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Acute and Chronic Role of 5-HT_3 Neuronal System on Behavioral and Neuroendocrine Changes Induced by Intravenous Cholecystokinin Tetrapeptide Administration in Humans

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The influence of single and multiple oral doses of ondansetron, a selective 5-HT_3 receptor antagonist, was evaluated against placebo on cholecystokinin tetrapeptide (CCK-4)-induced behavioral and neuroendocrine changes in humans. As compared to placebo, subjects receiving acute ondansetron treatment showed a significant decrease in the sum intensity of CCK-4-induced-panic symptoms (iPSS). Pre-CCK-4 neuropeptide Y (NPY) plasma levels were significantly higher and maximal changes in cortisol, growth hormone, and prolactin secretion from baseline (Δ_{max}) were significantly lower in the ondansetron group. After ondansetron and placebo chronic administration, there were no statistical differences in the iPSS between groups. Pre-CCK-4 NPY plasma levels were significantly higher; whereas, Δ_{max} for NPY significantly lower in the ondansetron group as compared to placebo. These results suggest a role for the 5-HT_3 receptor in the neurobiology of panic disorder through a possible interaction with CCK and NPY systems. Ondansetron chronic effect on CCK-4-induced behavioral changes needs further exploration.
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Currently, evidence suggests a role for cholecystokinin (CCK) in the neurobiology of panic disorder. Intravenous (IV) administration of cholecystokinin tetrapeptide (CCK-4) has been shown to induce panic attacks in patients suffering from panic disorder and in healthy subjects (Bradwejn et al. 1990, 1994b). In addition, panic disorder patients show an enhanced sensitivity to CCK-4 as compared to normal subjects suggesting anomalies in the CCK system (Bradwejn et al. 1991). The role of CCK in panic disorder is further supported by the finding of enhanced CCK-4-induced intracellular calcium mobilisation in patients with untreated panic disorder as compared to healthy subjects (Akiyoshi et al. 1997).

Investigations also suggest a role for the serotonin (5-HT) system in panic disorder. Anxiolytic activity has

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been reported for 5-HT_3 receptor antagonists in various animal models (Jones et al. 1988; Costall et al. 1988). In panic disorder patients, administration of ondansetron, a selective 5-HT_3 receptor antagonist, has been shown to reduce the intensity of panic attacks (Schneier et al. 1996). In contrast, comparative efficacy studies on buspirone, a 5-HT_{1A} partial agonist, fail to show statistical differences between buspirone and placebo (Pohl et al. 1989). Likewise, antagonists of the 5-HT_2 receptor, ritanserine and trazodone, are ineffective (Charney et al. 1986; den Boer and Westenberg 1990).

Experimental studies provide evidence supporting a relationship between the 5-HT and CCK systems. It has been observed that central injections of CCK-4 stimulate the metabolism of serotonin in the rat brain (Itoh et al. 1988). In the guinea pig, intraperitoneal administration of 10 μg/kg of butylocarbonyl (BOC)-CCK-4 amplifies the rise in extracellular 5-HT normally observed in the elevated plus-maze model of anxiety and produces anxiogenic effects. Pretreatment with L 365,260, a CCK_B receptor antagonist, opposes both effects. When administered alone, L 365,260 shows anxiolytic properties, decreases basal 5-HT levels, and prevents the rise in 5-HT induced by exposure to the elevated plus-maze test (Rex et al. 1994).

In addition, modulation of endogenous 5-HT_3 activity has been shown to influence the CCK system. Vasar et al. have shown that intraperitoneal pretreatment of rats with 10 μg/kg of ondansetron completely reverses the antiexploratory effect of subcutaneously injected 5 μg/kg of caerulein, a nonselective agonist of CCK_A/CCK_B, indicating the involvement of 5-HT_3 receptors in the regulation of anxiety (Vasar et al. 1993). Animal studies indicate that serotonin and 1-phenylbiguanide, a 5-HT_3 agonist, enhance the depolarisation-evoked release of CCK from synaptosomes of rat cerebral cortex or nucleus accumbens. This effect is not observed under basal conditions or after pretreatment with 5-HT_3 receptor antagonists such as MDL 72222, ICS 205-930, and ondansetron. In contrast, the 5-HT_1/5-HT_2 receptor blockade by methiothepin does not antagonize CCK release by serotonin, indicating that the effect is most likely mediated by 5-HT_3 receptors located on CCK-releasing nerve terminals (Paudice and Raiteri 1991; Raiteri et al. 1993). These animal findings further support the relationship between 5-HT_3 and CCK systems. In patients with panic disorder, chronically administered imipramine, a nonselective 5-HT/noradrenaline reuptake inhibitor, fluvoxamine, and citalopram, two 5-HT reuptake inhibitors, prevent the CCK-4-induced panicogenic-like symptoms (Bradwejn and Koszycki 1994a; Shlik et al. 1997; van Megen et al. 1997). Furthermore, it has been shown that acute tryptophan depletion intensifies the neuroendocrine changes produced by IV CCK-4 injection in healthy subjects (Koszycki et al. 1996). These results provide evidence for a role of the 5-HT system in the CCK-4-provoked neurobiological changes.

Based upon these findings, it seems likely that the 5-HT_3 receptor might be a mediator of the panicogenic effect produced by CCK-4. The aim of the present study was to evaluate the role of the 5-HT_3 system in CCK-4-induced panic symptoms. Thus, we studied the effect of a single oral dose (acute treatment) and multiple oral doses (chronic treatment) of ondansetron in the mediation of CCK-4-induced panic symptoms in humans. The evaluation of the acute versus chronic effect of ondansetron was used to assess possible neuroadaptation following chronic ondansetron treatment.

METHODS

Study Design

The influence of ondansetron on CCK-4-induced behavioral and neuroendocrine changes was investigated in 36 healthy male subjects using a double-blind, randomized, parallel-group, placebo-controlled design. Subjects were randomly assigned into one of the two treatment groups according to a 1:2 allocation scheme. Depending upon their assigned treatment group, subjects received IV CCK-4 challenge test after a single (2 mg) dose and multiple (2 mg twice daily for 28 days) oral doses of either ondansetron or placebo.

Materials

As previously described, CCK-4, consisting of an amino acid chain of TRP-MET-ASP-PHE-NH_2, was synthesized by Peninsula (Belmont, CA, USA) and prepared by GIS médicament (Nantes, France) as intravenous solutions (50 μg in 2.5 ml of NaCl 0.9%) (Bradwejn et al. 1991). Ondansetron (1 mg calculated as free base) and placebo were formulated as film-coated pink tablets identical in size, color, and weight. Ondansetron and placebo were prepared and quality assured by Glaxo Wellcome Inc., Mississauga, Ontario, Canada.

Experimental Procedures

The study followed the guidelines of the Declaration of Helsinki of 1989. It was approved by the Institutional Human Experimentation Committee of the University of Toronto (Toronto, Ontario) and the Health Protection Branch of Health and Welfare Canada (Ottawa, Ontario). Subjects gave written informed consent.

Subject Population. Male subjects between 18 and 55 years of age and within 20% of the ideal weight for height and body frame were allowed to participate in

the study. The screening assessments were performed on an out-patient basis and included a medical history, physical and psychiatric examinations, vital signs, and a 12-lead electrocardiogram tracing. Structure Clinical Interview for DSM-III-R (SCID for nonpatients), Hamilton Anxiety Rating Scale, and 90-point Symptom Checklist were completed to rule out any underlying psychiatric conditions. For the screening to be successfully completed, subjects had to be healthy as per the evaluating clinician and to show no clinically significant laboratory abnormality.

Behavioral and Neuroendocrine Evaluation. Two IV CCK-4 challenge test periods were performed 28 days apart. Subjects were instructed to maintain an overnight fast prior to each challenge period. Upon admission to the research unit in the morning of each period, subjects underwent a drug urine screen analysis and, immediately after, completed a visual analog scale (VAS) to assess their anxiety level prior to the beginning of the experiment. Thereafter, an IV catheter was installed into the antecubital vein of the right arm through which a normal saline solution was slowly infused. The IV catheter maintained an open vein to allow CCK-4 injection and blood samplings. A blood pressure cuff with a pulse monitor attached to a Dinamap® (Critikon, Canada) sphygmomanometer was installed on the left arm. Immediately after completion of these procedures, subjects received and ingested two tablets of study medication (either placebo or ondansetron) with 150 ml of water. Sixty minutes postdosing, subjects completed a second VAS assessment and, then, received a 50 μg bolus of CCK-4. Vital signs were recorded prior to and 20, 40, 60, 80, 100, and 120 seconds after CCK-4 administration. Subjects were instructed to describe any symptoms they experienced following the injection. The time to onset and duration of symptoms were recorded. Immediately after symptoms abated, the symptoms experienced by the subjects were evaluated with the Panic Symptom Scale (PSS) (Bradwejn et al. 1991, 1995; Bradwejn and Koszycki 1994a; Koszycki et al. 1996). This 23-item scale consisted of the criteria set for a panic attack described in the DSM-IV and included five panic-unrelated symptoms for validation. The intensity of symptoms was rated on a 5-point scale: 0 (not present) to 4 (extremely severe). All subjects were assessed as to whether they experienced a panic-like attack defined as per the DSM-IV criteria for a panic attack plus a score of two or more on the anxiety, fear and apprehension item of the PSS. Subjects were instructed to rate their anxiety on the VAS to reflect how they felt at peak effect after CCK-4 injection. At the end of the first challenge test period, subjects were dismissed from the research unit after instruction to continue taking study medication at breakfast and dinner times for the next 28 days, at which time the second CCK-4 challenge test was performed 60 minutes after ingestion of the last ondansetron dosing. Compliance and adverse event assessments were performed weekly.

Adrenocorticotropic hormone (ACTH), cortisol (CORT), growth hormone (GH), neuropeptide Y (NPY), and prolactin (PRL) were measured as endocrine indicators for stress response. Blood samples were drawn from the indwelling cannula kept patent with normal saline solution prior to and at 2, 5, 10, and 15 minutes after each CCK-4 injection. Blood samples for NPY measurements were collected into ice-chilled EDTA-containing test tubes. Immediately after blood collection, 0.3 ml of the enzyme inhibitor, aprotinin (10 000 KIU/ml, Sigma Chemical Company, St. Louis, MO, USA), was added to test tubes. Tubes were then inverted to ensure good mixing of the blood with the enzyme inhibitor. Blood was centrifuged, and plasma for ACTH and NPY levels was stored at −70°C until assayed; whereas, serum for CORT, PRL, and GH was immediately analyzed.

Hormone-Level Determinations

Plasma ACTH was determined at The Hospital-In-Common (Brampton, Ontario, Canada) by immunoradiometric assay (IRMA) using a commercially available kit (INCSTAR® Corporation, Stillwater, MN, USA). The lowest level of detection and within-run coefficient of variation were 1.9 pmol/l and less than 10%, respectively. Cortisol, PRL, and GH serum levels were analyzed at The Sick Childrens' Hospital (Toronto, Ontario). Cortisol serum levels were measured by competitive immunoassay with chemiluminescent detection (Access®, Sanofi Pasteur Diagnostics, Chaska, MN, USA). The lowest level of detection was less than 5.6 nmol/l. The within-run coefficient of variation was 10% at 75 nmol/l, 5% at 600 nmol/l, and 4% at 1,000 nmol/l. Growth hormone serum levels were measured by monoclonal/polyclonal two-site immunometric assay with chemiluminescent detection (Immulite®, Diagnostics Products Corporation, Los Angeles, CA, USA). The lowest level of detection was 0.003 μg/l. The within-run coefficient of variation was 5%. Prolactin serum levels were measured by enzyme immunometric assay with chemiluminescent detection, with a lowest level of detection of less than 0.25 μg/l (Access®, Sanofi Pasteur Diagnostics, Chaska, MN, USA). The within-run coefficient of variation was 5%. NPY plasma levels were analyzed by radioimmunoassay in the Department of Pharmacology, Faculty of Medicine, Université de Sherbrooke, using an antiserum to h-neuropeptide Y and ^{125}I-labeled NPY as a tracer. The assay method used was a modification of the methodology developed by Mouri et al. (1992). The intra- and interassay variations were 8 and 14%, respectively. The detection limit of the assay was 5 pg/ml of plasma.

Statistical Analysis

Because the sum intensity of symptoms (iPSS) and the number of symptoms (nPSS) are highly correlated (r = 0.9057), either variable can be considered to be the primary efficacy variable. The effect size calculated using Bradwejn and Koszycki (1994a) was 1.6. However, for a group of healthy subjects, the effect size was expected to be much less and, therefore, an effect size of 1.2 was chosen. For either iPSS or nPSS comparison with an effect size of 1.2, two-tailed α of 0.05 and a power of 90%, led to a sample size of 18 subjects per group for a total of 36 subjects. This power reduced to 88% when a 1:2 allocation scheme was used leading to a sample size of 12 subjects for the placebo group and 24 subjects for the ondansetron group. All other variables cannot be used in a confirmatory mode but rather as hypotheses generators. Protocol-defined secondary study end-points included the iPSS and nPSS symptoms experienced after acute treatment, time to onset and duration of symptoms, number of panic attacks, and change from baseline for VAS score, vital signs, and hormone levels for both acute and chronic treatment. The VAS score consisted of a distance in millimeters from the left-hand side of a 100-millimeter line to a perpendicular mark drawn by the subject. Change from baseline values (Δ_{max}) was calculated by subtracting the baseline value from the maximal value observed after IV CCK-4 administration.

Data were analyzed by using the SAS system for PC version 6.12 (Cary, NC, USA). The assumption of the normal distribution of data was tested with the Shapiro–Wilk statistic. For continuous variables without repeated measurements such as iPSS and nPSS scores, statistical comparisons were made by using a two-tailed *t*-test for unpaired samples. For continuous variables with repeated measurements such as VAS scores, vital signs, and neuroendocrine data, repeated measures analysis of variance models were used. Adjustment for baseline differences between treatment groups was done using analysis of covariance (ANCOVA) models. The Δ_{max} values were analyzed by a one-way (treatment) ANCOVA. Mann–Whitney U statistic test for two independent samples (two-tailed) was used for continuous data without repeated measurements that are not normally distributed, such as onset and duration of symptoms. Chi-square test was used for categorical data. For cells with expected frequency less than five, Fisher's exact test was used to analyze individual 2×2 tables, such as the number of panic-like attacks. For all analyses, a p value below .05 was considered statistically significant. Results are reported as mean ± SEM.

RESULTS

To ensure adequate clinical data from 36 subjects, a total of 41 subjects were randomized to one of the two treatment groups. One subject from the placebo group was discontinued because of protocol violation. From the ondansetron group, one subject was discontinued because of a severe allergic-type reaction to study medication and one withdrew his participation for reasons unrelated to the study. Therefore, 41 healthy male subjects completed the acute period of the study, 27 of whom received ondansetron treatment. Thirty-eight subjects completed both the acute and chronic treatments, 25 of whom received ondansetron. Baseline characteristics of subjects are summarized in Table 1. There was no statistical difference in baseline characteristics between ondansetron and placebo groups.

Primary Study End-Points

The primary outcome measures are summarized in Table 2. There were no statistical differences in the mean iPSS or nPSS symptoms experienced by subjects who received chronic treatment with ondansetron versus those who were administered placebo.

Secondary Study End-Points

Effect of Acute Treatment with Ondansetron on PSS Symptoms. The mean iPSS and nPSS symptoms experienced by subjects after a single oral dose of either ondansetron or placebo are shown in Table 2. A significant decrease in the mean iPSS score was observed in the ondansetron group, as compared to the placebo group ($p = .0306$). In line with this reduction, there was a decline in the mean nPSS score in the ondansetron group, which did not reach statistical significance ($p = .1577$). A student's *t*-test of the mean iPSS and nPSS measures between CCK-4 challenge periods within each treatment group showed significant differences within the ondansetron group (iPSS $p = .0192$, nPSS $p = .0211$) and the placebo group (iPSS $p = .0004$, nPSS $p = .0042$).

Effect of Ondansetron on Other Behavioral Measures. Individual PSS symptoms were further examined to identify those affected by the ondansetron treatment (Table 3). This statistical analysis was exploratory rather than confirmatory. Therefore, these results should be interpreted with caution. Nevertheless, the post hoc analysis revealed that, as compared to placebo, the intensity of dyspnea, choking feeling, anxiety, fear and/or apprehension, and fear of losing control was significantly reduced after acute treatment with ondansetron. There were no differences between individual PSS symptoms after chronic treatment with ondansetron. Neither CCK-4 nor placebo treatment affected the five panic-unrelated symptoms, namely earache, itchy nose, stuffy nose, low back pain, and itchy feet, included in the PSS to evaluate response bias.

Table 1. Demographic Data[a]

Variable	Acute Treatment[b] Placebo (n = 14)	Acute Treatment[b] Ondansetron (n = 27)	Chronic Treatment[c] Placebo (n = 13)	Chronic Treatment[c] Ondansetron (n = 25)
Age[d] (yrs)	27.1 ± 1.4	28.2 ± 1.0	27.3 ± 1.4	27.6 ± 1.0
Height[d] (cm)	176.1 ± 1.4	180.1 ± 1.1	176.3 ± 1.5	180.0 ± 1.5
Weight[de] (kg)	73.5 ± 2.4	77.6 ± 1.9	74.6 ± 2.3	77.8 ± 2.1
Ethnic origin (n)				
Caucasian	9 (64.3%)	24 (88.9%)	9 (69.2%)	22 (88.0%)
Black	2 (14.3%)	0	2 (15.4%)	0
Oriental	2 (14.3%)	2 (7.4%)	2 (15.4%)	2 (8.0%)
Hispanic	1 (7.1%)	1 (3.7%)	0	1 (4.0%)
Tobacco user (n)				
Never	9 (64.3%)	17 (63.0%)	8 (61.5%)	16 (64.0%)
Former	0	6 (22.2%)	0	5 (20.0%)
Current	5 (35.7%)	4 (14.8%)	5 (38.5%)	4 (16.0%)
Alcohol consumption[d] (unit/week)	3.7 ± 1.0	3.0 ± 0.6	3.8 ± 1.1	3.1 ± 0.6
Sitting SBP[d] (mmHg)	123.1 ± 3.2	121.8 ± 2.5	124.8 ± 3.0	122.4 ± 2.7
Sitting DBP[d] (mmHg)	70.4 ± 1.6	71.8 ± 1.0	70.5 ± 1.7	71.8 ± 1.0
Sitting heart rate[d] (bmp)	68.4 ± 2.3	68.0 ± 2.0	68.8 ± 2.4	68.8 ± 2.1
HAM-A score[d]	1.4 ± 0.4	1.3 ± 0.3	1.5 ± 0.4	1.4 ± 0.3
SCL-90 score[d]	9.1 ± 2.0	6.9 ± 1.5	9.0 ± 2.2	7.4 ± 1.5

[a]There was no statistical difference between treatment groups for the demographic variables.
[b]2 mg.
[c]2 mg twice daily for 28 days.
[d]Data are mean ± SEM.
[e]Body weights were within 20% of ideal body weight.
SBP, systolic blood pressure; DBP, diastolic blood pressure; HAM-A, Hamilton anxiety scale; SCL-90, 90-point symptom checklist score.

Although not statistically significant, there was a reduction in the number of panic-like attacks experienced by subjects who received a single dose of ondansetron as compared to those who received a single dose of placebo (11/27 [41%] vs. 9/14 [64%], p = .1526). The number of panic-like attacks was similar in both groups after chronic administration of either treatment (6/25 [24%] vs 4/13 [31%], p = .7092). The time to onset of symptoms showed a delay in the occurrence of symptoms in the ondansetron group after acute treatment, but the difference failed to reach statistical significance (23 vs. 20 s, p = .0747); there was no difference after

Table 2. Effect of Ondansetron on Intravenous CCK-4-Induced Behavioral Changes

Parameter	Acute Treatment[a] Placebo (n = 14)	Acute Treatment[a] Ondansetron (n = 27)	p-value	Chronic Treatment[b] Placebo (n = 13)	Chronic Treatment[b] Ondansetron (n = 25)	p-value
PSS scores						
iPSS	25.9 ± 2.7	18.4 ± 2.0	.0306	15.7 ± 2.2	15.0 ± 1.6	.8088
nPSS	11.3 ± 0.8	9.8 ± 0.6	.1577	8.5 ± 0.9	8.5 ± 0.7	.9870
VAS scores						
Baseline	1.8 ± 0.4	1.6 ± 0.2	.6076	1.1 ± 0.2	1.2 ± 0.2	.6942
Pre-CCK4	1.9 ± 0.4	1.7 ± 0.2	.7912	1.3 ± 0.2	1.4 ± 0.2	.7673
Post-CCK4	6.7 ± 0.6	5.2 ± 0.3	.0274	4.2 ± 0.5	4.7 ± 0.4	.4027
Δ_{max}	4.9 ± 0.8	3.5 ± 0.4	.0298	2.8 ± 0.5	3.3 ± 0.4	.4389

Data are mean ± SEM.
[a]2 mg.
[b]2 mg twice daily for 28 days.
PSS, panic symptom scale; iPSS, sum intensity of PSS symptoms; nPSS, number of PSS symptoms; VAS, visual analog scale; baseline, VAS anxiety score before ondansetron or placebo administration; pre-CCK4, VAS anxiety score 60 min after ondansetron or placebo administration that is immediately before intravenous CCK-4 administration; post-CCK4, VAS anxiety score at peak effect after intravenous CCK-4 administration; Δ_{max}, post-CCK4−pre-CCK4.

Table 3. Effect of Ondansetron on Intensity of PSS Symptoms Induced by CCK-4

	Acute Treatment[a]		Chronic Treatment[b]	
Panic Symptom Scale	**Placebo (n = 14)**	**Ondansetron (n = 27)**	**Placebo (n = 13)**	**Ondansetron (n = 25)**
Dyspnea	2.4 ± 0.3 (92.8)	1.5 ± 0.2[c](85.2)	1.2 ± 0.3 (69.2)	1.4 ± 0.2 (76.0)
Dizziness	0.9 ± 0.2 (71.4)	0.8 ± 0.2 (55.6)	0.5 ± 0.1 (53.8)	0.6 ± 0.2 (32.0)
Unsteady feeling	1.4 ± 0.3 (71.4)	1.1 ± 0.2 (66.7)	0.8 ± 0.2 (61.5)	0.9 ± 0.2 (60.0)
Faintness	1.4 ± 0.4 (64.3)	0.7 ± 0.2 (44.4)	0.5 ± 0.2 (38.5)	0.6 ± 0.1 (48.0)
Palpitations	2.2 ± 0.4 (78.6)	1.6 ± 0.2 (77.8)	1.6 ± 0.4 (76.9)	1.6 ± 0.2 (88.0)
Trembling/shaking	0.8 ± 0.3 (50.0)	0.6 ± 0.2 (40.7)	0.4 ± 0.1 (38.5)	0.2 ± 0.1 (20.0)
Sweating	0.6 ± 0.3 (28.6)	0.9 ± 0.2 (51.8)	0.6 ± 0.3 (38.5)	0.4 ± 0.2 (28.0)
Choking feeling	1.8 ± 0.4 (71.4)	0.7 ± 0.2[d](44.4)	1.4 ± 0.4 (53.8)	0.9 ± 0.2 (48.0)
Nausea	1.9 ± 0.4 (78.6)	1.1 ± 0.2 (55.6)	1.8 ± 0.3 (84.6)	1.0 ± 0.2 (48.0)
Abdominal distress	2.2 ± 0.4 (78.6)	2.2 ± 0.2 (92.6)	1.8 ± 0.4 (76.9)	2.2 ± 0.2 (92.0)
Feeling unreal or detached	0.6 ± 0.3 (35.7)	0.5 ± 0.1 (37.0)	0.2 ± 0.2 (15.4)	0.2 ± 0.1 (20.0)
Numbness/tingling	1.9 ± 0.4 (85.7)	1.7 ± 0.3 (70.4)	1.2 ± 0.3 (61.5)	1.3 ± 0.2 (64.0)
Hot flushes or cold chills	1.8 ± 0.4 (64.3)	1.8 ± 0.2 (85.2)	1.0 ± 0.4 (38.5)	1.2 ± 0.2 (60.0)
Chest pain or discomfort	1.9 ± 0.4 (71.4)	1.4 ± 0.2 (63.0)	1.6 ± 0.4 (61.5)	1.4 ± 0.2 (76.0)
Anxiety, fear, or apprehension	2.3 ± 0.3 (92.8)	1.2 ± 0.2[e](74.1)	0.9 ± 0.3 (53.8)	1.0 ± 0.2 (72.0)
Fear of dying	0.4 ± 0.2 (21.4)	0 ± 0	0 ± 0	0 ± 0
Fear of losing control	1.1 ± 0.2 (71.4)	0.4 ± 0.1[f](40.7)	0.2 ± 0.1 (23.1)	0.2 ± 0.1 (16.0)
Fear of going crazy	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Data are mean ± SEM scores on a scale of 0 to 4; data in parentheses are percentages of subjects reporting symptoms.

[a]2 mg.

[b]2 mg twice daily for 28 days.

[c–f]Unpaired t-test, two-tailed (post hoc analysis): [c]p = .010; [d]p = .025; [e]p = .004; [f]p = 0.011.

chronic treatment (19 vs. 16 s, p = .2167). There were no significant differences between ondansetron and placebo groups with respect to the duration of symptoms after either acute (136 vs. 156 s, p = .2263) or chronic (102 vs. 95 s, p = .6334) treatment.

Mean VAS anxiety scores are displayed in Table 2. Mean VAS anxiety scores at baseline and prior to CCK-4 administration, that are prior to and 60 min after study drug administration, respectively, were similar in both treatment groups after either acute or chronic exposure. There was no difference between mean VAS scores at baseline and mean pre-CCK-4 VAS scores. This allowed the use of mean pre-CCK-4 VAS values as the baseline in evaluating changes because of CCK-4 challenge tests. Subjects rated themselves as significantly less anxious at peak effect of CCK-4 after acute treatment with ondansetron, as compared to placebo (p = .0274). Likewise, mean change in the CCK-4-induced VAS score (Δ_{max}) from VAS score measured before CCK-4 administration significantly differed between the acute ondansetron treatment group and the acute placebo treatment group (p = .0298). The baseline value was a significant covariate (p = .0005). Once again, there was no difference between treatment groups after chronic administration. A Student's t-test of the VAS scores between CCK-4 challenge periods within each treatment group showed no significant differences, with the exception of the post-CCK-4 VAS score within the placebo group only (p = .0008).

Effects of Ondansetron on Cardiovascular Measures. Table 4 shows the influence of ondansetron on mean basal vital signs (systolic/diastolic blood pressures and heart rate) and on intravenous CCK-4-induced maximal vital sign mean changes from baseline (Δ_{max}). After acute treatment, there was no significant difference in mean basal and in Δ_{max} vital signs between ondansetron and placebo groups. After chronic treatment, there was a reduction, which did not reach statistical significance, in basal systolic blood pressure in subjects who received the ondansetron treatment, as compared to placebo (p = .0557); whereas, Δ_{max} for any vital signs did not significantly differ between groups. There was no significant change in vital signs over time, with the exception of the heart rate at the first CCK-4 challenge period (p = .0108). A repeated measures analysis of covariance of the changes in vital signs over time showed no significant differences between treatment groups with respect to systolic/diastolic blood pressures and heart rate at either CCK-4 challenge periods. A Student's t-test of the vital signs between CCK-4 challenge periods within each treatment group showed a significant

Table 4. Effect of Ondansetron on Intravenous CCK-4-Induced Vital Sign Changes

	Acute Treatment[a]			Chronic Treatment[b]		
Parameter	Placebo (n = 13)	Ondansetron (n = 27)	p-value	Placebo (n = 13)	Ondansetron (n = 25)	p-value
Sitting HR[c]						
Baseline	72.4 ± 3.4[d]	72.6 ± 2.7	.9774	73.6 ± 3.0	70.0 ± 2.6	.3855
Δ_{MAX}	28.3 ± 4.8	29.8 ± 2.7	.7791	28.7 ± 5.6	31.1 ± 1.6	.6494
Sitting SBP[c]						
Baseline	118.9 ± 3.6[d]	115.7 ± 2.3	.4451	126.3 ± 4.2	117.3 ± 2.5	.0557
Δ_{MAX}	17.0 ± 2.8	17.4 ± 2.4	.8739	15.2 ± 4.5	14.0 ± 2.0	.3062
Sitting DBP[c]						
Baseline	70.4 ± 1.7[d]	70.7 ± 1.4	.8669	72.4 ± 2.1	70.3 ± 1.1	.3398
Δ_{MAX}	8.5 ± 2.5	6.9 ± 1.2	.5269	9.2 ± 3.2	7.2 ± 1.5	.2849

Data are mean ± SEM.
[a]2 mg.
[b]2 mg twice daily for 28 days.
[c]HR, heart rate in bpm; SBP, systolic blood pressure in mmHg; DBP, diastolic blood pressure in mmHg.
[d]n = 14.
Δ_{MAX}, maximal change from baseline.

difference in the basal systolic blood pressure within the placebo group only (p = .0280).

Effects of Ondansetron on Neuroendocrine Measures. Mean basal plasma hormone levels and CCK-4-induced neuroendocrine mean changes from baseline after acute and chronic treatment with ondansetron and placebo are summarized in Table 5. Values below the limit of detection have been imputed using a value just below the limit of detection. The Shapiro–Wilk statistic test for normality indicated that the distribution of the neuroendocrine data violated the assumption of normality. A repeated measures analysis of covariance of the change in hormones over time showed no significant differences between the ondansetron and the placebo groups. The baseline value was a significant covariate with the exception of ACTH and PRL.

After acute treatment, there was a significant increase in the mean NPY plasma level at baseline in the ondansetron group, as compared to the placebo group

Table 5. Effect of Ondansetron on Intravenous CCK-4-Induced Neuroendocrine Changes

	Acute Treatment[a]			Chronic Treatment[b]		
Parameter	Placebo (n = 14)	Ondansetron (n = 27)	p-value	Placebo (n = 13)	Ondansetron (n = 25)	p-value
ACTH (pmol/l)						
Baseline	9.5 ± 2.0[c]	6.8 ± 0.8	.2146	8.6 ± 2.1	5.5 ± 0.5	.1786
Δ_{ACTH}	19.2 ± 3.1[c]	13.4 ± 2.7	.0911	13.8 ± 2.7	8.6 ± 1.4	.1320
CORT (nmol/l)						
Baseline	460.4 ± 51.5	447.3 ± 33.6	.8281	405.2 ± 40.7	355.2 ± 28.6	.3179
Δ_{CORT}	176.3 ± 25.1	128.8 ± 18.6	.0485	128.5 ± 35.4	128.9 ± 23.4	.4123
GH (μg/l)						
Baseline	0.34 ± 0.12	0.81 ± 0.27	.1185	0.42 ± 0.19	0.66 ± 0.37	.5802
Δ_{GH}	1.88 ± 0.71	0.21 ± 0.16	.0049	2.05 ± 1.40	0.09 ± 0.15	.0724
NPY (pg/ml)						
Baseline	17.2 ± 1.6	25.2 ± 1.9	.0024	17.9 ± 1.9	25.6 ± 2.2	.0103
Δ_{NPY}	13.6 ± 3.2	8.4 ± 3.0	.4136	14.4 ± 2.9	4.0 ± 1.8	.0097
PRL (μg/l)						
Baseline	8.6 ± 1.2	10.0 ± 1.1	.4436	7.8 ± 1.0	8.3 ± 0.6	.6705
Δ_{PRL}	5.9 ± 1.2	2.1 ± 0.9	.0323	5.4 ± 2.0	2.4 ± 0.6	.0645

Data are mean ± SEM.
[a]2 mg.
[b]2 mg twice daily for 28 days.
[c]n = 13.
ACTH, adrenocorticotropic hormone; CORT, cortisol; GH, growth hormone; NPY, neuropeptide Y; PRL, prolactin; Δ, maximal change from baseline.

($p = .0024$). Compared to placebo, there was a significant reduction in the mean maximal increase in cortisol ($p = .0485$), GH ($p = .0049$) and PRL ($p = .0323$) secretion from baseline (Δ_{max}) in the ondansetron group after intravenous CCK-4 administration. There was no differences in Δ_{max} ACTH and NPY between the acute ondansetron treatment group as compared to placebo after CCK-4 injection.

After chronic treatment, the mean basal NPY plasma level was still significantly higher in the ondansetron group, as compared to the placebo group ($p = .0103$). After CCK-4 injection, there was a significant reduction in Δ_{max} NPY in the ondansetron group ($p = .0097$). There was a decline in the Δ_{max} GH and PRL in the ondansetron group, as compared to the placebo group, but the difference failed to reach statistical significance. There was no difference between the ondansetron and the placebo groups with respect to the Δ_{max} ACTH and CORT.

The maximal rise in ACTH, PRL, and cortisol occurred 5, 10, and 15 minutes, respectively, after CCK-4 administration for both acute and chronic ondansetron- and placebo-treated subjects. GH reached peak concentrations 15 minutes after CCK-4 injection for acute and chronic ondansetron- and placebo-treated subjects. NPY profiles were described by smooth curves. The maximal rise in NPY occurred 10 minutes after CCK-4 administration in the acute placebo group; whereas, NPY peak plasma levels were reached 2 minutes after administration of CCK-4 in the chronic placebo group. The maximal rise in NPY occurred 2 minutes after CCK-4 administration for the acute and chronic ondansetron-treated subjects.

A Student's *t*-test of the mean ACTH and NPY plasma levels and CORT, PRL, and GH serum levels measured at baseline between CCK-4 challenge periods within each treatment group showed significant differences in the ondansetron group only. Compared to acute treatment, there was a significant decrease in mean basal ACTH ($p = .0482$) and CORT ($p = .0007$) levels after chronic administration of ondansetron. A reduction in the mean basal PRL serum level was observed between challenge periods but the difference failed to reach statistical significance ($p = .0517$). Mean basal neuroendocrine plasma or serum levels were not significantly different between challenge periods within the placebo group ($p > .35$).

Adverse Events

Regardless of relationship to treatment, 89% of subjects reported adverse events in the ondansetron group, as compared to 71% in the placebo group. Gastrointestinal symptoms, mostly constipation, diarrhea, and discomfort, were more frequently reported in the ondansetron group (67%), as compared to the placebo group (36%). In contrast, such neurological symptoms as headaches, tremors, dizziness, confusion, and paresthesia, were more frequent in the placebo group (50%) than in the ondansetron group (37%). Reports of cardiovascular-, psychiatric-, and musculoskeletal-related events were of similar incidence. The intensity of all reported adverse events were mild to moderate except for the allergic-type reaction, which was severe. The clinical symptoms of this reaction consisted of the swelling of feet with development of painful red nodules under the toes. Symptoms resolved upon discontinuation of study medication (ondansetron) without further intervention.

DISCUSSION

From a behavioral point of view, our results show that acute treatment with ondansetron significantly decreased the sum intensity of symptoms and the anxiety score at peak effect of CCK-4 on the VAS. This outcome was further supported by a reduction, although not significant, in the number of symptoms experienced by subjects of the ondansetron group, as compared to those of the placebo group. The four symptoms induced by CCK-4 that have been identified by the post hoc analysis; namely, dyspnea, choking feeling, anxiety/fear/apprehension, and fear of losing control, deserve special attention. These particular findings indicate that ondansetron attenuates not only somatic symptoms but also anxiety and cognitive symptoms induced by CCK-4. Ondansetron also decreased CCK-4-induced dyspnea, a symptom central to panic attacks (Klein 1993). Moreover, our findings show a significant reduction in the cortisol, GH, and PRL secretion in response to CCK-4 injection after acute treatment with ondansetron, as compared to placebo. These measures represent biological changes that coincide with the panicogenic effect of CCK-4. Hence, behavioral and biological results are strong evidence for an action of acute treatment with ondansetron on CCK-4 and support the role of the 5-HT_3 network in the mediation of the panicogenic effects of CCK-4.

The unexpected significant increase in pre-CCK-4 (basal) NPY plasma levels observed after both acute and chronic treatment with ondansetron is of interest. Similar effects have been observed in the frontal cortex and hypothalamus of rats after chronic oral administration of imipramine, a 5-HT and noradrenaline (through its metabolite) reuptake inhibitor (Heilig et al. 1988a). Because basal NPY plasma levels were measured just prior to intravenous CCK-4 administration, a contribution of anticipatory anxiety prior to receiving CCK-4 cannot be excluded. It has been shown that low concentrations of the C-terminal fragment $NPY_{13–36}$ increase, possibly through Y_2 receptors, the exploratory behavior of rats, which is interpreted as a decline in the animal

anxiety levels (Heilig et al. 1988b). Based on these experimental findings, we may suggest that ondansetron might activate the NPY anxiolytic system, leading to a rise in basal NPY plasma levels and, consequently, reducing the anxiety state in humans. The exact mechanism by which ondansetron increased NPY plasma levels cannot be elucidated by this study but may involve a previously postulated nonsynaptic interaction with NPY neurons (Guy et al. 1988). Whether the increase of NPY could suggest the existence of a presynaptic inhibitory regulation of NPY release by 5-HT is unknown. However, we now know that serotonin release in the brain cortex is inhibited by NPY and is increased by CCK (Schlicker et al. 1991; Kendrick et al. 1991). Hence, we may suggest the existence of a "cross-talk" between the 5-HT$_3$, CCK, and NPY systems that might have a role in the neurobiology of panic attacks.

Interestingly, our results show no difference in the iPSS scores, and the cortisol, GH, and PRL changes between the ondansetron and placebo groups after chronic exposure. This lack of effect contrasts with the significant difference observed in these parameters after single-dose administration of ondansetron as compared to placebo. Our results show also that the iPSS and nPSS values between challenge periods within each treatment group were significantly different. An increase in the iPSS and nPSS scores in the ondansetron group reaching those of the placebo group would likely reflect a neuroadaptation phenomenon occurring after chronic ondansetron exposure. However, our results show opposite trends. The reduction in the iPSS and nPSS values was further accentuated after the second relative to the first challenge period within the ondansetron group (iPSS $p = .0192$, nPSS $p = .0211$). Within the placebo group, values for both PSS parameters and the post-CCK-4 VAS score significantly decreased after chronic administration, as compared to acute administration reaching those observed in the ondansetron group (iPSS $p = .0004$, nPSS $p = .0042$, post-CCK-4 VAS $p = .0008$). This profile is more compatible with a placebo response theory, which is further substantiated by the absence of a difference in the neuroendocrine parameters between challenge periods within the placebo group.

A placebo response may not be the only explanation for this lack of effect. For example, an habituation effect cannot be excluded. Results observed in the placebo group after the first CCK-4 injection are comparable with the previously described CCK-4-induced behavioral and neuroendocrine changes in healthy subjects (Bradwejn et al. 1994b, 1995; Koszycki et al. 1996). A significant decline in iPSS and nPSS during the second, relative to the first, challenge injection has also been reported (Bradwejn et al 1994b). Our results show a similar decline between challenge periods within the placebo group. The 40% drop in the iPSS score noticed in the placebo group after the second, relative to the first, challenge period may have heavily influenced the outcome of the study. Therefore, summation of both a placebo response and an habituation effect along with other unknown confounding factors may have contributed to this lack of effect. Nevertheless, results from the acute treatment with ondansetron taken together with significant NPY plasma level changes and the significant period effect observed within the ondansetron group for the behavioral changes as well as for the ACTH ($p = .0482$) and cortisol ($p = .0007$) levels indicate that some of the acute action of ondansetron might be maintained over time.

There was no difference in CCK-4-induced ACTH secretion between groups after either acute or chronic treatment. With respect to cortisol, the time course of release remains to be established because of a continued increase beyond the collection time. Hence, no conclusive observations can be drawn from the cortisol profile. Circulating cortisol levels began rising after ACTH levels reached peak values. This observation may suggest that circulating cortisol followed, at least in part, the ACTH plasma level profile. The significant decline in ACTH ($p = .0482$) and cortisol ($p = .0007$) baseline values between challenge periods within the ondansetron group may suggest a decrease of anxiety in anticipation of the CCK-4 injection. These results can be contrasted with those observed in the placebo group that show no significant change in ACTH and cortisol baseline values between both challenge periods.

The maximal change in NPY secretion from baseline (Δ_{max}) provides some indication of a significant treatment difference in favor of ondansetron after chronic treatment. Findings show a significant reduction in the NPY secretion in response to CCK-4 injection after chronic treatment with ondansetron, as compared to placebo ($p = .0097$). The significant decline in the CCK-4-induced NPY release in the ondansetron group taken together with the significant increase in the pre-CCK-4 NPY release detected in the ondansetron group, as compared to the placebo group may be viewed as paradoxical: ondansetron administered prior to CCK-4 challenge procedure significantly increases basal NPY plasma levels but antagonizes CCK-4-induced NPY release. The underlying mechanisms for such effects are unknown. A series of potential interactions seem to occur in a very complex system. Therefore, many more observations are necessary to understand these findings.

Regulation of adenohypophyseal hormone secretion involves numerous transmitters that play controversial roles as both stimulatory and inhibitory effects have been reported. Experimental studies indicated the existence of complex interactions between CCK and dopaminergic D_2 receptors apparently controlled by the nature of the D_1 activation state (Li et al. 1994). In addition, it has been shown that such 5-HT$_3$ antagonist agents as

ICS 205,930 and ondansetron prevented the increase of drug-induced dopaminergic neuronal activities (Christoffersen et al. 1988; Carboni et al. 1989). Based on these findings, GH and PRL responses observed after acute treatment with ondansetron may have resulted from an interaction between CCK, 5-HT_3, and dopamine networks. Moreover, part of changes in GH may be secondary to NPY changes. NPY has been shown to stimulate the basal secretion of somatostatin that inhibits the GH release (Milgram et al. 1990). Therefore, the significant increase in the NPY plasma levels produced by ondansetron prior to CCK-4 injection could theoretically have promoted the secretion of somatostatin and, consequently, inhibited GH secretion. However, differences in PRL and GH secretion between groups failed to show significance after chronic exposure of either ondansetron or placebo despite the significant increase in basal NPY plasma levels in the ondansetron group. Further evidence on the existence of these hypothetical mechanisms is required to assess the mechanistic involved in these findings.

In conclusion, results from this study suggest the following. First, decrease in behavioral and neuroendocrine responses provide strong evidence for an action of acute treatment with ondansetron on CCK-4 and support a role for receptors in CCK-4-induced panic attacks in healthy subjects. Second, acute and chronic treatment with ondansetron affects basal NPY and CCK-4-induced changes in NPY. Hence, we suggest that 5-HT_3 may serve as an important regulator of basal and CCK-4-stimulated NPY release. Finally, our results suggest a role for 5-HT_3 receptors in the neurobiology of anxiety and panic attacks through its interaction with CCK and NPY systems. Chronic effect of ondansetron on CCK-4-induced panic symptoms still needs exploration.

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