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Endocrinological Effects of High-Dose *Hypericum perforatum* Extract WS 5570 in Healthy Subjects

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Key Words

St. John's wort *(Hypericum perforatum)* extract WS 5570 · Hyperforin · Cortisol · Adrenocorticotropic hormone · Growth hormone · Prolactin

Abstract

In this single-blind study, the effects of acute oral administration of high-dose *Hypericum perforatum* extract WS 5570 on the cortisol (COR), adrenocorticotropic hormone (ACTH), growth hormone (GH), and prolactin (PRL) secretions were examined in 12 healthy male volunteers. In a randomized order, the subjects received placebo or WS 5570 at several dosages (600, 900, and 1,200 mg) at 08.00 h on 4 different days. After insertion of an intravenous catheter, blood samples were drawn 1 h prior to administration of placebo or WS 5570 (600, 900, or 1,200 mg), at the time of administration, and during 5 h thereafter at intervals of 30 min. The serum concentrations of COR, GH, and PRL as well as the plasma levels of ACTH were determined in each blood sample by means of double antibody radioimmunoassay, fluoroimmunoassay, and chemiluminescence immunometric assay methods. The area under the curve value was used as parameter for COR, ACTH, GH, and PRL responses. Repeated-measures Anova revealed a significant stimulatory effect of WS 5570 on the ACTH secretion, whereas COR and PRL secretions were not significantly influenced. Moreover, there was a stimulatory peak of GH release 240 min after challenge with WS 5570 in some but not all volunteers, without reaching statistical significance in comparison with placebo. Mean arterial blood pressure and heart rate remained unchanged after administration of WS 5570. Apparently, WS 5570 at the dosages given in this study inconsistently causes endocrinological effects in healthy subjects by influencing central neurotransmitters.

Introduction

Preparations from *Hypericum perforatum* (St. John's wort; SJW) extracts have been shown in most studies to be efficacious in mild to moderate depression [1, 2]. However, the exact mechanism of action has not been sufficiently clarified yet. Earlier findings suggesting monoamine oxidase inhibiting effects of the constituents hyper-

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icin or pseudohypericin [3] could not be replicated in later studies [4]. In more recent research, the focus of attention has been directed towards the role of the components hyperforin and adhyperforin for antidepressant efficacy. While neither hypericin nor the flavonol glycoside kaempferol show any reuptake-inhibiting properties, hyperforin and its structural analogue adhyperforin have been identified as the only reuptake-inhibiting constituents of SJW extracts in synaptosomal preparations [5]. Apparently, hyperforin and adhyperforin have a broad inhibitory profile [reuptake inhibition of serotonin (5-HT), noradrenaline, and dopamine, but also of γ-aminobutyric acid and *L*-glutamate] and do not act as competitive inhibitors at the neurotransmitter-binding sites of the transporter proteins, but affect the sodium gradient, thereby causing an uptake inhibition [6, 7]. Moreover, according to a clinical study performed in depressive patients, an important role of hyperforin for the antidepressant action of SJW extracts has been suggested [8].

To further examine the effects of SJW extracts on the central aminergic systems in vivo, our research group investigated the neuroendocrine effects of the hyperforin-containing SJW extract WS 5570 at lower dosages (300 and 600 mg p.o.) in a former study [9], using the neuroendocrine challenge paradigm. This paradigm is based on the involvement of monoamine pathways in the control of anterior pituitary hormone secretion [10] and has been described extensively [11]. In the former investigation [9], WS 5570 caused a weak but significant cortisol (COR) stimulation in a dose-dependent manner (COR stimulation after 600 mg, but not after 300 mg WS 5570). In contrast to a study performed by Franklin et al. [12], who administered the SJW extract LI 160 at a high dosage (2,700 mg p.o.), in our former investigation [9], the stimulatory effects of WS 5570 on the growth hormone (GH) secretion were inconsistent, and no inhibitory influence of WS 5570 on the prolactin (PRL) secretion was seen. To test the hypothesis that these conflicting and inconsistent endocrinological findings are due to the different dosages used and to answer the question whether increased dosages of WS 5570 would also cause an enhanced stimulatory impact on COR and/or GH secretion in a dose-dependent manner, we performed the present study using WS 5570 at higher dosages (600, 900, and 1,200 mg) in healthy male volunteers.

Subjects and Methods

Study Population and Study Design

Twelve healthy male subjects having a normal weight, age range 26–41 (mean ± SEM 32.08 ± 4.80) years, were included upon receipt of their informed consent, following a clinical examination (psychiatric and medical history, physical examination) and establishment of normal laboratory and clinical parameters: hemoglobin, K^+, Na^+, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, γ-glutamyltransferase, blood glucose, bilirubin, serum creatinine, heart rate, electrocardiogram and electroencephalogram. Alcohol abstinence 24 h prior to each experiment and abstinence from medication beginning 4 weeks before the study were mandatory.

Each subject took part four times in the trial. In a randomized order, the volunteers received placebo or the *H. perforatum* extract WS 5570 (600, 900, or 1,200 mg) orally on 4 different days. At 07.00 h, an intravenous catheter was inserted into the antecubital vein and kept open with physiological saline solution. The subjects rested in bed throughout the experiments (up to t = 300 min). At t = –60 min (07.00 h), at t = 0 min (08.00 h; administration of placebo or WS 5570), and at intervals of 30 min thereafter up to t = 300 min (13.00 h), blood was drawn. The subjects fasted from completion of the evening meal the day before until conclusion of the experiment.

The study was carried out according to the fifth revision of the Declaration of Helsinki [13] and had been approved by an ethics committee.

Endocrinological and Blood Pressure Measurements

Serum and plasma samples were separated by centrifugation as soon as possible, frozen at –80 °C, and stored for determination of the hormone concentrations. The serum concentrations of COR, GH, and PRL and the plasma concentrations of adrenocorticotropic hormone (ACTH) were determined in each blood sample. The COR, GH, and PRL levels were determined by means of double-antibody radioimmunoassay (RIA) and fluoroimmunoassay methods. The sensitivity ('minimal detectable dose') of the commercially available immunoassay kits was approximately 6.1 nmol/l for the COR RIA (Diagnostic Products Corporation, Los Angeles, Calif., USA), 4.5 pmol/l for the GH fluoroimmunoassay (DELFIA® hGH; Perkin-Elmer, Torrrance, Calif., USA), and 1.74 pmol/l for the PRL fluoroimmunoassay (DELFIA® Prolactin). The specificity was very high for the immunoassays with an extremely low cross-reactivity to other natural hormones; the percent cross-reactivity to other naturally occurring steroids in quality control tests (apparent concentrations related to the amount added in the experiment) was up to 6.8% for the COR RIA (although some steroids exhibit slight cross-reactivity, their normal phyiological concentrations are low as compared with COR so as not to significantly interfere in the double-antibody COR procedure), 0.1% for the GH fluoroimmunoassay, and <0.01% for the PRL fluoroimmunoassay. The total variation (% CV) was 6.6% for the COR RIA, 3.9% for the GH fluoroimmunoassay, and 2.7% for the PRL fluoroimmunoassay. ACTH was measured using a chemiluminescence immunometric assay (Nichols, San Juan Capistrano, Calif., USA); the lower detection limit of this assay is 0.11 pmol/l, intra- and interassay CVs are below 4 and 7%, respectively.

The blood pressure (diastolic pressure = RR_{dia}; systolic pressure = RR_{sys}) was measured by the Riva-Rocci method every 30 min. The mean arterial blood pressure (MAP) was calculated according to the equation $MAP = RR_{dia} + 1/3 (RR_{sys} - RR_{dia})$ and served as parameter

Table 1. Influence of placebo and WS 5570 on COR, ACTH, GH, and PRL secretions and on MAP and heart rate in 12 healthy male subjects (mean ± SEM)

	COR $AUC_{0–300}$ nmol/l × min	ACTH $AUC_{0–300}$ pmol/l × min	GH $AUC_{0–300}$ pmol/l × min	PRL $AUC_{0–300}$ pmol/l × min	MAP $AUC_{0–300}$ mmHg × min	Heart rate $AUC_{0–300}$ beats/min × min
Placebo	60,911.58 ± 4,800.52	1,578.26 ± 126.11	8,001.09 ± 1,974.80	61,346.46 ± 7,766.26	26,553.89 ± 566.37	18,342.50 ± 883.41
600 mg WS 5570	61,537.36 ± 3,628.78	1,858.43 ± 166.67	16,156.72 ± 6,198.09	56,877.87 ± 8,146.21	27,306.89 ± 631.11	18,409.17 ± 752.56
900 mg WS 5570	60,593.40 ± 3,330.35	1,856.21 ± 141.90	15,699.57 ± 6,333.69	56,586.61 ± 7,245.91	26,781.10 ± 782.42	18,333.33 ± 986.83
1,200 mg WS 5570	64,108.54 ± 3,359.15	1,953.42 ± 130.69	13,475.55 ± 5,039.50	60,018.42 ± 9,496.78	26,771.26 ± 737.08	18,507.50 ± 899.83

for assessing the blood pressure effects of WS 5570. Furthermore, the heart rate (beats/min) was recorded every 30 min.

Data Analysis

In addition to the descriptive and graphical evaluation of the mean curves (hormonal concentrations, MAP, heart rate), the areas under the curve (AUCs, 0–300 min) were calculated according to the method of Friedman [14], representing the total COR, ACTH, GH, and PRL secretions and total MAP and heart rate following oral administration of placebo or WS 5570 (600, 900, and 1,200 mg). For statistical comparisons of hormonal secretions (COR, ACTH, GH, and PRL AUCs) and vital parameters (MAP, heart rate), analysis of variance (Anova) with a repeated-measures design (Wilks' multivariate tests of significance) was performed, considering 'treatment' (administration of placebo or WS 5570 at the different dosages) as within-subjects factor with four levels (placebo, 600 mg WS 5570, 900 mg WS 5570, and 1,200 mg WS 5570). For those hormonal or vital parameters with a significant 'treatment' effect, additionally tests with contrasts were performed. As a nominal level of significance, alpha = 0.05 was accepted. To keep the type I error equal to 0.05, tests with contrasts were performed at a reduced level of significance (alpha adjusted according to the Bonferroni procedure).

Results

COR and ACTH Secretions

Using Anova with a repeated-measures design (Wilks' multivariate tests of significance), no significant influence of 'treatment' (placebo, 600 mg WS 5570, 900 mg WS 5570, and 1,200 mg WS 5570) on the COR secretion could be demonstrated (F = 0.634; d.f. = 3, 9; significance of F = 0.612; table 1, fig. 1). Nevertheless, there was a significant 'treatment' effect on the ACTH secretion (F = 4.061; d.f. = 3, 9; significance of F = 0.044), i.e., the ACTH release depended significantly on the type of treatment (placebo and WS 5570 at the different dosages), being the lowest after administration of placebo (ACTH $AUC_{0–300}$ 1,578.26 ± 126.11 pmol/l × min) and the highest after 1,200 mg WS 5570 (ACTH $AUC_{0–300}$ 1,953.42 ± 130.69 pmol/l × min; table 1, fig. 1). However, using the Bonferroni procedure for alpha adjustment post hoc tests with contrasts did not show any significant differences in ACTH $AUC_{0–300}$ values between the four treatment conditions.

GH Secretion

Descriptively, both the GH $AUC_{0–300}$ values and the mean values were higher after administration of WS 5570 at all dosages (600, 900, and 1,200 mg) as compared with placebo (table 1, fig. 1). In most cases, the GH stimulation was observed more than 150 min after administration of WS 5570. However, GH-stimulatory effects occurred inconsistently after WS 5570 was given. When regarding 50 pmol/l GH as minimum peak value, only part of the subjects reached this GH peak concentration after administration of WS 5570 (600 mg: 5 out of 12 subjects; 900 mg: 7 out of 12 subjects; 1,200 mg: 8 out of 12 subjects). Repeated-measures Anova did not reveal a significant 'treatment' effect on GH $AUC_{0–300}$ values (F = 0.560; d.f. = 3, 9; significance of F = 0.655). Nor was there any significant 'treatment' effect, if only GH concentrations from t = 150 min up to t = 300 min after administration of placebo or WS 5570 were taken into consideration (GH $AUC_{150–300}$ values: F = 1.113; d.f. = 3, 9; significance of F = 0.394).

PRL Secretion

PRL $AUC_{0–300}$ values and PRL mean values were comparable after administration of placebo and WS 5570 (600, 900, and 1,200 mg; table 1, fig. 1). No significant 'treatment' effect could be demonstrated (F = 0.334; d.f. = 3, 9; significance of F = 0.802).

MAP and Heart Rate

Neither the MAP nor the heart rate were significantly influenced by administration of placebo or WS 5570 at the different dosages (MAP $AUC_{0–300}$: F = 0.673; d.f. = 3, 9; significance of F = 0.590; heart rate $AUC_{0–300}$: F = 0.144; d.f. = 3, 9; significance of F = 0.931).

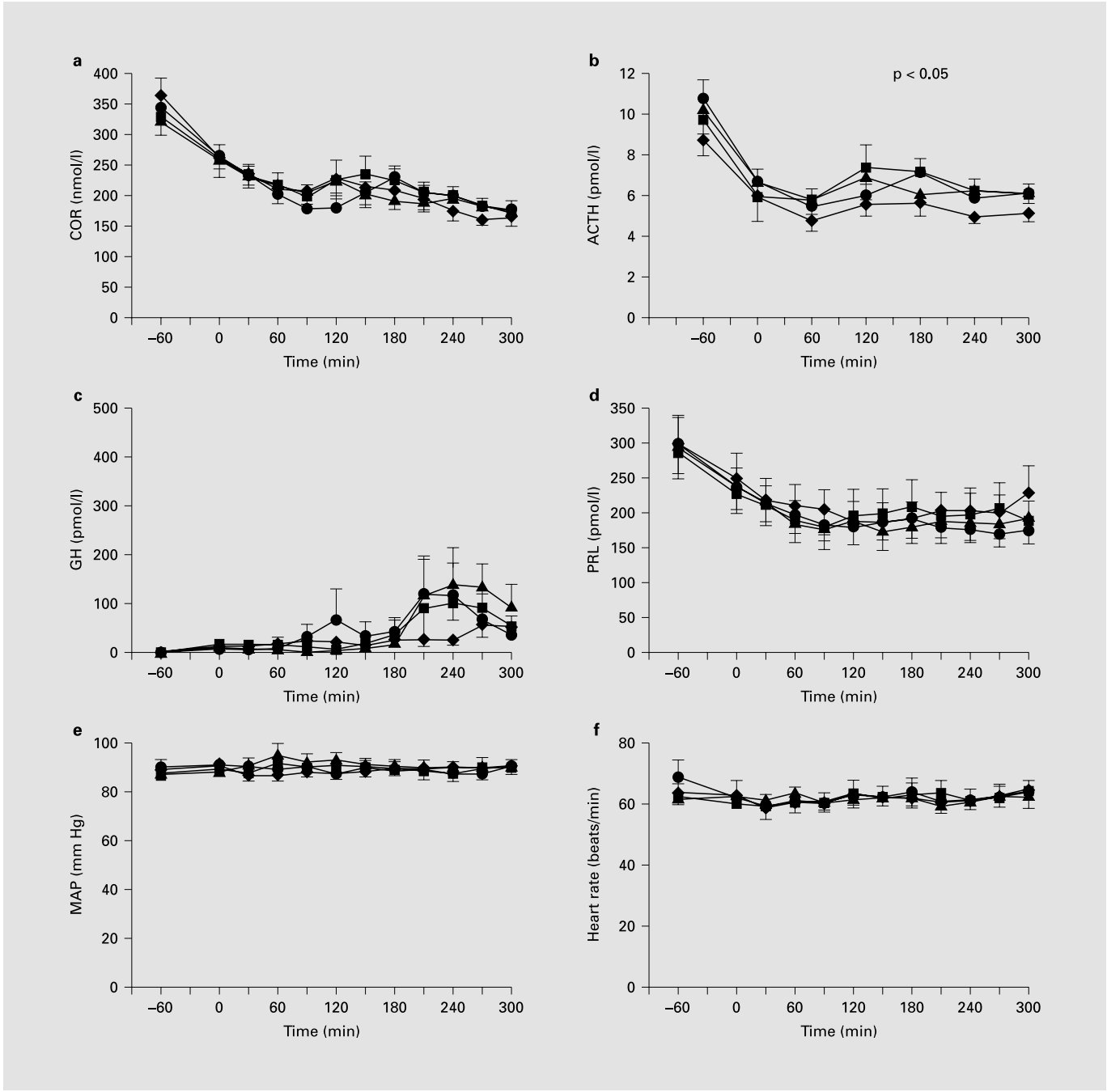

Fig. 1. Mean values ± SEM of COR (**a**), ACTH (**b**), GH (**c**), and PRL (**d**) concentrations and of MAP (**e**) and heart rate (**f**) after oral administration of placebo (♦), 600 mg WS 5570 (▲), 900 mg WS 5570 (●), and 1,200 mg WS 5570 (■) in 12 healthy male subjects. Statistical significance at the $p < 0.05$ level in repeated-measures Anova indicated.

Discussion

In the present study, the impact of the hyperforin-containing SJW extract WS 5570 at higher dosages (600, 900, and 1,200 mg) on anterior pituitary hormone secretion and on vital parameters was investigated in 12 healthy male subjects. We could only partially replicate our former finding of stimulatory effects of WS 5570 on the hypothalamic-pituitary-adrenocortical axis activity [9], in that there was a significant stimulatory 'treatment' effect of WS 5570 on the ACTH secretion but not on the COR secretion in the present study. Since hyperforin and adhyperforin have been identified as the reuptake-inhibiting components of SJW extracts in in vitro investigations using synaptosomal preparations [6, 7], one may assume that these constituents are responsible for influencing aminergic systems and causing endocrinological effects in the neuroendocrine challenge paradigm. According to animal [15] and human [11] studies, ACTH and COR stimulation by noradrenaline and/or 5-HT reuptake-inhibiting antidepressants may be mediated via agonistic effects of noradrenaline and 5-HT on hypothalamic α_1, 5-HT_1, and 5-HT_2 receptors, thereby enhancing hypothalamic corticotropin-releasing hormone and/or vasopressin release. Apparently, the dosages of WS 5570 used in the present study (600, 900, and 1,200 mg) were not sufficient to cause consistently a stimulatory impact on ACTH and COR secretions. Similarly, Franklin and coworkers found stimulatory effects of the hyperforin-containing SJW extract LI 160 on the COR secretion in some but not all healthy volunteers. In an unblinded pilot study of this research group [16], a significant increase of the salivary COR concentrations in 6 healthy males after oral administration of 2,700 mg LI 160 was found. However, when giving 2,700 mg LI 160 orally to 12 healthy subjects in a double-blind placebo-controlled study, elevated COR levels were seen only in 4 out of the 12 subjects, without reaching statistical significance [12].

As in our former study [9], some but not all volunteers were prone to show a certain increase of the GH secretion after administration of WS 5570 which may be due to α_2-adrenergic or dopaminergic actions at the hypothalamic level, leading to an enhanced GH-releasing hormone release. However, neither statistical significance nor dose dependency could be demonstrated in our new investigation. Interestingly, the weak GH-stimulatory effects in some participants in our study occurred more than 150 min after administration of WS 5570, with a maximum at t = 240 min (fig. 1). Franklin et al. [12] also found GH stimulation after the SJW extract LI 160 was given (2,700 mg p.o.); in contrast to our investigation, the GH-stimulatory effects reached statistical significance in comparison with placebo. In this context, pharmacokinetic observations have to be considered. First, in man the hyperforin plasma levels began to increase not until 60 min after administration of the respective SJW extract, reaching a peak maximum at t = 210 min [12], which may explain the delay of some stimulatory effects, e.g., the weak GH stimulation in some individuals of our study more than 150 min after the challenge. Second, after administration of 2,700 mg LI 160 to healthy volunteers, the mean hyperforin plasma peak was nearly 1,500 ng/ml [12], whereas in a pharmacokinetic study performed by Biber et al. [17], administration of 300, 600, and 1,200 mg WS 5570 led to lower maximal plasma hyperforin concentrations in the subjects (mean peak values 153, 302, and 437 ng/ml, respectively), suggesting that the inconsistent GH stimulation in our study may be a matter of dosage and bioavailability of hyperforin.

As opposed to the study performed by Franklin et al. [12], no significant inhibitory effects of the SJW extract WS 5570 on the PRL secretion could be demonstrated in our investigation. Whereas the PRL secretion is increased by enhancement of the serotonergic function [11], the PRL release is reduced by dopamine as a potent PRL release inhibiting factor [18]. Since hyperforin has been proven to be a reuptake inhibitor of both 5-HT and dopamine [4], opposing effects with regard to the PRL secretion may play a role. The presumption that the relevance of serotonergic and dopaminergic mechanisms for the regulation of PRL secretion is dose dependent may be the explanation for the different results in our study and in the investigation performed by Franklin et al. [12]. Moreover, it is conceivable that in our study, the dosage was too low to cause serotonergic and/or dopaminergic effects on the PRL release.

The missing of significant changes in vital parameters (blood pressure, heart rate) and the missing of side effects or adverse events confirm the good tolerability of the SJW extract WS 5570 at the dosages given in the present study (up to 1,200 mg p.o.). Additionally, no case of photosensitivity was observed in our study which is one of the possible side effects of SJW extracts reported in the literature [19].

Taken together, the SJW extract WS 5570, given at dosages which were in part higher than the dose usually administered in the treatment of depressive symptoms (3 × 300 mg per day), caused some weak and inconsistent endocrinological effects in healthy subjects. Our findings raise the question whether the therapeutically recom-

mended dosages of SJW extracts are adequate to lead to a sufficient impact on central neurotransmitter systems. Moreover, there is a need for standardization of possibly therapeutically active constituents of SJW extracts such as hyperforin or adhyperforin to establish evidence-based dosage guidelines.

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