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

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CHAPTER 5

Enhanced tolerability of the 5-hydroxytryptophane challenge test combined with granisetron

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Abstract

BACKGROUND A recently developed oral serotonergic challenge test consisting of 5-Hydroxytryptophane (5-HTP 200mg) combined with carbidopa (CBD 100mg + 50mg) exhibited dose-related neuroendocrine responsiveness and predictable pharmacokinetics. However, its applicability is limited by nausea and vomiting (Smarius et al.).

OBJECTIVES A randomized, double blind, placebo-controlled, four-way cross-over trial was performed in 12 healthy male volunteers. The 5-HTP/CBD-challenge was combined with two oral anti-emetics (granisetron 2mg or domperidone 10mg) to investigate its reliability when side-effects are suppressed. The neuroendocrine response (serum cortisol and prolactin), the side-effect profile (Visual Analogue Scale Nausea (VAS)) and vomiting subjects per treatment were the main outcome measures.

RESULTS Compared to 5-HTP/CBD/placebo, 5-HTP/CBD/granisetron had no impact on cortisol [% change with 95% confidence interval: -7.1% (-19; 6.5)] or prolactin levels [-9.6% (-25.1; 9.1)]; 5-HTP/CBD/domperidone increased cortisol [+13.0% (-4.2; 33.4)] and increased prolactin extensively [+336.8% (245.7; 451.9)]. Compared to placebo, VAS Nausea increased non-significantly with granisetron [+7.6mm(-1.3; 16.5)], as opposed to domperidone [+16.2mm(7.2; 25.2)] and 5-HTP/CBD/placebo [+14.7mm(5.5; 23.8)]. No subjects vomited with granisetron, compared to two subjects treated with 5-HTP/CBD/placebo and five subjects with domperidone. Compared to 5-HTP/CBD/placebo, granisetron addition decreased C_{MAX} of 5-HTP statistically significantly (from 1483 ng/ml to 1272 ng/ml) without influencing $AUC_{0-\infty}$.

CONCLUSIONS Addition of granisetron to the combined 5-HTP/CBD challenge suppresses nausea and vomiting without influencing the neuroendocrine response or pharmacokinetics, enhancing its clinical applicability in future psychiatric research and drug development.

Introduction

Various pharmacological challenge tests have been utilized to quantify the integrity and function of serotonergic pathways in the human central nervous system (CNS). These may be helpful in innovative central nervous system drug development and in delineating potential biological markers associated with psychiatric disorders. A commonly used serotonergic challenge test encompasses single oral administration of 5-Hydroxytryptophane (5-HTP), the immediate precursor of serotonin (5-HT). 5-HT is formed centrally following carboxylation of 5-HTP in various serotonergic neurons. Subsequently, serotonergic neurons with hypothalamic projections induce cortisol and prolactin release in peripheral blood, probably indirectly via increased corticotrophin-releasing hormone (CRH) release. Based on this assumption, serum cortisol and prolactin 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 unclear pharmacokinetics, and a narrow window between neuroendocrine responses and side effects (nausea and vomiting). Combined with carbidopa (CBD) to prevent peripheral carboxylation, Smarius *et al*. recently found a 5-HTP (100mg, 200mg and 300mg) challenge test to have a dose-dependent neuroendocrine responsivity and predictable pharmacokinetics (Smarius et al.). However, a wider use of this challenge test in psychiatric drug development and research was thwarted by the frequent dose-dependent occurrence of nausea and vomiting. 100mg 5-HTP combined with CBD 100mg + 50mg seemed to be the best tolerated and most reproducible oral serotonergic challenge test, but this causes a suboptimal stimulation of central serotonergic pathways that may be too small to show differences between patient groups or treatment effects. It would therefore be preferable to have a challenge test with a larger window between neuroendocrine effects and side-effects.

To investigate this objective, two pharmacologically distinct anti-emetics were combined with a challenge test consisting of 200mg 5-HTP administered together with CBD in the present study.

Methods

Study design

This study is a randomized, double blind, placebo controlled, four-way crossover study with the oral administration of 5-HTP with carbidopa (CBD) co-treatment, combined with two different anti-emetics with washout periods of at least 4 days. The study protocol was approved by the Medical Ethics Committee of Leiden University Medical Centre and performed according to Good Clinical Practice and International Conference on Harmonisation guidelines.

Drug administration

The four treatments were:

1. 5-HTP$_{200\ MG}$/CBD$_{100+50\ MG}$ and placebo anti-emetic (5-HTP/CBD/placebo);
2. 5-HTP$_{200MG}$/CBD$_{100+50\ MG}$ and 10 mg domperidone orally (5-HTP/CBD/domperidone);
3. 5-HTP $_{200\ MG}$/CBD$_{100+50MG}$ and 2 mg granisetron orally (5-HTP/CBD/granisetron);
4. matched double-dummy placebo.

The neuroendocrine responses (serum cortisol and serum prolactin) and the side-effect profile (Visual Analogue Scale Nausea, Somatic subscale of the Symptoms Check List and number of vomiting subjects per occasion) were the main outcome measures.

Subjects

Twelve healthy, male volunteers participated in the study. After providing their written informed consent subjects received a full medical examination during a pre-study screening. 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 et al.). Subjects with a current average use of alcohol of more than 3 units a

day or smoking more than 5 cigarettes a day were not allowed to participate in this study. No xanthine or tryptophan containing foods or beverages, tobacco or alcohol were allowed during the stay in the research unit. Concomitant medication other than paracetamol was not permitted during the study period.

5-HTP and CBD

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

Study days

Volunteers arrived at the Centre for Human Drug Research (CHDR) in the morning after an overnight fast. Subjects received the first oral dose of CBD (100 mg) or placebo (t=-3 hr), which was followed by a standardized breakfast. Subsequently, a cannula was inserted into the antecubital vein of one arm for blood sampling. Administration of either granisetron or domperidone, or its placebo, took place at 10.00 hours (t=-1 hr). At 11.00 hours (t=0 hr), 5-HTP or placebo was administered. The second dose of CBD (50 mg) was given at t=3 hr after which volunteers received a light lunch and dinner (t=7 hr). All challenges were performed under hospital conditions and a research physician attended all study occasions.

Vital signs and biochemical measurements

Vital signs were monitored at 1 hour before and at 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., Tokyo, Japan). For Electrocardiography (12 lead) a Nihon Kohden Cardiofax with ECaps 12 software (Nihon Kohden Co., Tokyo, Japan) was used. Oral temperature

was measured with a digital thermometer (Terumo Corporation, Tokyo, Japan). On each study day at 1 hour, 30 minutes and 5 minutes before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6 and 8 hours after 5-HTP administration, 6 ml whole blood was drawn in a Greiner Vacuette EDTA-tube, tilted, and immediately stored on ice water (0°C). Within one hour the blood was centrifuged at 4°C for 10 minutes at 2000 g. Plasma was divided into three portions, with at least 0.5 ml for cortisol and prolactin and at least 1 ml for the 5-HTP assay. Any remaining plasma is stored as reserve. Plasma was stored directly at -20°C. The determination of cortisol in serum was performed with a competitive electrochemiluminescence immunoassay (ECLIA) using a Roche Elecsys 1010 immunoassay analyzer (Mannheim, Germany). The sample volume was 20 µL. The detection limit was 0.5 nmol/l and the total precision in the measuring range was 2%. The prolactin assay was performed in serum by electrochemiluminescence immunoassay (ECLIA) on a Roche Elecsys 1010 immunoassay analyzer (Mannheim, Germany). The assay employed two monoclonal antibodies specifically directed against prolactin. One of the antibodies carried a ruthenium complex label and the other was biotinylated. The 5-HTP assay was performed at the Biochemical Laboratory of the Rijngeestgroep, Oegstgeest, the Netherlands according to methods described previously (Gijsman et al. 183-89).

Side-effects

Adverse events were registered from spontaneous reports and hourly inquiries. 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 study day.

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 somatization-subscale of the Dutch Translation and adaptation of the

Symptom Check List (SCL-90) (Arrindell WA) and Visual Analogue Scales as originally described by Norris, for the four factors corresponding to alertness, mood, calmness and nausea (Bond A and Lader M 211-18) were used for follow-up of subjective effects. The volunteers practised all four visual Analogue Scales three times during the pre-study screening.

Pharmacodynamic analysis

All pharmacodynamic data were LOG transformed except VAS-Nausea and SCL-90. Within the ANOVA design, estimated means (Least squared means; LSM's) of the neuroendocrine parameters (cortisol and prolactin) and the side-effect profile (VAS-Nausea and SCL-90) were calculated over eight hours using subject, subject by treatment and subject by time as random factors and treatment, time, study day and treatment by time as fixed factors and average pre-value as covariate. Estimated difference from placebo for all treatments were estimated and presented with 95% confidence intervals. Additionally, difference from 5-HTP/CBD/placebo for 5-HTP/CBD/granisetron and 5-HTP/CBD/domperidone was calculated. Since vomiting could have had an effect on the PD neuroendocrine effects, study days on which subjects had vomited were excluded from neuroendocrine analysis. VAS-Nausea and SCL-90 were analyzed including all study days on which subjects had experienced nausea and emesis.

Pharmacokinetic analysis

C_{MAX}, T_{MAX}, $AUC_{0-\infty}$ and the terminal half-life of 5-HTP were calculated over 8 hours with non-compartmental analysis using SAS9.1.2. (SAS Institute Inc., Cary, USA) The hypothesis that antiemetics had no influence on PK parameters of 5-HTP was tested on $AUC_{0-\infty}$ and C_{MAX} with a mixed model analysis of variance, with treatment as fixed factor and subject as random factor and an unstructured covariance structure. Past experience suggests that $AUC_{0-\infty}$ and C_{MAX} are distributed as log-normal, therefore parameters were LOG transformed before analysis. Since vomiting could have influenced the PK, occasions on which subjects vomited were excluded from pharmacokinetic analysis.

Results

Demographic data and subject disposition

Twelve subjects initially provided informed consent and were included in the study (mean age 24 years; range 18 -38 years). One subject dropped out during the 5-HTP/CBD/placebo treatment due to intolerable dizziness and was subsequently replaced. There were no drop-outs due to nausea or vomiting on any occasion.

Adverse events

The most common side-effect during placebo treatment was headache (6/12 study days). Vomiting occurred in 0/13, 5/12 and 2/13 study days for the 5-HTP/CBD/granisetron, 5-HTP/CBD/domperidone and 5-HTP/CBD/placebo treatments, respectively. Nausea occurred more frequently than vomiting (3/13, 8/12 and 6/13 study days, respectively). The main other reported side effects during active treatment were headache (2/13, 5/12 and 5/13 study days, respectively) and dizziness (2/13, 2/12 and 5/13 study days, respectively).

Self report questionnaires

Granisetron seemed effective in suppressing 5-HTP induced nausea. Compared to placebo VAS-Nausea did not increase significantly during the 5-HTP/CBD/granisetron treatment, while this was the case for both 5-HTP/CBD/placebo and 5-HTP/CBD/domperidone treatments (Table 2). Moreover, SCL-90 Nausea was significantly lower during the 5-HTP/CBD/granisetron treatment compared to the 5-HTP/CBD/placebo treatment (Table 2, Figure 4).

Pharmacodynamic effects

Mean cortisol response differed significantly from placebo for all three active treatments. The 5-HTP/CBD/granisetron induced a cortisol response that differed significantly from placebo but did not differ significantly from that induced by 5-HTP/CBD/placebo (Table 2 and Figure 1). Compared with placebo, mean prolactin response

increased significantly for the 5-HTP/CBD/domperidone and 5-HTP/CBD/placebo treatments, but not the 5-HTP/CBD/granisetron treatment. However, the difference between 5-HTP/CBD/granisetron and 5-HTP/CBD/placebo was not statistically significant (Table 2 and Figure 2). Moreover, the mean increases in cortisol and prolactin during the 5-HTP/CBD/ placebo treatment were comparable to our previous findings on the same challenge test (Smarius et al.).

Pharmacokinetics

Pharmacokinetic parameters for 5-HTP are presented in Table 1 and the average concentration-time-curves for 5-HTP, 5-HTP combined with domperidone and 5-HTP combined with granisetron are displayed in *Figure 1*.

Discussion

We have demonstrated enhanced tolerability of a serotonergic challenge test consisting of $5\text{-HTP}_{200MG}/\text{CBD}_{100+50MG}$ with addition of the selective 5-HT_3 receptor antagonist granisetron. Moreover, the addition of granisetron had no impact on the 5-HTP induced serum cortisol response or pharmacokinetics of 5-HTP.

After its conversion from 5-HTP, serotonin (5-HT) is available for non-specific post-synaptic neurotransmission via the 5-HT_{1C}, 5-HT_2, 5-HT_3 and 5-HT_4 receptors. As a result, nausea and vomiting ensue as the most intolerable untoward effects. 5-HT induced nausea and vomiting are hypothesized to result from stimulation of both peripheral (vagal fibres and chemoreceptor trigger zone (CTZ)) and central (medulla oblongata and solitary tract nucleus) 5-HT receptors (Endo et al. 189-201; Sanger and Andrews 3-16). Granisetron strongly and selectively binds to 5-HT_3 receptors and since it readily crosses the blood-brain-barrier (BBB) it is believed to exert its anti-emetic action both centrally and peripherally (Tan 1563-71). Normally, domperidone does not effectively cross the BBB and predominantly antagonizes D_2 receptors in the pontine vomiting centre in the area postrema, which lies outside the BBB. The dopamine (DA) receptor antagonists are believed to exert their anti-emetic action by antagonism of the D_2-receptor (D_2) in the area postrema (Mitchelson 295-315) but the actual source of dopamine release

modulating nausea and emesis is unknown. Therefore, although there is a clear rationale on how D_2 antagonists prevent emesis evoked by dopamine agonists (eg. L-DOPA in Parkinson's disease), it is not clear how they inhibit emesis associated with other conditions (eg. chemotherapy, post-operative nausea). Domperidone was not effective in curbing nausea and vomiting, indicating that these symptoms are not mediated by (indirect) serotonergic stimulation of pontine dopamine systems.

Compared with previous investigations of our serotonergic challenge (Smarius et al.) the addition of granisetron in this trial to the combined 5-HTP/CBD challenge test did not significantly influence 5-HTP induced serum cortisol release. Also, the mean maximal serum cortisol concentration attained with5-HTP_{200MG}/$CBD_{100+50MG}$/granisetron (465.8 nmol/l) was very similar to that attained with5-HTP_{200MG}/$CBD_{100+50MG}$ without anti-emetic (455.9 nmol/l) in our previous trial (Smarius et al.). This concentration approaches the maximal one attained with a previous (close to maximum tolerated) intravenous meta-chlorophenylpiperazine (mCPP) dose of 0.5 mg/kg, which lead to an average maximum serum cortisol concentration of around 630 nmol/l (Gijsman et al. 289-95). Thus, it seems that the combined 5-HTP/CBD/granisetron challenge covers a major part of the maximum range for serum cortisol release that can be attained in healthy volunteers, without being hampered by nausea and vomiting. Challenge tests are used to show changes in responsivity of a particular system, in this case the HPA-axis, during pathological conditions or treatments. Therefore, it is essential that the level of stimulation of the HPA-axis with this newly developed challenge (5-HTP/CBD/granisetron) is neither too small to show decreases, nor too large to show increases.

The combined 5-HTP/CBD/domperidone challenge was associated with significant hyperprolactinemia in comparison with the 5-HTP/CBD/placebo and 5-HTP/CBD/granisetron treatments. Prolactin release is believed to depend not only on the stimulation of the postsynaptic 5-HT_2 receptors (Van de Kar et al. 203-08), but also on tonic inhibition by the neurotransmitter dopamine (DA). Pretreatment with the D_2 antagonist domperidone caused a mean increase in prolactin of roughly 400% compared to 32% and 17% for the 5-HTP-challenge with placebo and granisetron respectively. Prolactin release is a well-known effect of D_2-antagonism, but the

limited (albeit statistically significant) prolactin increase by 5-HTP with or without granisetron demonstrates that prolactin can also be stimulated directly by serotonergic mechanisms.

Compared to 5-HTP/CBD/placebo, pretreatment with granisetron lead to a statistically significant decrease in C_{MAX} of 5-HTP of 14 ng/ml (from 1483 to 1272 ng/ml) without influencing its $AUC_{0-\infty}$. It would perhaps have been expected that an anti-emetic would improve absorption, rather than reduce it. However, the difference was small and it could be spurious or due to a more erratic absorption of 5-HTP without an anti-emetic, as a manifestation of gastrointestinal side-effects.

There is some controversy regarding the pharmacological mechanisms of serotonergic stimulation of the HPA axis. Our studies show that serotonin-induced HPA axis activation is not critically dependent on 5-HT_3-receptors. Furthermore, previous experiments found no effects of granisetron on 5-HTP induced ACTH release (Gartside and Cowen 103-09). Studies in experimental animals and healthy humans suggest that activation of central post-synaptic 5-HT_1 or 5-HT_{1C}) receptors is involved (Jorgensen et al. 788-95).

The current challenge is composed of three different medications, to improve the pharmacokinetic stability and tolerability of the test. Despite this complexity, we believe that the improved reproducibility and reduced side effect profile of the combined 5-HTP/CBD/granisetron-test renders it more acceptable as a practical serotonergic challenge test in clinical research. Such a test could be used to show biochemical/neuroendocrine differences between healthy individuals and individuals (at risk of) suffering from HPA-axis related psychiatric disease. It could also be useful in innovative drug development, to study the effects of neuromodulatory agents that may have few specific pharmacological CNS-effects of their own in healthy subjects, but which are expected to indirectly affect serotonergic functionality. For example, specific serotonin reuptake inhibitors (SSRI's) have few consistent effects in healthy subjects (Dumont et al. 495-510), but cause clear changes in sensitivity to serotonergic challenges. SSRI's like paroxetine (Sargent, Williamson, and Cowen 49-52) or citalopram (Lowe et al. 473-84) cause an increase in 5-HTP-induced cortisol release. Furthermore, this sensitivity changes over time. Initially, 5-HTP-induced cortisol release is augmented in combination with

paroxetine, but this diminishes during three weeks of continued SSRI-treatment (Sargent, Williamson, and Cowen 49-52). These changes may indicate a decrease in serotonergic sensitivity during chronic SSRI-dosing. This may be related to down-regulation of (post synaptic 5HT2) serotonin receptors, which has also been implicated in the delayed therapeutic action of SSRIs.

An improved serotonergic challenge may be a very useful instrument to investigate the physiology of serotonergic systems in health and disease, and its changes over time under different conditions or treatments. Addition of granisetron to the oral 5-HTP/CBD challenge (Smarius et al.) has enlarged the window between the neuroendocrine pharmacodynamic effects and the adverse side-effects of this serotonergic challenge.

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Table 1 **Pharmacokinetic parameters for 5-HTP: Least square mean (SE) of C_{MAX} (ng/ml) and $AUC_{0-\infty}$ (min*ng/ml) with estimated difference from 5-HTP/CBD with 95% confidence interval for 200 mg 5-HTP/CBD/domperidone and 200 mg 5-HTP/CBD/granisetron.**

Pharmacokinetic parameter		**5-HTP/CBD n=13**	**5-HTP/CBD/ domperidone n=12**	**5-HTP/CBD/ granisetron n=12**
5-HTP C_{MAX} (ng/ml)	LSM (SE) Estimated difference from 5-HTP/CBD (95% CI)	1483	1383 -7 (-29;22) p=0.58	1272 -14 (-26;0) p=0.05
5-HTP $AUC_{0-\infty}$ (min*ng/ml)	LSM (SE) Estimated difference from 5-HTP/CBD (95% CI)	549207	495371 -10 (-28;13) p=0.34	519492 -5 (-31;29) p=0.70

Table 2 **Pharmacodynamic parameters over 8 hours: Estimated means (LSM's) of serum cortisol (nmol/l), serum prolactin (ug/l), VAS-Nausea (mm) and SCL-90 Nausea (5 point scale) for the treatments placebo, 5-HTP/CBD/placebo, 5-HTP/CBD/domperidone and 5-HTP/CBD/granisetron. Estimated difference from placebo (%) with 95% confidence interval for placebo, 5-HTP/CBD/placebo, 5-HTP/CBD/domperidone and 5-HTP/CBD/granisetron and estimated difference from 5-HTP/CBD for 5-HTP/CBD/domperidone and 5-HTP/CBD/granisetron.**

	Least Square Means (vomiting subjects excluded)					**Estimated difference (%)**				
Parameter	plac n=12	5-HTP + plac n=11	5-HTP +dom n=7	5-HTP +gran n=13	treatment P-value	5-HTP + plac vs plac	5-HTP + dom vs plac	5-HTP + gran vs plac	5-HTP + dom vs 5-HTP + plac	5-HTP + gran vs 5-HTP + plac
Serum cortisol (nmol/l)	167.6	315.0	356.1	292.7	p <0.0001	+88.1 (63.5;116.4) p < 0.0001	+112.6 (80.7;150.2) p < 0.0001	+74.8 (53.0;99.7) p < 0.0001	+13.0 (-4.2;33.4) p=0.1398	-7.1 (-18.9;6.5) p=0.2775
Serum prolactin (µg/l)	4.3	5.5	24.0	5.0	p <0.0001	+28.5 (4.9;57.3) p =0.0180	+461.1 (339.3;616.7) p < 0.0001	+16.1 (-4.0;40.5) p=0.1162	+336.8 (245.7;451.9) p<0.0001	-9.6 (-25.1;9.1) p=0.2750
	Least Square Means (vomiting subjects included)					**Estimated difference (%)**				
Parameter	plac n=12	5-HTP + plac n=12	5-HTP +dom n=12	5-HTP +gran n=13	treatment P-value	5-HTP + plac vs plac	5-HTP + dom vs plac	5-HTP + gran vs plac	5-HTP + dom vs 5-HTP + plac	5-HTP + gran vs 5-HTP + plac
SCl-90 Nausea (5 point scale)	+1.0	+1.7	+1.7	+1.4	p=0.0002	+0.7 (0.4;1.0) p=0.0001	+0.7 (0.4;1.0) p=0.0001	+0.3 (0.0;0.7) p=0.0389	0.0 (-0.3;0.3) p=0.9861	-0.4 (-0.7;0.0) p=0.0276
VAS-Nausea (mm)	+6.8	+21.5	+23.0	+14.4	p=0.0034	+14.7 (5.5;23.8) p=0.0027	+16.2 (7.2;25.2) p=0.0009	+7.6 (-1.3;16.5) p=0.0915	-1.5 (-7.6;10.7) p=0.7343	-7.1 (-16.0;1.9) p=0.1164

plac= Placebo; 5-HTP= 200mg 5-hydroxytryptophane/100mg + 50mg carbidopa; dom= domperidone; gran= granisetron

Figure 1 Average time profile of serum 5-HTP (ng/ml) over 8 hours for the treatments 5-HTP/CBD (n=13), 5-HTP/CBD/domperidone (n=12) and 5-HTP/CBD/granisetron (n=12) with SD error bars.

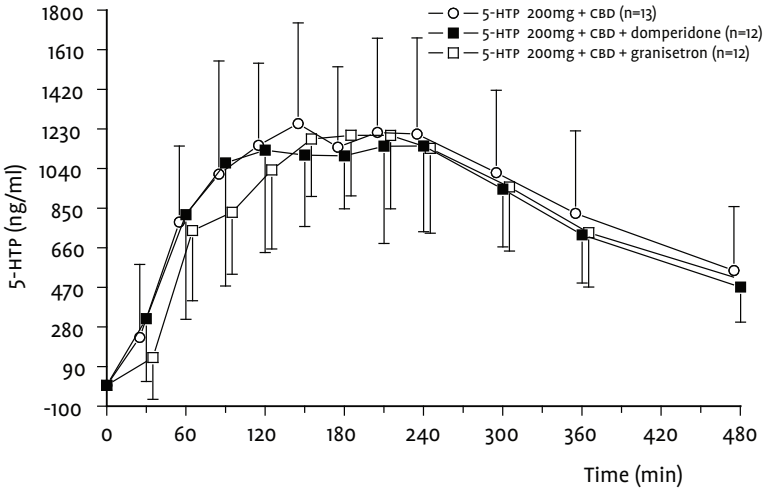


Figure 2 Estimated means (nmol/l) with SD error bars and estimated change from placebo (%) for serum cortisol after administration of placebo (n=12), 5-HTP/CBD (n=11), 5-HTP/CBD/domperidone (n=7), 5-HTP/CBD/granisetron (n=13), excluding data of study days on which subjects vomited.

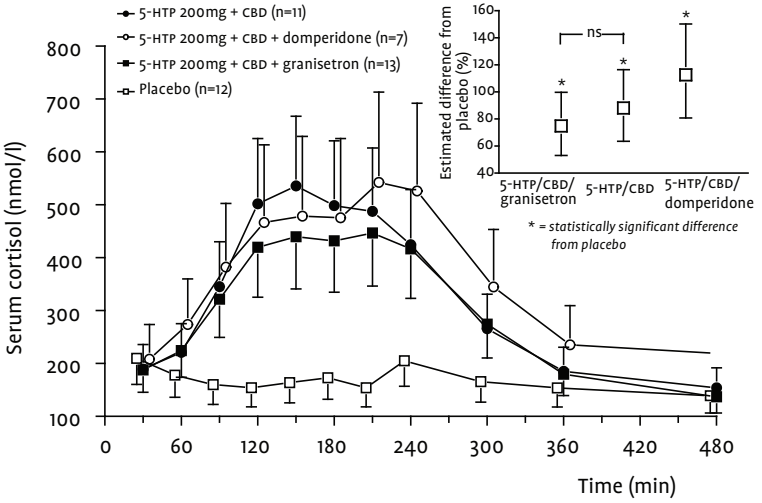


Figure 3 **Estimated means (µg/l) with SD error bars and estimated change from placebo (%) for serum prolactin after administration of placebo (n=12), 5-HTP/CBD (n=11), 5-HTP/CBD/domperidone (n=7), 5-HTP/CBD/granisetron (n=13), excluding data of study days on which subjects vomited.**

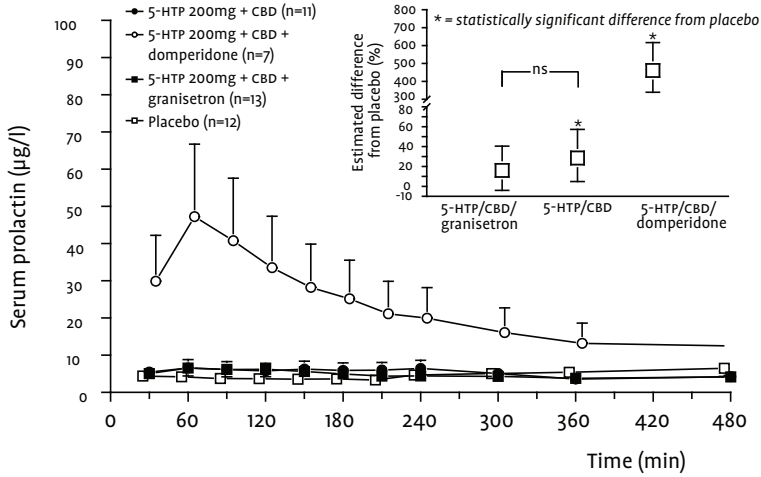
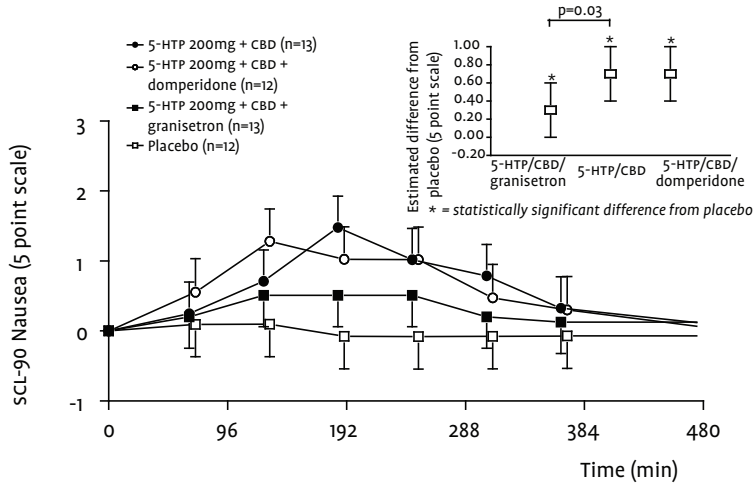


Figure 4 **Estimated mean change from baseline (5 point scale) and estimated change from placebo for SCL-90 Nausea after administration of placebo (n=12), 5-HTP/CBD (n=12), 5-HTP/CBD/domperidone (n=12), 5-HTP/CBD/granisetron (n=13) with SD error bars, including all data.**



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