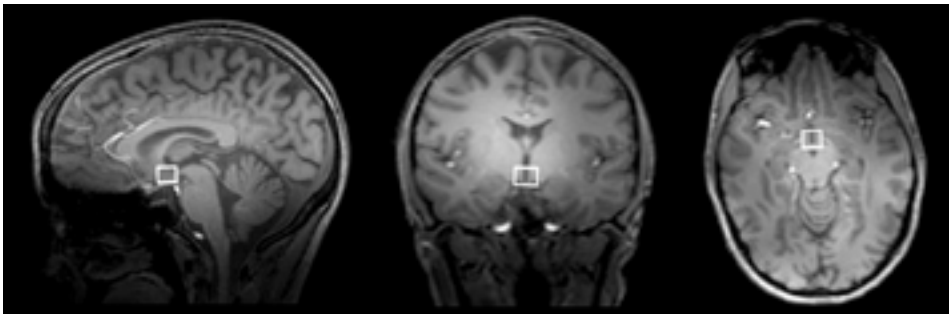


Figure 2 Example of the typical MRS spectra for choline (chol); creatine (creat); glutamate-glutamine (Glx) and N-acetylaspartate (NAA) derived from a volunteer in the present study.

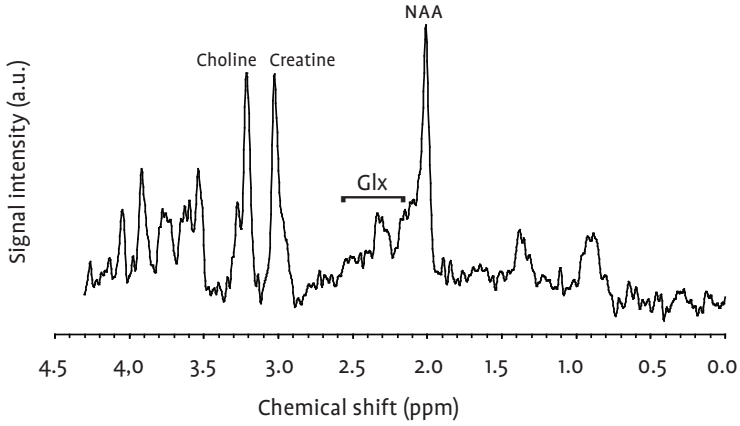


Figure 3 Estimated means with 95% CI error bars for MRS Glx: Creat over the period 0 to 180 minutes, after administration of placebo (n=12) and the 200mg 5-HTP function test (n=8).

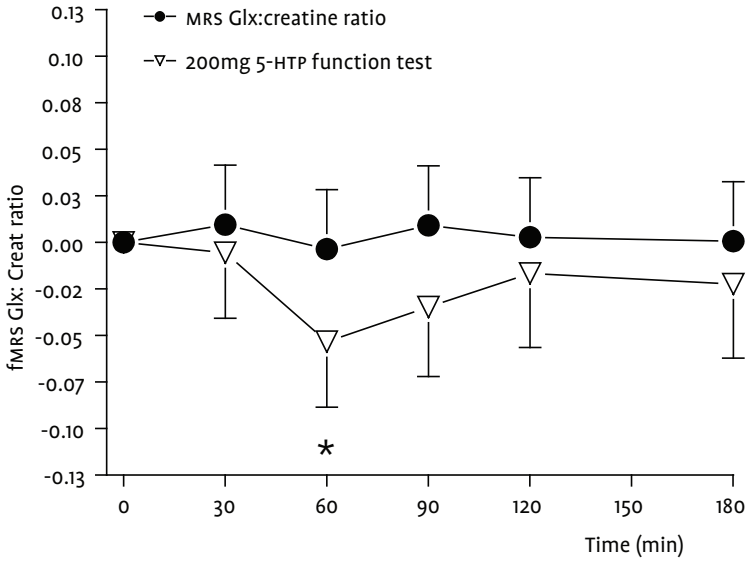


Figure 4 Estimated means (ng/l) with 95% CI error bars for plasma adrenocorticotrophic hormone (ACTH) over the period 0 to 240 minutes, after administration of placebo (n=12) and the 200mg 5-HTP function test (n=8).

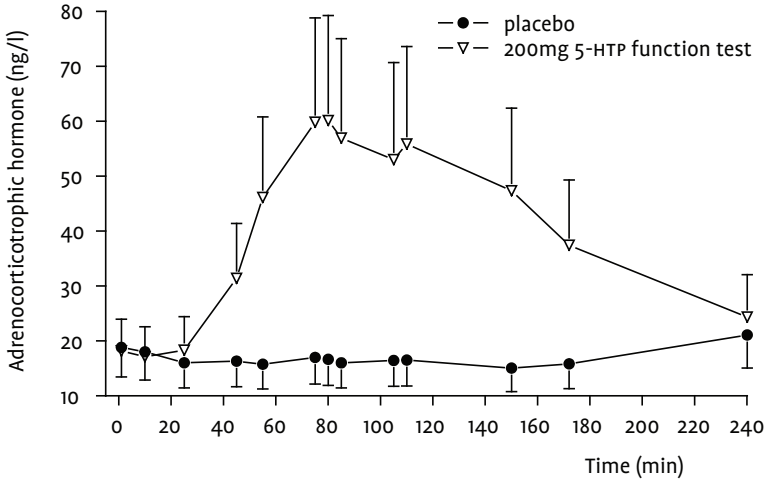


Figure 5 Estimated means (ng/ml) with 95% CI error bars for serum cortisol over the period 0 to 240 minutes, after administration of placebo (n=12) and the 200mg 5-HTP function test (n=8).

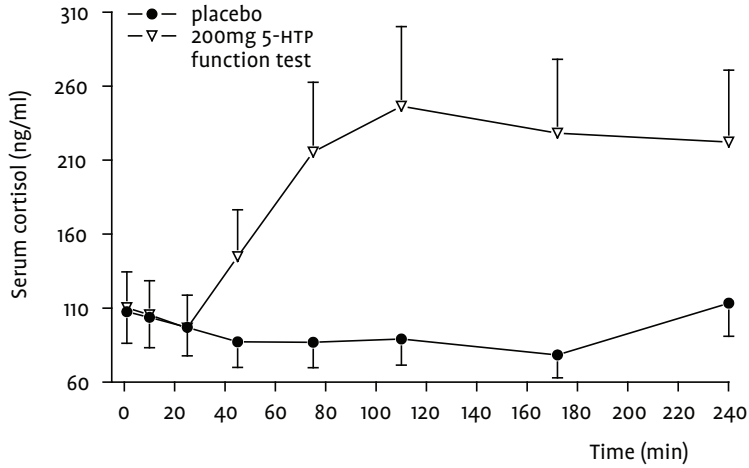


Figure 6 Average time profile with SD error bars of serum 5-HTP (ng/ml) over 300 minutes for the 200mg 5-HTP function test (n=10).

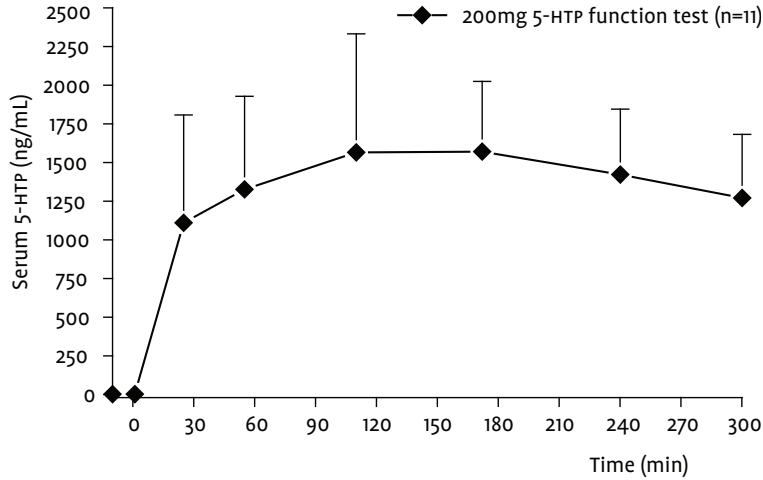
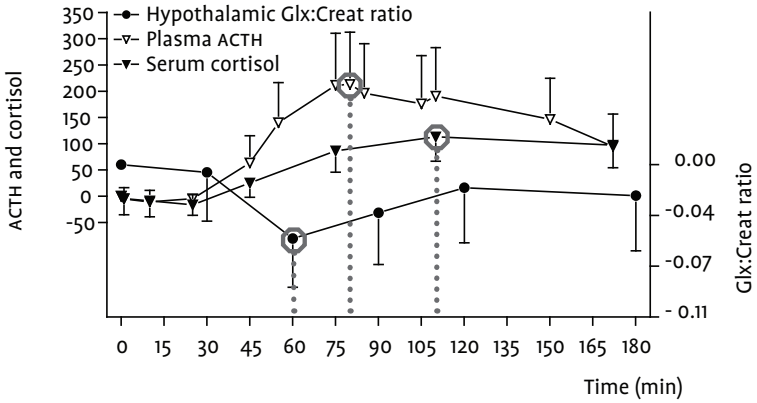


Figure 7 Combined graphical representation of the estimated mean percentual change from placebo (%) with 95% CI error bars for adrenocorticotrophic hormone (ACTH) and cortisol (left Y-axis) and the mean change relative to placebo with error bars for MRS Glx: Creat (right Y-axis), after administration of the 200mg 5-HTP function test. The grey circles and dotted lines represent the respective E_{MAX} for Glx: Creat at 60 minutes, ACTH at 80minutes and cortisol at 110minutes.



REFERENCE LIST

Anderson I M, Clark L, Elliott R, Kulkarni B, Williams S R, Deakin J F (2002) 5-HT(2C) receptor activation by m-chlorophenylpiperazine detected in humans with fMRI. Neuroreport 13: 1547-1551.

Anderson I M, McKie S, Elliott R, Williams S R, Deakin J F (2008) Assessing human 5-HT function in vivo with pharmacoMRI. Neuropharmacology 55: 1029-1037.

Danbolt N C (2001) Glutamate uptake. Prog Neurobiol 65: 1-105.

de Kloet E R, Joels M, Holsboer F (2005) Stress and the brain: from adaptation to disease. Nat Rev Neurosci 6: 463-475.

De Stefano N, Filippi M, Miller D et al. (2007) Guidelines for using proton MR spectroscopy in multicenter clinical MS studies. Neurology 69: 1942-1952.

Dinan T G (1996) Serotonin and the regulation of hypothalamic-pituitary-adrenal axis function. Life Sci 58: 1683-1694.

Gartside S E, Cowen P J (1990) Mediation of ACTH and prolactin responses to 5-HTP by 5-HT2 receptors. Eur J Pharmacol 179: 103-109.

Gijsman H J, van Gerven J M A, de Kam M L et al. (2002) Placebo-controlled comparison of three dose-regimens of 5-hydroxytryptophan challenge test in healthy volunteers. Journal of Clinical Psychopharmacology 22: 183-189.

Iversen L L, Iversen S D, Bloom F E, Roth R H (2009) Introduction to Neuropsychopharmacology. 85-103

Jacobs G, Kamerling I, de K M et al. (2008) Enhanced tolerability of the 5-hydroxytryptophane challenge test combined with granisetron. J Psychopharmacol

Leonard B E (2005) The HPA and immune axes in stress: the involvement of the serotonergic system. Eur Psychiatry 20 Suppl 3: S302-S306.

Mason G F, Krystal J H (2006) MR spectroscopy: its potential role for drug development for the treatment of psychiatric diseases. NMR Biomed 19: 690-701.

Singh S, Mistry S, Jefferson S et al. (2009) A magnetic resonance spectroscopy study of brain glutamate in a model of plasticity in human pharyngeal motor cortex. Gastroenterology 136: 417-424.

Smarius L J, Jacobs G E, Hoeberechts-Lefrandt D H et al. (2008) Pharmacology of rising oral doses of 5-hydroxytryptophan with carbidopa. J Psychopharmacol

Soares D P, Law M (2009) Magnetic resonance spectroscopy of the brain: review of metabolites and clinical applications. Clin Radiol 64: 12-21.

Sotty F, Folgering J H, Brennum L T et al. (2009) Relevance of dorsal raphe nucleus firing in serotonin 5-HT(2C) receptor blockade-induced augmentation of SSRIs effects. Neuropharmacology 57: 18-24.

Stark J A, Davies K E, Williams S R, Luckman S M (2006) Functional magnetic resonance imaging and c-Fos mapping in rats following an anorectic dose of m-chlorophenylpiperazine. Neuroimage 31: 1228-1237.

Stark J A, McKie S, Davies K E, Williams S R, Luckman S M (2008) 5-HT(2C) antagonism blocks blood oxygen level-dependent pharmacological-challenge magnetic resonance imaging signal in rat brain areas related to feeding. Eur J Neurosci 27: 457-465.

Taylor M, Murphy S E, Selvaraj S et al. (2008) Differential effects of citalopram and reboxetine on cortical Glx measured with proton MR spectroscopy. J Psychopharmacol 22: 473-476.

Wise R G, Tracey I (2006) The role of fMRI in drug discovery. J Magn Reson Imaging 23: 862-876.