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Pharmacology of rising oral doses of 5-hydroxytryptophan with carbidopa

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PHARMACOLOGICAL ASPECTS OF CORTICOTROPHINERGIC AND VASOPRESSINERGIC FUNCTION TESTS FOR HPA AXIS ACTIVATION

Abstract

BACKGROUND 5-hydroxytryptophan (5-HTP) is a direct 5-hydroxytryptamine (5-HT) precursor used to assess central serotonergic function. Its use has been limited by a narrow window between neuroendocrine changes and side effects, and variable kinetics related to inconsistent administration modes. By combining 5-HTP with carbidopa (CBD), increased bioavailability for brain penetration, and decreased peripheral side effects would be expected, due to reduced peripheral decarboxylation of 5-HTP to 5-HT.

OBJECTIVES A double blind, placebo-controlled, single rising dose, four-way crossover trial with placebo randomization was performed in fifteen healthy male volunteers to investigate the neuroendocrine dose-response relationship at various 5-HTP levels, the tolerability and subjective effects of oral 5-HTP at 100, 200 and 300 mg combined with CBD and the pharmacokinetic properties of the 5-HTP/CBD-challenge.

RESULTS Dose-dependent increases in average cortisol concentrations were observed. Mean response (AUC) over the first 4 hours (SD): 172.0 nmol/l (22.3) for placebo; 258.3 nmol/l (72.6) for 100 mg; 328.47 nmol/l (84.6) for 200 mg; and 387.3 nmol/l (82.4) for 300 mg 5-HTP. Similar dose-dependent increases for prolactin were seen while ACTH-response was more variable. 5-HTP kinetics were adequately described using a one-compartment model with first order absorption and a lag time (mean oral clearance 28L/hr ± inter-individual coefficient of variation 31%). Nausea and vomiting occurred dose-dependently as most frequent side effects, resulting in dose-related dropout of 6.6% at 100 mg and 45.5% at 300 mg 5-HTP.

CONCLUSIONS Orally administered 5-HTP combined with CBD is an effective serotonergic challenge test, exhibiting dose-related plasma concentrations and neuroendocrine responsiveness. Frequent occurrence of nausea and vomiting limits the applicability of this challenge at 5-HTP doses above 100 mg.

Introduction

Pharmacological stimulation tests, using challenges with receptor agonists, release stimulators or re-uptake inhibitors, are used to quantify the integrity and function of serotonergic pathways in the central nervous system (CNS) (Gijsman et al., 2002b). These challenges may provide a tool for understanding the mechanisms underlying psychiatric disorders, or to predict the efficacy of novel therapies with a direct or indirect effect on serotonergic systems (Meibohm and Derendorf, 1997). One of the more frequently used serotonergic challenge tests consists of a single dose of L-5-Hydroxytryptophane (5-HTP), the immediate precursor of serotonin. Its decarboxylation into serotonin in raphé neurons with hypothalamic projections induces increases in cortisol and prolactin in peripheral blood, which are frequently used neuroendocrine endpoints of serotonergic challenges. However, there is little standardisation of 5-HTP-challenge tests, and their use has been hampered by a narrow window between neuroendocrine responses and side effects, mainly nausea and vomiting. The doses used in 5-HTP challenge tests vary extensively, and include oral doses of 10, 20 and 40 mg (den Boer and Westenberg, 1990), 60 mg (van Vliet et al., 1996), 100 mg (Vlasses et al., 1989), 200 mg (Maron et al., 2004; Schruers et al., 2002b; Schruers et al., 2002a), and intravenous doses of 0.8 mg/kg (Birmaher et al., 1997; Ryan et al., 1992) . These doses were established by trial and error. In some studies, oral 5-HTP has been administered together with the peripheral L-aromatic decarboxylase inhibitor carbidopa (CBD). The rationale of CBD co-administration is that it prevents peripheral decarboxylation of 5-HTP, leading to more 5-HTP being available to enter the brain, and less peripheral serotonin being formed to cause systemic side effects. In practice, different doses of CBD have been used, varying from one single dose to three daily doses for one week before the challenge test (Birmaher et al., 1997; Magnussen and Nielsen-Kudsk, 1979; Magnussen and Engbaek, 1978; van Vliet et al., 1996; Vlasses et al., 1989). Recently, the pharmacokinetics and pharmacodynamics of a 5-HTP challenge test, consisting of two different doses of 5-HTP with or without pre-treatment with CBD, were studied (Gijsman et al., 2002a). A dose of 100 mg 5-HTP plus CBD (100 mg before and 50 mg after 5-HTP administration) induced a significant cortisol

and prolactin response, and the elimination half-life of 5-HTP was doubled compared to 5-HTP 100 mg without CBD. However, the neuroendocrine effects, which are known to be relatively sensitive to serotonergic challenges, were smaller after 5-HTP 100 mg with CBD than with usual challenge doses of dexfenfluramine or mCPP. 5-HTP 100 mg with CBD produced few clinically significant adverse events in this study (3 out of 12 cases of nausea of which 1 vomited) compared to mCPP. This suggests that the 100 mg dose of 5-HTP may be too low to exert a full serotonergic response, despite its combination with CBD. The optimal dosing regimen of 5-HTP that leads to a simple, reliable and tolerable challenge test of pre-synaptic serotonergic transmission thus remains to be established. Therefore, the current study was aimed at examining the neuroendocrine effects and the tolerability of single doses of 5-HTP with CBD co treatment, in a rising dose design. A second aim was to reproduce some of the findings of the previous 5-HTP-study, to develop an impression of the intersubject variability and to establish the pharmacokinetic properties of the 5-HTP/CBD-challenge test.

Methods

This study was a double blind, placebo controlled, four-way cross-over, single rising dose investigation of oral 5-HTP with co-administration of carbidopa (CBD) with interspersed placebo randomization. The study protocol was approved by the Medical Ethics Committee of Leiden University Medical Centre (LUMC) and all subjects gave written informed consent. The four dose regimens were placebo (including CBD placebo); 5-HTP 100 mg; 5-HTP 200 mg; and 5-HTP 300 mg, the last three with co-administration of 100 mg CBD 3 hours before and 50 mg CBD 3 hours after administration of 5-HTP. Based on the pharmacokinetic characteristics of CBD, we expected this regimen to result in a relatively constant level of CBD during the first hours after administration of 5-HTP.

Subjects

Twelve healthy, male volunteers were recruited from the CHDR database and from the local student population by means of

advertisements. Dropouts were to be replaced with subjects assigned to the same treatment randomisation order. A Dutch translation of the Structured Clinical Interview for DSM-IV axis I (SCID I), was used to exclude any subject with a past or present psychiatric disorder, including substance abuse (Groenestijn MAC and Akkerhuis GW, 1999). Other exclusion criteria were abnormalities on physical or laboratory examination or ECG; participation in a clinical study within three months prior to the study, or in (more than) two studies in the past year; blood donation within three months before the study period; a self-admitted chance of a HIV positive state; the use of medication other than occasional paracetamol in the last months; smoking of more than 5 cigarettes per day; an average intake of xanthene containing fluids of more than 3 units per day; current average use of alcohol of more than 3 units per day; or an irregular day-night-rhythm.

Study days

Volunteers arrived at the Centre for Human Drug Research (CHDR) at 8.00 in a fasted state, and remained on site for the following 12 hours. On arrival a urine drug screen (morphine, benzodiazepines, cocaine, amphetamine, THC, methamphetamines including MDMA) was performed. Subjects with a negative drug screen received the first oral dose of CBD or placebo (t=-3 hr). This was followed by a standardized breakfast, which, like all other supplied meals and snacks during their stay, did not contain tryptophan rich foods. Subsequently, a cannula was inserted into the antecubital vein of one arm to allow withdrawal of blood, an ECG was mounted and all baseline measurements were made. At 11.00 hours (t=0 hr), 5-HTP or its placebo was administered and the second dose of CBD was given at t=3 hr. At t= 2 hr the volunteers received a glucose drink, after CBD administration (t=3hr) a light lunch and at t=7 hr a dinner. All challenges were performed under hospital conditions. A research physician attended all study occasions. Vital signs were monitored at 1 hour before and at t=1, 2, 3, 4, 5 and 6 hours after 5-HTP administration. Blood pressure was measured using a non-invasive oscillometric system, the Nihon Kohden Lifescoop EC BSM-1101J/K (Nihon Kohden Co., Oss). For Electrocardiography (12 lead) a Nihon Kohden Cardiofax with ECaps 12 software (Nihon Kohden Co.,

Japan) was used. Oral temperature was measured with a digital thermometer (Terumo Corporation, Tokyo, Japan. Occasions were separated by a washout period of at least 6 days.

5-HTP and CBD

5-HTP and CBD were obtained from BUFA b.v. (Uitgeest, The Netherlands) and prepared by the Department of Clinical Pharmacy of the Leiden University Medical Centre. Capsules containing 100 mg 5-HTP and capsules containing 50 mg CBD were made with matching placebos.

Biochemical measurements

On every challenge day at 1 hour before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6 and 8 hours after 5-HTP administration, 9 ml whole blood was drawn in a Greiner Vacuette EDTA-tube, tilted, and immediately stored on ice water (4°C). Within one hour blood was centrifuged at 4°C for 10 minutes at 2000G. Plasma was divided into four portions, with two portions of at least 1,0 ml for the 5-HTP assay, at least 1.0 ml for cortisol and prolactin and 0.5 ml for ACTH. Plasma was stored directly at -20°C. The 5-HTP assay was performed at the Biochemical Laboratory of the Rijngeestgroep, Oegstgeest, The Netherlands. After addition of buffer and internal standard, a solution with 1.55 mol/l trichloroacetic acid, 13.4 mmol/l EDTA, and 50 mmol/l sodium bisulphite ($Na_2S_2O_5$) was added to remove proteins. After centrifuging at 3,000 g for 32 minutes, the supernatant was assayed by high-pressure liquid chromatography (HPLC) with electrochemical detection using 650 millivolts. The HPLC contained a Merck LiChroSpher 60, RP Select B5 _m, 125 _4 mm internal diameter plus 1 cm guard column. For the mobile phase, we used a solution (pH 3,60) with 50 mmol/l sodium acetate (NaAc), 50 mmol/l citric acid, 0,27 mmol/l EDTA, 1,17 mmol/l 1-octanesulfonic acid sodium salt, and 1.5% volume- to-volume ratio acetonitrile. The lower limits of detection and quantification were 0.5 ng/ml and 1.7 ng/ml, respectively. The coefficients of variability for precision and reliability were 2.6% and 7.9%, respectively. Cortisol and prolactin were analysed by an electrochemiluminescention immunoassay (ECLIA) on the Roche Elecsys 1010, and analysis of ACTH

was performed by radio-immuno-assay, according to methods described previously (Gijsman et al., 2002a).

Side-effects

Adverse events were registered from spontaneous reports and hourly inquiries. For each symptom, the relationship (definite, probable, possible, unknown, definitely not) with the treatment was assessed by the research physician. Only side effects judged definitely, probably or possibly related to the treatment were considered in the analyses. Side effects did not necessarily lead to dropout from the study. If deemed safe by the research physician, subjects who experienced side effects were allowed to participate in the following occasion.

Self report questionnaires

The volunteers scored self report questionnaires at 1 hour before and 1, 2, 3, 4, 5, 6 and 8 hours after 5-HTP administration. The short version of the Dutch translation of the Profile of Mood States (POMS) (McNair DM et al., 1971; Wald FDM and Mellenbergh GJ, 1990), the Dutch version of the State scale of the Spielberger State-Trait Anxiety Inventory (STAI-DY-1) (Spielberger CD et al., 1983; Wald FDM and Mellenbergh GJ, 1990), the somatization-subscale of the Dutch Translation and adaptation of the Symptom Check List (SCL-90) (Arrindell WA, 1986; Derogatis et al., 1973) and Visual Analogue Scales (as originally described by Norris, for the three factors corresponding to alertness, mood and calmness (Bond A and Lader M, 1974; Norris, 1971) were used for follow up of subjective effects. All Visual Analogue Scales were practised three times during the pre-study screening.

Pharmacodynamic analysis

Occasions on which subjects vomited were excluded from pharmacodynamic analysis, since this would have interfered with the neuroendocrine treatment effects. Analyses were performed on subjects of whom the data of at least two non-vomiting occasions were available. For each individual, the AUC over 4 hour and 8-hour

periods were calculated and divided by the corresponding time-span for all parameters. The resulting time-weighted average effect was analyzed using ANOVA with factors subject, treatment and occasion. Contrasts of all treatments with placebo were estimated within the ANOVA design and presented with 95% confidence intervals. The 4 hour period was chosen based on the results of a previous study, indicating that most of the effects of 5-HTP 100 mg with CBD had dissipated after 4 hours (Gijsman et al., 2002a). All variables were analyzed untransformed.

Pharmacokinetic analysis

The 5-HTP data were analysed using nonlinear mixed effect modelling as implemented in the NONMEM program (Version 5, Globomax LLC, Hanover, MD, USA). This 'population approach' allows the description of the entire data set using a common structural model, with allowance for interindividual kinetic variability. Based on the population estimates, individual empirical Bayes estimates can be generated by NONMEM that can be used to generate individual concentration time profiles. First order conditional estimation was used throughout. Data were described using a one-compartment model with first order absorption and a lag-time. Various models and parameterisations were attempted. In the final model absorption half-life, elimination half-life, oral clearance (Cl/F) and lag-time were estimated. Inter-individual variability was modelled using a multiplicative model with a correlation between absorption and elimination half-life. An additive residual error model best described the data.

Occasions on which subjects vomited were excluded from primary pharmacokinetic analysis. Analysis was performed on subjects for whom the data of at least one non-vomiting occasion were available.

Results

Demographic data

Twenty-three volunteers were recruited originally, eight of whom were excluded at screening. To replace dropouts, three volunteers

were recruited in addition to the anticipated 12 subjects. It was decided not to recruit more replacements, when blinded interim safety analyses suggested that the dropout rate approached 50% at the highest dose. The mean age of the 15 participants was 23.2 years (range 19 – 29 years).

Subject disposition

Study dropouts occurred solely due to nausea and vomiting, at different stages of the study. Of 15 subjects who started, 1/15 dropped out after 100 mg (6,6%), 4/14 after 200 mg (28,6%), and 5/11 after 300 mg (45,5%) 5-HTP respectively. Two subjects opted to continue the study despite having vomited on the 100 mg and 200 mg occasions respectively, and dropped out after the next higher dose. In total nine out of 15 subjects commenced all four occasions, six of whom completed all doses without vomiting. The subject disposition is represented in *Figure 1*. Different numbers of subjects per dosing group were evaluable for the different analyses. For analyses of safety and tolerability, all subjects who started with a dose were included. Vomiting may have had an impact on both the pharmacokinetics of study medication (since the absorption of 5-HTP and CBD could have been influenced, also considering that in eight out of 10 cases of vomiting subjects did not receive their second dose of CBD), and on neuroendocrine responses (enhanced pharmacokinetic variability, stress-related cortisol increase and nausea-induced prolactin elevation). Therefore, subject occasions on which a subject vomited were excluded from the primary pharmacokinetic and pharmacodynamic endocrine analyses. A secondary analysis was performed to investigate whether subjects who vomited were more susceptible to the effects of 5-HTP or had higher drug levels. For this, the neuroendocrine and pharmacokinetic results at 100 mg were compared between subjects who never vomited at all (Subgroup A), and those who did not vomit at 100 mg but did at a higher dose (Subgroup B). This approach eliminated the direct influence of vomiting on the neuroendocrine reactions or plasma concentrations, but allowed us to identify inherent differences in drug sensitivity or pharmacokinetic characteristics between subjects who were prone to vomiting and those who were not.

Adverse events

No side effects were reported during the placebo occasion. During all 5-HTP occasions, nausea occurred most frequently (6/15; 12/14; 9/11 occasions for 100, 200 and 300 mg respectively). Vomiting occurred less frequently but in a dose-related manner (1/15; 4/14; 5/11 occasions respectively). The main other reported side effects were epigastric discomfort (5/15; 5/14; 7/11 occasions respectively), dizziness/lightheadedness (1/15; 4/14; 4/11 occasions respectively) and drowsiness/sleepiness/tiredness (4/15; 2/14; 0/11 occasions respectively). On two occasions during an episode of nausea and vomiting at 300 mg, mild disorientation in place and euphoria with déja-vu's occurred respectively.

Self report questionnaires

There were no significant effects on the STAI, POMS and visual analogue scales, except for a significant decrease on the POMS vigor scale (p=0.008), in the 4-hour period after the 200 mg dose. The SCL-90 subscale somatization showed a significant effect on the nausea score for 100 mg (p=0.045), 200 mg (p=0.002), and 300 mg (p=0.040) in the 4-hour period. There also was a significant effect on the warm/cold score for the 300 mg dose for the 4-hour period (p<0.001).

Pharmacokinetics

The results from the NONMEM model as described in the Methods section are shown in *Table 1*. The average concentration-time-curves for the three different doses of 5-HTP are displayed in *Figure 2*. 5-HTP has dose-proportional pharmacokinetics. The absorption and elimination half-lives, which are highly correlated, show a large variability. However, these parameters do not determine the level of exposure of 5-HTP as much as mean oral clearance (Cl/F), which has a limited inter-individual variability of 31%. Subjects who vomited at higher doses of 5-HTP may have had inherently different pharmacokinetic characteristics, leading to higher plasma levels at the 100 mg dose, although this dose did not yet lead to vomiting. To examine this possibility, we performed a secondary

comparison of the 5-HTP concentrations at 100 mg between Sub-group A (n=6) and Subgroup B (n=6), at 4 and 8 hours. No significant differences in concentrations could be found.

Pharmacodynamic effects

There was a significant dose effect on cortisol and prolactin. The AUC of cortisol differed significantly from placebo on all three occasions for the 4-hour period, which covered the main part of the response (Table 2). Figure 3 shows the plasma cortisol responses (nmol/l) after the four dose regimens. The effect of ACTH was short lasting and only statistically significant after 200 mg in the 4-hour period (Figure 4). The prolactin response reached statistical significance only for 100 mg and 200 mg in the 4-hour period, but not in the small number of subjects who completed the 300 mg dose (Figure 5). To examine the possibility that vomiting subjects were more sensitive to the effects of 5-HTP, the pharmacodynamic responses at the 100 mg dose were compared between Subgroup A (n=6) and Subgroup B (n=6). Vomiting subjects showed a larger average increase in the AUC of the SCL Nausea score for the 8-hour period after the 100 mg dose, but there were no relationships with the cortisol or prolactin responses.

Discussion

The lack of a standardized serotonergic challenge procedure hampers the application of this potentially useful paradigm in biological psychiatry and CNS drug development (Gijsman et al., 2004). The variability of different serotonergic challenge tests also contributes to the confusion that surrounds many aspects of the role of serotonergic systems in psychiatric disease. We have therefore set up a series of experiments, with the aim of identifying a reproducible and practical serotonergic challenge procedure. Such a procedure would have to be well tolerated, show little pharmacokinetic variability, and cause clear reproducible dose-related responses of meaningful functions of the central nervous system (CNS) (Gijsman et al., 2004).

One of the main aims of this study was to determine the pharmacokinetic (PK) properties of the 5-HTP/CBD-challenge. The study

also allowed us to create an impression of the inter- and intra-subject variability of the challenge. Although the study did not systematically evaluate all aspects of reproducibility, it did allow an assessment of dose-proportionality, and inter- and intra-subject variability of different pharmacokinetic properties. The 5-HTP/CBD-challenge displays clear dose proportional increases in plasma concentrations, and the variability of the main pharmacokinetic parameter that determines drug exposure, mean oral clearance (Cl/F), shows an acceptable inter-individual coefficient of variation (IICV) of 31%. Another aspect of the reliability of the challenge can be derived from a comparison with a previous study in which a lower dose range of 5-HTP was examined, which also included 100 mg 5-HTP combined with CBD (Gijsman et al., 2002a). Both studies showed quite similar pharmacokinetic characteristics for this challenge: Mean oral clearance (Cl/F) was 36 L/hr with a 30% IICV in the first study, and 28 L/hr with 31% IICV in the current one. The average elimination $T_{1/2}$ was 3.0 hr in both studies. After oral administration, absorption and elimination of 5-HTP run in parallel and cannot be reliably separated by the PK models used in this study. Consequently, these two parameters were highly correlated, and their added intersubject variability was considerable. It could not be assessed whether variability was largest for absorption or for elimination. An inter-individual kinetic variability of about 30% seems acceptable for a challenge test, particularly since a non-invasive challenge will be more acceptable for depressed patients than an intravenous infusion, which may also cause stress-related cortisol increases.

It seems reasonable to assume that vomiting may have reduced drug absorption, also because this usually interfered with the ingestion of the second carbidopa-dose. Subjects who vomited were therefore excluded from the primary pharmacokinetic analyses. It is possible that subjects who vomited after 5-HTP had higher plasma levels or were more sensitive to the drug's effects. To explore this possibility, we compared the effects of 5-HTP 100 mg between subjects who never vomited at any dose of 5-HTP (n=6), and those who did not vomit at 100 mg but only at a higher dose (n=6). Although both subgroups are too small for statistical comfort, they showed quite similar pharmacokinetic profiles of 5-HTP at the 100 mg dose. Neuroendocrine responses were also very

comparable. However, subjects who vomited at a higher dose had statistically significant larger nausea scores at the 100 mg dose, which had not yet caused vomiting. This suggests that nausea and vomiting in these subjects are not due to higher plasma levels, but to a larger sensitivity of the pertaining gastrointestinal and/or medullar chemoreceptors. This does not seem to be accompanied by an increased sensitivity of the other (central) serotonergic systems that are involved in the release of cortisol or prolactin, since these neuroendocrine responses appeared very similar in these small subgroups. Before vomiting occurs, nausea does not seem to have an influence on the pharmacokinetics of 5-HTP. This contributes to the pharmacokinetic reproducibility of the different dose regimens of the 5-HTP/CBD-challenge.

5-HTP 100, 200 and 300 mg plus CBD caused dose-related increases in cortisol release, the most reliable response parameter for serotonergic challenges. A comparable estimated difference from placebo of 91 nmol/l was obtained with the 100 mg dose of 5-HTP plus CBD in the 4 hour period in a previous study (Gijsman et al., 2002a). Thus, it seems that the pharmacodynamic reproducibility of the 5-HTP/CBD-challenge is adequate for 100 mg, but this remains to be proven for the higher dose range.

The ACTH response for the 5-HTP 200 mg treatment was statistically significant compared to placebo. However, at 300 mg the average response was lower and the increase not did reach statistical significance. This could signify a lack of statistical power, due to dropouts at the highest dose; an assay variability that was higher than for cortisol; and somewhat conservative estimates of treatment effects (ie. overall AUCs rather than individual timepoints, relatively few measurements etc.). It is unlikely that 5-HTP 200 mg approached its ceiling effects on CRH-ACTH activation, since this would have led to a congruent reduction in cortisol at the highest dose. Alternatively, a dose-related cortisol-increase despite a lack of a dose-propotional ACTH-increase could indicate that 5-HTP-induced cortisol-release is partly independent from CRH-ACTH activation; ie. that at higher doses it occurs at the level of the adrenal cortex. This cannot be fully excluded from this study, but there are several arguments from previous studies pointing to the contrary. ACTH-release was shown to be induced by 5-HTP alone or combined with CBD (Imura et al., 1973; Lee et al., 1991), although not all studies

confirmed this finding (Gijsman et al., 2002a). Several pharmaco-logical studies in experimental animals and healthy humans suggest that serotonergic stimulation of cortisol-release involves activation of hypothalamic 5-HT_2 (5-HT_{1C}) receptors (Gartside and Cowen, 1990; Jorgensen et al., 2002). In all these studies, the ACTH-response preceded the cortisol-response, whereas a primary adrenal cortisol-release would have resulted in an ACTH-reduction. Nonetheless, our results cannot fully exclude that the slightly lower average ACTH-response at the highest dose of 5-HTP-induced was due to negative feedback from an ensuing 5-HTP-induced adrenal cortisol release. The data itself and most of the literature results are compatible with a mixed hypothalamic/pituitary and peripheral/adrenal effect for 5-HTP, and more research is needed to separate these two potentially concomitant processes

The average maximum cortisol level caused by the highest 5-HTP dose was 609.5 nmol/l. This approaches the effect of a (close to maximum tolerated) intravenous mCPP dose of 0.5 mg/kg, which leads to an average maximum concentration of around 630 nmol/l (Gijsman et al., 1998). Thus, it seems that the 5-HTP/CBD-challenge with doses of 100-300 mg covers a major part of the maximum effect range for cortisol release that can be attained in healthy volunteers. The administration of higher doses of both mCPP and 5-HTP was unfortunately impeded by adverse events, including nausea and vomiting. These side effects prevent a wider use of the 5-HTP dosing regimens employed in this study, although these otherwise satisfy many of the methodological criteria of an adequate challenge test. Only the lowest dose of 100 mg 5-HTP plus CBD seems to be tolerated well enough to be of practical use. Dose related nausea and vomiting are also the most frequently reported side effects in the literature (den Boer and Westenberg, 1990; Magnussen and Nielsen-Kudsk, 1979; Westenberg et al., 1982). Generally, it seems that plasma concentrations above 1000 mg/l cause high rates of nausea and vomiting, which clearly limits the applicability of the 5-HTP/CBD-challenge at doses at or above 200 mg.

In practice, the current 5-HTP/CBD-challenge is limited to a maximum oral dose of 100 mg. Although this produces cortisol responses with an acceptable reproducibility, these are quite limited compared to the maximum response that is generated by other (less practical or less well tolerated) serotonergic challenge

tests. In principle, a challenge does not always have to produce a maximum response to show treatment effects or differences between patient groups. In fact, (supra)maximal challenges may even obscure changes or differences in serotonergic sensitivity between different groups by means of ceiling effects or unintended neuroendocrine activation through other (less specific or adverse effect related) mechanisms. On the other hand, it is also difficult to detect differences between patient groups or treatment effects, if the challenge test produces a suboptimal stimulation. Therefore, it would be preferable to have a challenge test with a larger therapeutic window, covering the main part of the dose response curve without unacceptable side effects. One possibility to achieve this may be to combine a high-dose 5-HTP/CBD-challenge with an anti-emetic to suppress nausea and vomiting. The most appropriate anti-emetic agent needs to be established, because several different neurotransmitter systems are involved in drug-induced nausea and vomiting, notably serotonin and dopamine, which are also involved in the neuroendocrine challenge-responses. Not every anti-emetic may be efficacious, and others may interfere with the desired serotonergic effects. It remains important to develop a serotonergic challenge with all the proper methodological characteristics, to provide a reliable tool for the study of serotonergic functions in health and disease. So far, 5-HTP 100 mg combined with carbidopa 100+50 mg seems to be the best tolerated and most reproducible oral serotonergic challenge test.

PHARMACOLOGICAL ASPECTS OF CORTICOTROPHINERGIC AND VASOPRESSINERGIC FUNCTION TESTS FOR HPA AXIS ACTIVATION

Table 1   Population pharmacokinetic parameters of 5-HTP

Parameter	Mean	SEM	IICV
Oral clearance (Cl/F, l/hr)	28.0	2.06	31%
Absorption half-life (hr)	0.358	0.0963	22%*
Elimination half-life (hr)	3.02	0.222	122%*
lag-time (hr)	0.407	0.0353	19%
Residual variability (SD)	142		

*Correlation coefficient between absorption and elimination; half-life = -0.75; Mean = population average;
SEM = standard error of the population average; IICV= interindividual coefficient of variation.

Table 2   Pharmacodynamic effects over 4 hours: Least square mean (SD) AUC over 4 hours of cortisol (nmol/l), prolactin (μU/ml), ACTH (ng/l) and SCL-90 Nausea score and estimated difference from placebo with 95% confidence interval for placebo, 100 mg 5-HTP, 200 mg 5-HTP and 300 mg 5-HTP.

| Pharmacodynamic parameter		placebo n=10	100 mg n=12	200 mg n=10	300 mg n=6	Overall treatment effect
Cortisol (nmol/l)	LSM AUC (SD) Estimated difference from placebo (95% CI)	187.0 (22.5) 84 (17, 151) p=0.016	272 (28.1) 157 (89, 226) p=0.000	345 (26.4) 162 (57, 267) p=0.004	350.1 (40.38)	p <0.001
Prolactin (mU/l)	LSM AUC (SD) Estimated difference from placebo (95% CI)	246 (18.33) 60 (6,114) p=0.032	306 (22.9) 85 (29,140) p=0.004	330 (21.5) 38 (-47,123) p=0.370	284 (32.9)	p= 0.021
ACTH (ng/l)	LSM AUC (SD) Estimated difference from placebo (95% CI)	23.31 (7.13) 10.8 (-10.3,31.9) p=0.300	34.1 (8.9) 24.2 (2.5,45.8) p=0.031	47.5 (8.4) 15.2 (-18.0,48.4) p=0.350	38.5 (12.8)	p= 0.159
SCl-90 Nausea	LSM AUC (SD) Estimated difference from placebo (95% CI)	1..1 (0.10) 0.3 (0.01,.60) p=0.05	1.4 (0.13) 0.51 (0.21,0.82) p=0.002	1.6 (0.12) 0.49 (0.02,0.96) p=0.04	1.6 (0.18)	p=0.008

Figure 1   Subject disposition for dropout rate, primary and secondary pharmacokinetic analysis, primary and secondary neuroendocrine analyses and safety analyses.
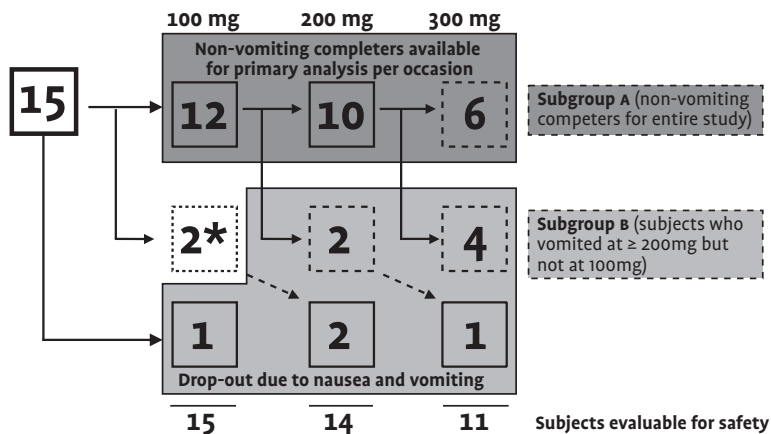
* PK failed for 100mg in one subject due to technical reasons, another subject vomited at 100mg; both subjects proceeded to the next occasion and subsequently dropped out.
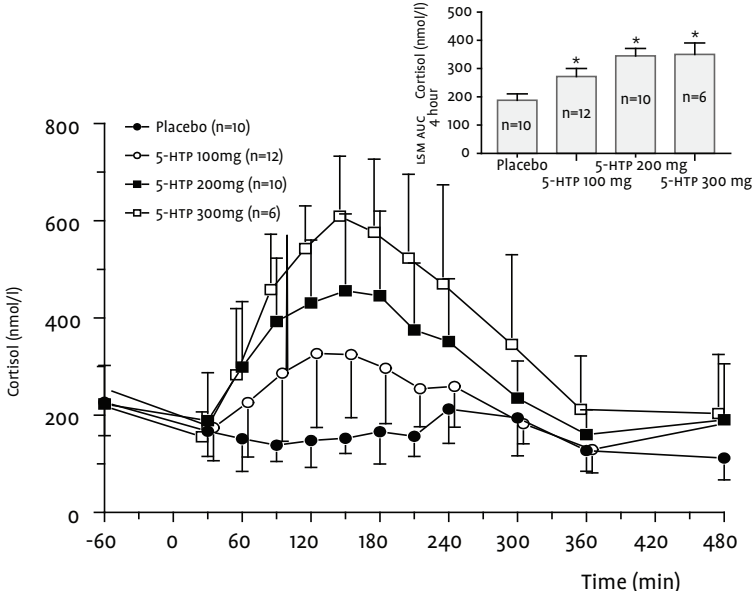
Figure 2   Average graph of predicted and observed 100 mg (n=12), 200 mg (n=10) and 300 mg (n=6) 5-HTP (ng/ml) and CBD co-treatment with SD error bars
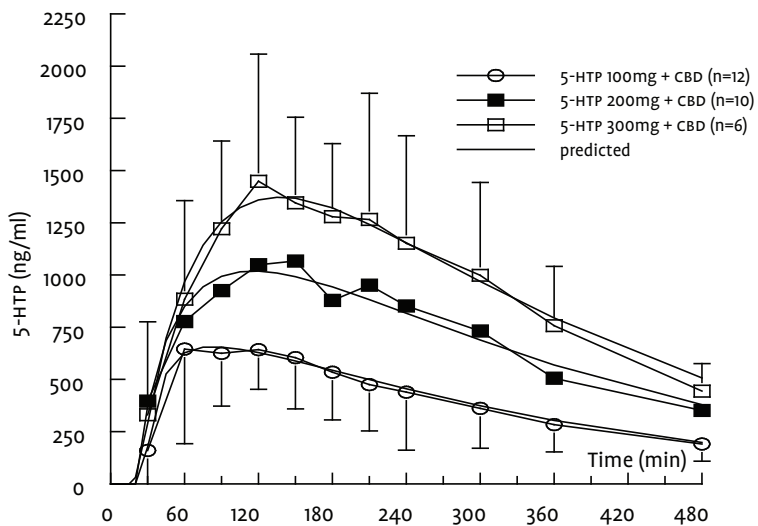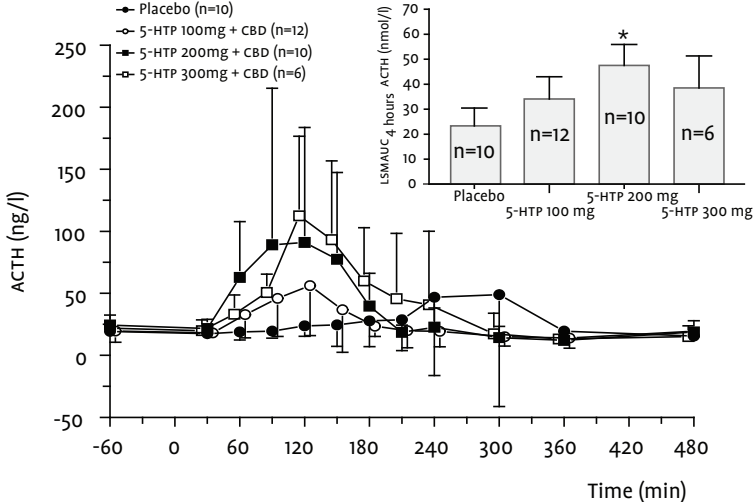
Figure 3   Average plasma cortisol (nmol/l) time-course profile after administration of placebo (n=10), 100 mg (n=12), 200 mg (n=10) and 300 mg (n=6) 5-HTP and CBD co-treatment with SD error bars, and least square mean AUC's over 4 hours.


Figure 4   Average plasma ACTH (ng/l) time-course profile after administration of placebo (n=10), 100 mg (n=12), 200 mg (n=10) and 300 mg (n=6) 5-HTP and CBD co-treatment with SD error bars, and least square mean AUC's over 4 hours.

Figure 5

Average plasma prolactin (µU/l) time-course profile after administration of pla-
cebo (n=10), 100 mg (n=12), 200 mg (n=10) and 300 mg (n=6) 5-HTP and CBD co-
treatment with SD error bars, and least square mean AUC's over 4 hours.
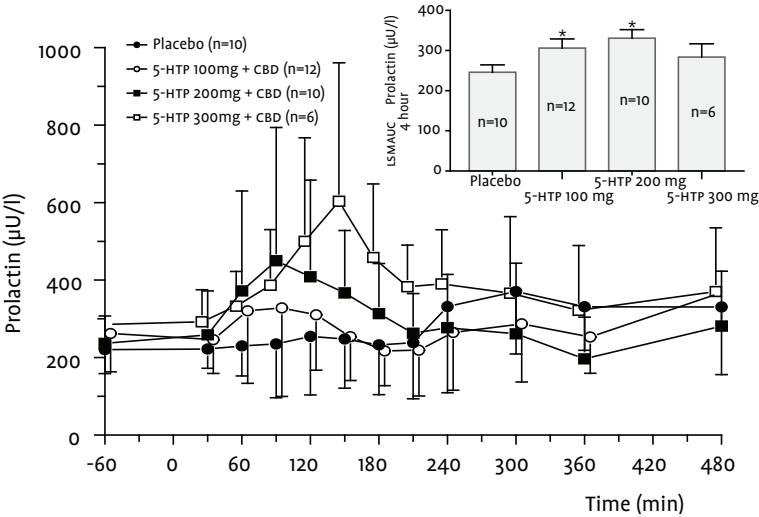
*correlation coefficient between absorption and elimination half-life: -0.75 / Mean= Population Average /
SEM= Standard Error of the Population Average / IICV= Inter Individual Coefficient of Variation*

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