Cerebral Blood Flow Changes After Treatment of Social Phobia with the Neurokinin-1 Antagonist GR205171, Citalopram, or Placebo

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Background: *Evidence is accumulating that pharmacological blockade of the substance P preferring neurokinin-1 (NK1) receptor reduces anxiety. This study compared the effects of an NK1 receptor antagonist, citalopram, and placebo on brain activity and anxiety symptoms in social phobia.*

Methods: *Thirty-six patients diagnosed with social phobia were treated for 6 weeks with the NK1 antagonist GR205171 (5 mg), citalopram (40 mg), or matching placebo under randomized double-blind conditions. GR205171 was administered for 4 weeks preceded by 2 weeks of placebo. Before and after treatment, regional cerebral blood flow (rCBF) during a stressful public speaking task was assessed using oxygen-15 positron emission tomography. Response rate was determined by the Clinical Global Impression Improvement Scale.*

Results: *Patients improved to a larger extent with the NK1 antagonist (41.7% responders) and citalopram (50% responders), compared with placebo (8.3% responders). Within- and between-group comparisons showed that symptom improvement was paralleled by a significantly reduced rCBF response to public speaking in the rhinal cortex, amygdala, and parahippocampal-hippocampal regions. The rCBF pattern was corroborated in follow-up analyses of responders and subjects showing large state anxiety reduction.*

Conclusions: *Short-term administration of GR205171 and citalopram alleviated social anxiety. Neurokinin-1 antagonists may act like serotonin reuptake inhibitors by attenuating neural activity in a medial temporal lobe network.*

Key Words: Brain, NK1 antagonist, rCBF, social anxiety, SSRI, substance P

Peptide neurotransmitters like substance P (SP) have recently attracted considerable interest in the field of anxiety (Griebel 1999). For example, it has been demonstrated that pharmacological blockade of the SP preferring neurokinin-1 (NK1) receptor yields significant antianxiety and antidepressant effects in patients suffering from major depression (Kramer et al 1998, 2004). In animals, NK1 receptor antagonists have an anxiolytic profile in various models of anxiety such as the rat elevated plus maze, social interaction test, and tests of transient maternal separation (File 2000; Kramer et al 1998; Varty et al 2002). Genetic disruption of the NK1 receptor in mice also reduces anxiety and stress-related behaviors (Santarelli et al 2001). Intracerebral injections of SP agonists provoke anxiety in animal trials (Aguiar and Brandao 1996; Kramer at al 1998; Krase et al 1994), whereas administration of SP antagonists have anxiolytic effects (File 1997; Teixeira et al 1996). Moreover, in rats, central SP is released during aversive or noxious conditions (Brodin et al 1994; Rupniak and Kramer 1999). Thus, it has been proposed that anxiety is associated with increased levels of central SP (Hasenohrl et al 2000).

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Kramer et al (1998) reported that the anxiolytic and antidepressant effects of an NK1 receptor antagonist were comparable to those produced by a selective serotonin reuptake inhibitor (SSRI). Selective serotonin reuptake inhibitors have rapidly become the pharmacological treatment of choice for major depressive disorder and also for various anxiety conditions (Gorman and Kent 1999). For instance, several placebo-controlled studies have shown that SSRIs are effective in social phobia, also known as social anxiety disorder (Van Ameringen et al 1999). This is a highly common (Furmark 2002), disabling (Wittchen et al 2000), and enduring (Yonkers et al 2001) condition, characterized by a fear of scrutiny or humiliation in social performance and interactional situations. Even though current pharmacological treatments of social phobia are helpful, they often produce only partial improvement (Ameringen et al 2000). A better understanding of the neurofunctional changes that underlie the beneficial effects on mood and anxiety could facilitate the development of new anxiolytic agents. The drug-brain interaction can be studied by functional neuroimaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI).

Recently, we reported that citalopram and cognitive-behavioral therapy significantly reduced public speaking anxiety in patients with social phobia and that symptom improvement was associated with reduced regional cerebral blood flow (rCBF) mainly in the medial temporal lobe (MTL), including the amygdala, hippocampus, and the surrounding rhinal and parahippocampal cortices (Furmark et al 2002). Congruently, it has been observed that citalopram reduces resting-state neuronal activity in the left temporal cortex in patients with social phobia (van der Linden et al 2000). Several neuroimaging studies also point to a pivotal role for the MTL in the modulation of social anxiety. For instance, we showed that rCBF in the amygdaloid complex increased significantly more in patients with social phobia than

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in nonanxious control subjects during anxiety induced by a public speaking task (Tillfors et al 2001). Further, increased neural activity was observed in the left amygdaloid-hippocampal region and inferior temporal cortex during anticipation of the speaking task (Tillfors et al 2002). Activation of the MTL during speech anticipatory social anxiety was recently confirmed in a fMRI study (Lorberbaum et al 2004). Moreover, fMRI studies have reported increased amygdala and hippocampal activation during aversive conditioning (Schneider et al 1999) and enhanced amygdalar reactivity to social cues such as neutral faces in patients with social phobia relative to control subjects (Birbaumer et al 1998; Veit et al 2002). Stein et al (2002) demonstrated that the MTL, including the amygdala, was more reactive to angry and contemptuous facial expressions than to happy or neutral expressions in patients with generalized social phobia compared with healthy control subjects. These neuroimaging results are, in turn, consistent with a wealth of data from animals and humans indicating that the MTL, especially the amygdala and hippocampus, is crucially involved in the regulation of anxiety-related behaviors (Davidson et al 2000; Davis and Whalen 2001; Gray and McNaughton 1996; LeDoux 1996, 2000).

Neurobiological data imply that the MTL is a potential target for SSRIs in the treatment of anxiety disorders. Neurokinin-1 antagonists may also act at the MTL level, since NK1 receptors are highly expressed in the amygdala and hippocampus (McLean et al 1991). In mammals, psychological stress such as maternal separation cause a release of SP in the amygdala (Kramer et al 1998), whereas anxiolytic and antidepressant drugs reduce central levels of SP, e.g., in the amygdala and hippocampus (Hasenohrl et al 2000; Shirayama et al 1996). However, it remains to be elucidated whether NK1 receptor antagonists are effective in the treatment of anxiety disorders in humans and whether these drugs act on unique or common neural networks compared with the SSRIs.

GR205171 is a selective NK1 receptor antagonist developed by GlaxoSmithKline (Gardner et al 1996). The GR205171 compound has shown good penetration to the brain and high affinity to NK1 receptors in rats (Rupniak et al 2003). Evaluation of GR205171 binding kinetic in monkeys, using PET, has confirmed the possibility to achieve high levels (>90%) of central NK1 receptor occupancy (Zamuner et al 2002). The aim of the present experimental study was to evaluate the effects of short-term treatment with GR205171, compared with a SSRI, on brain activity (rCBF) in patients diagnosed with social phobia. Patients received daily doses of GR205171, citalopram, or placebo under randomized and double-blind conditions during a 6-week period. Before and after this period, patients were exposed to a stressful public speaking task during which alterations in rCBF were studied by means of PET and oxygen-15 (^{15}O) labeled water. We hypothesized that anxiety reduction, following active drug administration, would be associated with decreased neural activity in the MTL region.

Methods and Materials

Screening

Participants were recruited through newspaper advertising. Initial screening included a brief telephone interview and social anxiety questionnaires returned by mail. Structured clinical diagnostic interviews (Structured Clinical Interview for DSM-IV [SCID]) (First et al 1998) were thereafter administered by a clinical psychologist and a public speaking behavioral test was performed. In addition, a psychiatrist (K.W.) administered the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al 1998) to exclude other serious psychiatric disorders. Finally, medical examinations were performed.

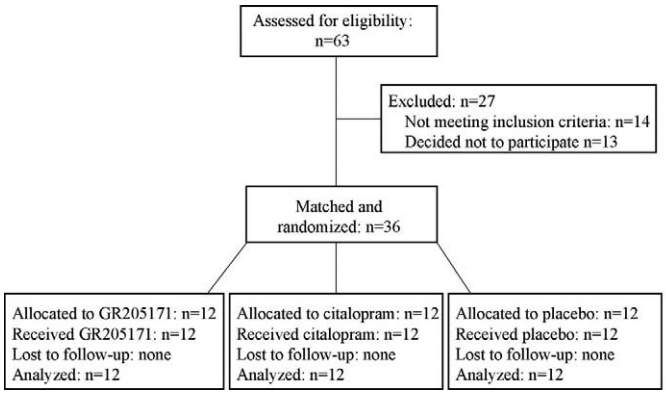

Figure 1. Flow diagram of subject eligibility from screening to statistical analysis.

Main criteria for exclusion were treatment of social anxiety in the past 6 months, current serious or dominant psychiatric disorder other than social phobia (e.g., psychosis, major depressive or bipolar disorder), neurological disorders, somatic disease, chronic use of prescribed medication, abuse of alcohol/narcotics, pregnancy, menopause, left handedness, previous PET examination, and positive family history of cancer.

Approvals were obtained from the Uppsala University Medical Faculty Ethical Review Board, the Uppsala University Isotope Committee, and the Swedish Medical Products Agency. A written informed consent was obtained from all participants.

Study Population

Thirty-six patients (17 men and 19 women; mean age ± SD: 31.6 ± 7.7 years; range 19–48) were included. All participants met the DSM-IV (American Psychiatric Association 1994) criteria for social phobia and exhibited marked public speaking anxiety. Nineteen (52.8%) patients were diagnosed with generalized social phobia and eight qualified for a comorbid diagnosis (three with specific phobia, four with generalized anxiety disorder, and one with both disorders).

Prior to the first PET investigation, patients were matched for severity in triplets based on the Social Phobia Screening Questionnaire (Furmark et al 1999) and also, as far as practically possible, for sex and age. Patients were thereafter randomly allocated to one of three groups: NK1 antagonist, SSRI, or placebo (n = 12 per group). Mean age [$F(2,33)$ = .87, ns], sex (χ^2 = .89, ns), and subtype (χ^2 = 1.56, ns) distributions did not differ significantly across study groups. Patients with comorbid anxiety disorders were distributed equally across the NK1 and SSRI groups (four each). The progress of eligible subjects from screening to analysis is described in Figure 1.

Treatment Procedure

The study was double blind. GlaxoSmithKline (Verona, Italy) supplied the study drugs for a 6-week treatment period. The NK1 group received a daily oral dose of 5 mg GR205171, which started after 14 days of placebo because of limited available safety data on repeated dosing. GR205171 was taken as 4 mL solution made up to 100 mL in orange juice. The SSRI group was treated with 40 mg citalopram (one tablet), starting with 20 mg (half tablet) during the first week. To maintain study blindness, the NK1 and SSRI groups received tablets and solution as dummy

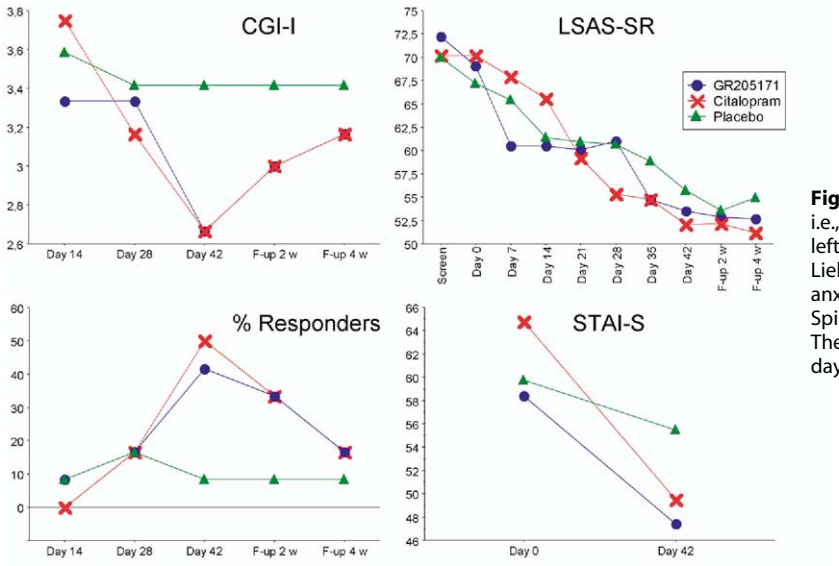

Figure 2. Change scores for primary clinical outcome measures, i.e., the Clinical Global Impression improvement subscale (top left), percentage of responders over time (bottom left), the Liebowitz Social Anxiety Scale (LSAS-SR, top right) and state anxiety during public speaking challenge measured by the Spielberger State-Trait Anxiety Inventory (STAI-S, bottom right). The treatment started postscreening on day 0, terminated on day 42, and was followed up 2 and 4 weeks later (F-up 2/4 w).

treatments, respectively. The placebo group received dummy treatments matching GR205171 solution and citalopram tablets. All subjects started with half a tablet the first week.

In all groups, the first dose was given immediately after the first PET examination and the final dose was administrated 2 to 4 hours before the second PET assessment on day 42. Subjects did not receive any other form of treatment than the one allocated in the study and no systematic exposure instructions were given.

Patients visited the clinic weekly for assessments of compliance and side effects and to receive new supplies of medication. Vital signs (heart rate, blood pressure) were checked, laboratory safety tests (hematology, biochemistry, and urine analysis) were performed, and self-report questionnaires were administered. Pregnancy tests and electrocardiography were performed twice. Screenings for alcohol and nonallowed drugs were performed at a randomly selected visit.

Follow-up assessments were performed 2 and 4 weeks after the treatment period. Checking of vital signs, safety tests, and questionnaire administration were then repeated. After completion, patients were offered further psychiatric consultation and additional therapy with market drugs.

PET Assessments

Investigations were performed using a 32-ring ECAT EXACT HR+ camera (Siemens/CTI, Knoxville, Tennessee). The camera enables acquisition of 63 contiguous planes of data with a distance of 2.46 mm, resulting in a total axial field of view of 155 mm.

Subjects were positioned in the scanner with the head gently fixated, and a venous catheter for tracer injections was inserted. Patients were instructed to prepare a 2.5-minute speech about a vacation or travel experience about 20 minutes before the initial emission scan. A 10-minute transmission scan was performed using three retractable germanium (^{68}Ge) rotating line sources. The ^{15}O-water tracer, approximately 10 MBq/kg body weight, was thereafter injected intravenously. The emission scan started automatically in three-dimensional (3-D) mode when the bolus reached the brain (50,000 counts/second) and consisted of three 30-second frames.

Immediately following tracer injection, patients were asked to start speaking and continue until they received instructions to stop. The speech was performed in the presence of a silently observing audience of six to eight persons. Patients were instructed to observe the audience. The speech was recorded from close distance with a portable video camera to increase observational anxiety and document verbal performance. Heart rate was recorded simultaneously. Directly after the speech, state anxiety scales (see below) were administered to estimate retrospectively how anxious patients felt during scans.

Emission scans were reconstructed with a filter back projection using an 8-mm Hanning filter, resulting in a spatial resolution of about 5 mm in the field of view. The matrix included 128 × 128 pixels. Data were corrected for photon attenuation, decay, scattered radiation, and random coincidences. After reconstruction, a summation image of the three frames was made to obtain a better statistical reference for realignment and subsequent analyses.

Participants fasted 3 hours and refrained from tobacco, alcohol, and caffeine for 12 hours before PET investigations. The PET procedure was the same after treatment but with altered speech topic.

Clinical Outcome Measures

Primary Outcome Measures. Response rate was determined by the Clinical Global Impression improvement item (CGI-I) (Zaider et al 2003) administered by a psychiatrist (K.W.) at weeks 2, 4, and 6 and at follow-ups. Patients having a score of 1 or 2 (i.e., very much or much improved) on the CGI-I on day 42 were classified as responders, whereas those having scores of 3 (minimally improved) or higher were considered as nonresponders.

Changes in state anxiety from pretreatment to posttreatment were evaluated using the Spielberger State-Trait Anxiety Inventory (STAI-S) (Spielberger et al 1970), administered after each public speaking challenge. Additional changes in the social phobia symptom profile over the treatment course were evaluated by the self-report version of the Liebowitz Social Anxiety Scale (LSAS-SR) (Baker et al 2002).

Secondary Outcome Measures. The CGI severity subscale (CGI-S) was administered in addition to the global improvement item (CGI-I). Further, patients completed a battery of questionnaires at screening and day 42: Social Phobia Screening Questionnaire (SPSQ) (Furmark et al 1999), the Social Phobia Scale (SPS) and Social Interaction Anxiety Scale (SIAS) (Mattick and Clarke 1998), Global Assessment of Functioning (GAF) self-

Table 1. Temporal Lobe Regions Showing Decreased Within-Group Activation After Treatment of Social Phobia

Group/Brain Region[a]	Coordinate[b] x	y	z	*z*-Score	Voxel *p* Value[c]	Cluster *p* Value[c]
GR205171 ($n = 12$)						
Left Inferior Temporal Cortex, BA36	−20	−6	−33	4.18	.009	.047
BA28	−20	2	−30	3.09		
Amygdala	−18	−3	−22	2.31		
Citalopram ($n = 12$)						
Left Inferior Temporal Cortex, BA 20	−24	−8	−38	3.84	.029	.010
BA36	−24	−6	−33	3.51		
BA28	−20	1	−29	2.31		
BA35	−20	−9	−26	2.08		
Amygdala	−26	−3	−22	2.30		
Left Parahippocampal Cortex, BA 27	−14	−31	−4	4.06	.014	ns
Right Superior Temporal Cortex, BA38	36	−1	−10	3.81	.031	.004
BA36	28	−7	−33	3.28		
BA21	42	−2	−10	3.10		
BA13	40	5	−10	2.96		
BA38	30	16	−34	2.30		
BA20	30	−11	−30	2.09		
BA28	28	−11	−30	2.00		
Amygdala	28	−8	−13	2.22		
Placebo ($n = 12$)					ns	ns
Responders ($n = 12$)						
Left Inferior Temporal Cortex, BA36[d]	−18	−1	−29	2.63	ns	.040
BA28	−16	−1	−25	2.47		
BA35	−22	−5	−25	2.03		
BA34	−18	3	−22	2.00		
Right Inferior Temporal Cortex, BA36[e]	30	−3	−27	3.52	.075	.022
BA20	40	−21	−28	2.29		
Amygdala	28	−3	−22	2.96		

BA, Brodmann area; NK1, neurokinin-1.

[a]Location of maximum voxel value (presented first) and spatial extention of significant clusters are listed. GR205171 is a NK1-antagonist. Responders = scores 1–2 on the Clinical Global Impression improvement item.

[b]Coordinates in millimeters correspond to the stereotactic atlas of Talairach and Tournoux (1988).

[c]Corrected for multiple comparisons.

[d]Left amygdala implicated at lower threshold (−22 −1 −22; *z*-score = 1.80).

[e]Right hippocampus implicated at lower threshold (28 −9 −21; *z*-score = 1.78).

report scale (Bodlund et al 1994), Personal Report on Confidence as a Speaker (PRCS) (Paul 1966), and Sheehan Disability Inventory (SDI) (Leon et al 1992). Heart rate (HR), calculated from the interbeat interval and expressed in beats per minute, was recorded during all public speaking tasks by means of the PSYLAB6 integrated system for psychophysiology (Contact Precision Instruments, London, United Kingdom). Subjects also rated levels of fear and distress, associated with the speaking tasks, on 0 to 100 (minimum to maximum) visual analogue scales (Furmark et al 2002). These secondary measures were included to obtain a more complete clinical picture and for long-term research purposes.

Statistical Analyses

Positron Emission Tomography Data Analyses. Positron emission tomography images were realigned to correct for different positions between scans and normalized to the Montreal Neurological Institute's (MNI) stereotactic template (ICBM 152), using the Statistical Parametric Mapping (SPM99) software (Wellcome Department of Cognitive Neurology, London, United Kingdom). Images were then smoothed using a 12-mm Gaussian kernel. Positron emission tomography data were statistically evaluated using within-group and between-group comparisons defined in SPM99 with rCBF data fitted to the general linear model (Friston et al 1995). Between-group differences were evaluated by group × time interactions in the form of double subtractions, such as $(NK1_{post}\text{-}\ NK1_{pre}) - (Placebo_{post}\text{-}\ Placebo_{pre})$. Differences in global blood flow were corrected for using the proportional scaling method within SPM99. Contrasts generated *t*-maps, subsequently converted to *z*-scores, for interpretation. Brain locations are described as xyz coordinates in the Talairach space, obtained by mathematical transformation of the MNI coordinates in SPM99 (www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html). Anatomical localization was supported by searches in the Talairach atlas (Talairach and Tournoux 1988) and the Talairach Daemon (Lancaster et al 2000).

In line with our a priori hypothesis, primary analyses were focused on the medial temporal lobe. A circumscribed search volume for the right and left MTL was created by defining a 26 × 46 × 46 mm box containing 6877 voxels (1 voxel = 2 × 2 × 2 mm) in each hemisphere at the level of the inferior hippocampus. Treatment effects on rCBF were evaluated at the voxel level by examining statistically significant changes ($p < .05$) corrected for multiple comparisons in the defined volume. The spatial extent of voxels exceeding the significance threshold was also examined when motivated by significant cluster *p*-values (corrected) in the volume of interest. In addition, exploratory whole-brain analyses were performed

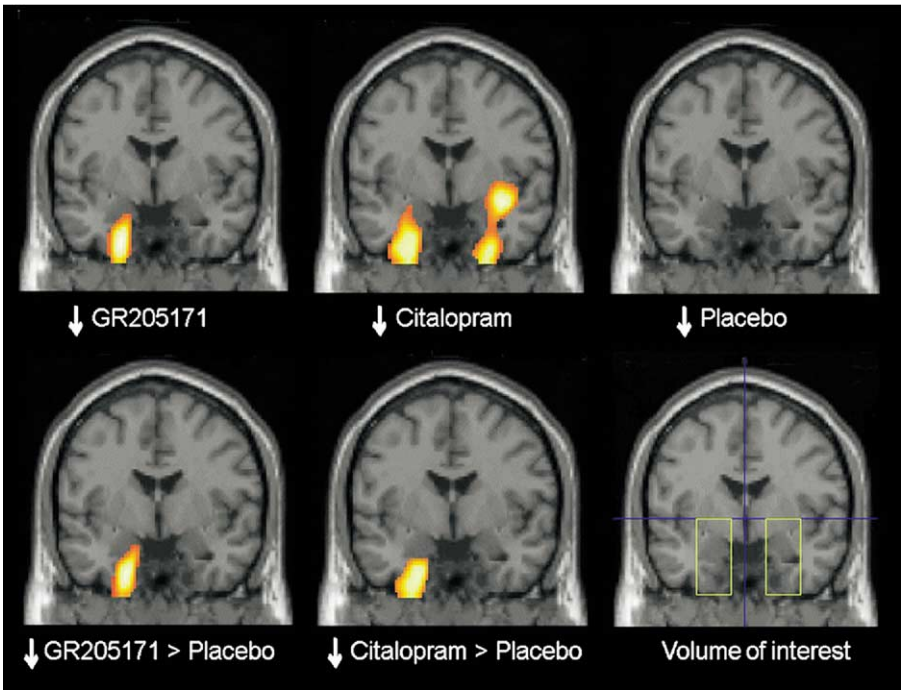

Figure 3. Coronal PET images of patients with social phobia showing clusters of significantly reduced rCBF in the medial temporal lobe during public speaking, after as compared with before treatment, within the NK1 antagonist GR205171 (top left), citalopram (top central), and placebo (top right panel) groups. All groups included 12 subjects each. Between-group comparisons revealed a significantly larger reduction of rCBF in subjects treated with GR205171 (n = 12; bottom left) and citalopram (n = 12; bottom middle) compared with placebo (n = 12). Bottom right panel illustrates the volume of interest used for all hypothesis-driven analyses of rCBF changes in the left and right medial temporal lobe. PET, positron emission tomography; rCBF, regional cerebral blood flow.

evaluating activity changes exceeding $p < .05$, corrected for multiple comparisons.

Clinical Outcome. Data were scanned for violations of normality and heterogeneity of variance and between-group differences at pretreatment were tested by analysis of variance (ANOVA). The distribution of responders/nonresponders, according to the CGI-I on day 42, was evaluated using exact single-cell tests (Bergman and El-Khouri 1987). Planned t tests (paired, two-tailed) were used to detect within-group changes from pretreatment to posttreatment. Between-group differences were tested by pairwise comparisons of the adjusted mean values following analyses of covariance (ANCOVA) with posttreatment score as dependent variable and pretreatment score as covariate in the statistical model. Repeated measurement ANOVA was used to evaluate the LSAS-SR. Analyses were performed using StatView 5.0.1 (SAS Institute Inc., Cary, North Carolina) and Statistica 6.0 (StatSoft Inc, Tulsa, Oklahoma). The alpha-level used was $p < .05$ in all tests.

Verbal Performance. Verbal performance was evaluated by comparing the number of spoken syllables during the first 10 seconds of each videotaped speech, using a repeated measurement ANOVA.

Results

Pretreatment Evaluation

There were no significant differences between groups before treatment on any primary ($.027 < F < 1.38$; $.27 < p < .93$) or secondary ($.22 < F < 3.20$; $.054 < p < .80$) clinical outcome measure.

Primary Clinical Outcome Measures

Response Rate. On day 42 (end of treatment), the numbers of CGI responders were 5 (41.7%) in the NK1 group, 6 (50%) in the citalopram group, and 1 (8.3%) in the placebo group (Figure 2). Eleven nonresponders (NK1/citalopram/placebo = 4/4/3) were "minimally improved" (CGI-I = 3), whereas 13 (NK1/citalopram/placebo = 3/2/8) were categorized as "no change" (CGI-I = 4). Exact single-cell tests showed evidence of a statistically significant association between CGI responders and group ($p = .026$). The two drug groups deteriorated after treatment withdrawal on day 42, whereas placebo subjects did not change.

State Anxiety. Both the NK1 [$t(11) = 3.87$, $p = .0026$] and citalopram [$t(11) = 7.26$, $p < .0001$] groups improved significantly on the STAI-S from pretreatment to posttreatment, whereas the placebo group did not [$t(11) = 1.53$, ns]. A significant effect of group was noted in the ANCOVA of posttreatment scores [$F(2,32) = 4.13$, $p = .025$] and pairwise comparisons showed that both the NK1 ($p = .031$) and citalopram ($p = .013$) groups were significantly more improved than placebo on the STAI-S (Figure 2). Speech ratings were always higher than ratings during a preceding control assessment ($p < .0001$).

Liebowitz Social Anxiety Scale. All groups improved significantly on the LSAS-SR ($2.94 < t < 3.97$, $df = 11$, $.0022 < p < .014$) from screening to day 42 (Figure 2). Repeated measure ANOVAs of the LSAS-SR scores revealed a significant main effect of time [$F(2,33) = 17.0$, $p < .0001$] but no significant effect of group or time × group interaction.

Regional Cerebral Blood Flow

Medial Temporal Lobe Analyses. Within the NK1 and citalopram groups, the rCBF response was significantly lower after treatment in the perirhinal, entorhinal, and parahippocampal cortices, as well as the amygdala. This pattern was bilateral in citalopram subjects but localized mainly to the left

Table 2. Temporal Lobe Regions Showing Decreased Between-Group Activation After Treatment of Social Phobia

Comparison/Brain Region[a]	Coordinate[b] x	y	z	*z*-Score	Voxel *p* Value[c]	Cluster *p* Value[c]
GR205171 vs. Placebo (n = 12/12)						
Left Inferior Temporal Cortex, BA36[e]	−18	−7	−32	4.42	.004	.036
BA28	−20	−11	−30	3.72		
BA35	−18	−11	−25	3.09		
BA34	−16	−7	−22	2.73		
BA38	−22	6	−32	2.37		
Amygdala	−18	−5	−22	2.46		
Citalopram vs. Placebo (n = 12/12)						
Left Inferior Temporal Cortex, BA36[d]	−20	−7	−32	3.84	.028	.018
BA28	−20	−11	−31	3.46		
BA35	−22	−11	−25	2.80		
Hippocampus	−24	−11	−21	2.32		
Responders vs. No change (n = 12/13)						
Right Parahippocampal Cortex, BA28	28	−1	−25	3.53	.072	.034
BA38	26	5	−25	2.84		
BA28	18	5	−25	2.79		
BA36	18	−1	−27	2.01		
Amygdala	28	−3	−22	3.41		
Hippocampus	30	−24	−9	2.11		

BA, Brodmann area; NK1, neurokinin-1.

[a]Location of maximum voxel value (presented first) and spatial extention of significant clusters are listed. GR205171 is a NK1-antagonist. Responders = scores 1–2 and No change = 4 on the Clinical Global Impression improvement item.

[b]Coordinates in millimeters correspond to the stereotactic atlas of Talairach and Tournoux (1988).

[c]Corrected for multiple comparisons.

[d]Left amygdala implicated at lower threshold (−24 −5 −22; *z*-score = 1.81).

[e]Left hippocampus implicated at lower threshold (−24 −11 −21; *z*-score = 1.75).

hemisphere in the NK1 group. No significant changes were observed in the placebo group (Table 1, Figure 3). Between-group comparisons confirmed that rCBF in the MTL region was significantly more reduced after drug treatment compared with placebo (Table 2, Figure 3). Decreases of rCBF in the hippocampus proper were also noted in these between-group comparisons. The NK1 and citalopram groups did not differ significantly.

Follow-up analyses revealed that responders, irrespective of treatment modality, exhibited significantly lower rCBF after treatment bilaterally in the rhinal and parahippocampal cortices, as well as the amygdala region. A between-group comparison showed that rCBF in the previously noted MTL domain was more reduced in responders (CGI-I ≤ 2) than in patients that did not change (CGI-I = 4), but this pattern was significant only in the right hemisphere (Table 2, Figure 4). In the active drug groups, rCBF alterations were further characterized by comparing subjects that differed in state anxiety reduction. Subjects were ranked within each of the state anxiety measures (STAI-S, HR, Fear, and Distress) using change scores from pretreatment to posttreatment. The four rankings were summed and a median split of the summed rank was used to define subgroups showing either a large (n = 6) or small (n = 6) anxiety reduction. Both in NK1 and citalopram subjects, the rCBF decrease in the MTL region was significant only in the subgroups exhibiting large anxiety reduction (Table 3, Figure 4).

Whole Brain Analyses. In the NK1 group, rCBF increased significantly in a cluster located in the left occipital cortex (Brodmann area [BA] 17) (-22 -87 -1; *z*-score 4.08, p = .008). Citalopram subjects exhibited a significant decrease of rCBF in a cluster in the posterior cingulate cortex (BA 31) (-18 -31 38; *z*-score 4.11, p = .032). In placebo subjects, rCBF increased significantly in the left cerebellum (-2 -76 -13; *z*-score 4.64; p = .039).

There were no significant effects of group [$F(2,33) = 1.11$, ns], time [$F(1,33) = 1.14$, ns], or group × time interaction [$F(2,33) = 1.48$, ns] with regard to global flow.

Secondary Clinical Outcome Measures

Results on the secondary outcome measures are presented in Table 4. The NK1 group improved significantly on eight (CGI-S, SPSQ, SPS, SIAS, GAF, Fear, Distress, HR), the citalopram group on six (CGI-S, SPSQ, SPS, SIAS, GAF, Distress), and the placebo group on four (CGI-S, SPSQ, SPS, Fear) measures. The PRCS and SDI scales were insensitive to changes in all groups (data not shown). At posttreatment, the ANCOVAs did not reveal significant effects of group on any of the secondary measures [$F(2,32)$ = .02–1.07, ns] and the pairwise comparisons of the adjusted means remained insignificant.

Verbal Performance

No significant effects of group [$F(2,30)$ = .59, ns], time [$F(1,30)$ = .06, ns], or group × time interaction [$F(2,30)$ = .55, ns] were noted regarding the number of spoken syllables.

Adverse Events

There were 26, 43, and 23 drug-related adverse events in the NK1, citalopram, and placebo groups, respectively, the most common being headache, tiredness, insomnia, nausea, irritability, and somnolence. Events were generally mild or moderate and all were resolved. No subject expressed a wish to discontinue the study.

Discussion

This study explored changes in rCBF following short-term treatment with the NK1 antagonist GR205171, compared with

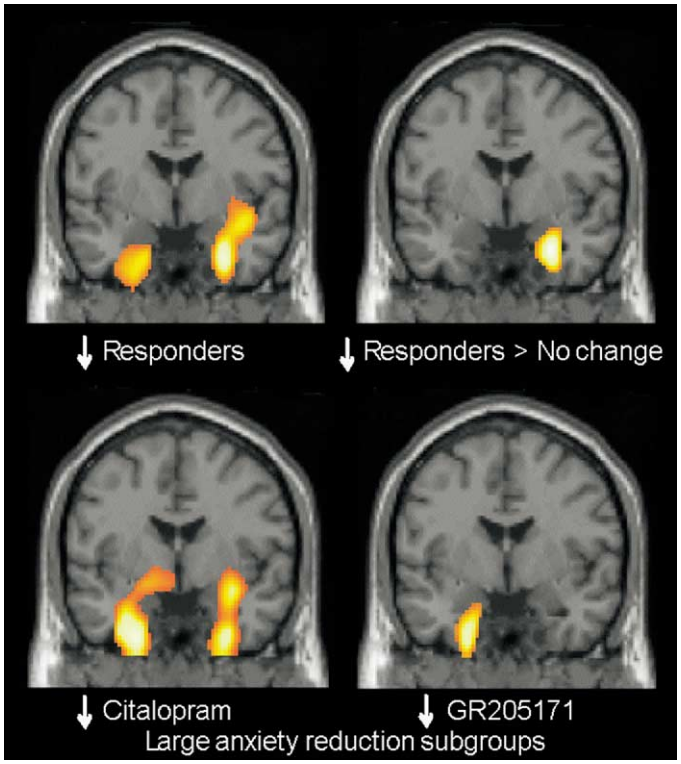

Figure 4. Follow up PET analyses. Coronal images of significantly reduced rCBF during public speaking in the medial temporal lobe after treatment in responders on the Clinical Global Impression improvement scale (n = 12; top left) and in responders compared with subjects that did not change on this scale (n = 13; top right). Further analyses showed significantly decreased rCBF in patients that exhibited the largest reduction of state anxiety from pretreatment to posttreatment within the citalopram (bottom left) and the NK1 antagonist GR205171 (bottom right) groups. No significant rCBF changes were observed in the remaining subjects exhibiting a smaller anxiety reduction (not illustrated). PET, positron emission tomography; rCBF, regional cerebral blood flow.

citalopram and placebo, in patients with social phobia. Posttreatment assessments (day 42) suggested that the NK1 and SSRI treatments reduced the neural response to public speaking in the MTL, including the perirhinal, entorhinal, and parahippocampal cortices, as well as the amygdala. This effect was also observed in responders as defined by the CGI-I, regardless of treatment modality, but not in the placebo group or in subjects that did not change clinically. Between-group comparisons confirmed larger rCBF decrement in the MTL, including the hippocampus proper, in both active drug groups relative to placebo and in responders relative to subjects that did not change. Within the NK1 and SSRI groups, the rCBF decrease was mediated predominantly by subjects showing a large reduction of public speaking state anxiety from pretreatment to posttreatment.

The clinician's ratings (CGI-I) and differential reduction of state anxiety during the public speaking task (STAI-S) suggested that both the NK1 antagonist and the SSRI were superior to placebo. In both drug groups, symptoms deteriorated 2 and 4 weeks after treatment withdrawal, supporting a pharmacological effect. Significant within-group improvement was observed on the majority of secondary measures after active drug treatment, although the placebo group also improved on some scales. On all meausures, the anxiolytic effect of the NK1 antagonist was similar to that of citalopram, even though it was administered for a shorter period, i.e. 4 as compared with 6 weeks. Verbal performance, indexed by number of spoken syllables, was similar before and after treatment in all groups. This pattern supports that the observed rCBF alterations were specifically related to social anxiety reduction following active drug treatment.

The present results are in good agreement with our previous study of social phobia in which symptom improvement with 9 weeks of either citalopram or cognitive-behavioral therapy was associated with reduced rCBF during public speaking in the MTL region (Furmark et al 2002). Both studies strongly indicate that down-regulation of MTL neural activity is an important mechanism in the alleviation of social anxiety. The present findings are also congruent with previous neuroimaging studies in social phobia that have suggested a role for the MTL, or the amygdala, in situationally elicited (Tillfors et al 2001) and anticipatory (Lorberbaum et al 2004; Tillfors et al 2002) anxiety, aversive conditioning (Schneider et al 1999), and in the perception of harsh (Stein et al 2002) and neutral (Birbaumer et al 1998; Veit et al 2002) facial expressions. Taken together, these imaging studies suggest exaggerated responsiveness of the MTL in social phobia and that effective treatment attenuates anxiety-related neural activation in this region. In depressed patients, some investigators have noted that antidepressant drug treatment reduces resting state amygdala hypermetabolism (Drevets et al 2002) and exaggerated amygdala responses to masked fearful faces (Sheline et al 2001). This could imply that the amygdala is a general target for treatments of disorders characterized by negative affect.

The amygdala has long been implicated in the acquisition and expression of fear-related behavior (LeDoux 1996, 2000). It may be particularly important in attention and vigilance, or when the meaning of stimuli is detected, in aversive or ambiguous contexts (Davis and Whalen 2001). The amygdala also has a role in social perception and judgment (Adolphs 2003), which may have specific relevance for social phobia (Amaral 2002). Increased hippocampal activation in frightening situations might be attributed to cognitive processes or contextual evaluation, whereas the surrounding perirhinal, entorhinal, and parahippocampal cortices could be an important transit area for sensory and/or memory information into the subcortical structures (LeDoux 1996). The amygdala is not necessarily the main site of action in the alleviation of anxiety. Neuroimaging studies of anxiety pathways often report conjoint activation of a larger MTL region, comprising both subcortical and cortical areas, rather than an isolated activation of the amygdala (Furmark et al 2002; Stein et al 2002; Tillfors et al 2001, 2002). It is plausible that MTL structures function collectively as an affect-sensitive network that is triggered by threatening stimulation. Selective serotonin reuptake inhibitors might attenuate this network either directly or indirectly, e.g., by balancing median raphe nucleus firing or through interactions with other transmitter systems and pathways (Grove et al 1997). Enhanced serotonergic tone after SSRI treatment could have inhibitory influences on thalamic and cortical inputs to the amygdala (Gorman et al 2000; Stein and Stahl 2000) and presumably other MTL areas. However, the role of serotonin in anxiety is complex since it may have opposite effects in different neural pathways (Graeff 2002). Serotonergic modulation of anxiety may involve both presynaptic and postsynaptic processes and numerous receptor subtypes (Kent et al 2002b; Sandford et al 2000). Studies of serotonin transporter functions could also help to unravel the

Table 3. Temporal Lobe Regions Exhibiting Decreased Activation After Treatment of Social Phobia in Subjects that Showed Either Large or Small Reduction in Public Speaking State Anxiety

Group/Brain Region[a]	Coordinate[b] x	y	z	*z*-score	Voxel *p* Value[c]	Cluster *p* Value[c]
GR205171: Large Reduction ($n = 6$)						
Left Inferior Temporal Cortex, BA36	−22	−2	−34	3.22	ns	.049
BA38	−22	4	−34	2.89		
Amygdala	−18	−3	−22	2.18		
Citalopram: Large Reduction ($n = 6$)						
Left Inferior Temporal Cortex, BA28	−30	1	−29	4.13	.011	.002
BA36	−24	−4	−33	3.81		
BA20	−26	−4	−35	3.77		
BA38	−24	2	−35	3.04		
Amygdala	−26	−1	−18	2.47		
Right Inferior Temporal Cortex, BA20[d]	28	−10	−37	3.60	.060	.001
BA36	30	−6	−33	3.39		
BA38	24	2	−35	2.12		
Amygdala	28	−5	−13	2.48		

BA, Brodmann area; NK1, neurokinin-1.

[a]Location of maximum voxel value (presented first) and spatial extention of significant clusters are listed. GR205171 is a NK1-antagonist. No significant changes were noted in the small reduction subgroups.

[b]Coordinates in millimeters correspond to the stereotactic atlas of Talairach and Tournoux (1988).

[c]Corrected for multiple comparisons.

[d]Right hippocampus implicated at lower threshold (30 −11 −20; *z*-score = 1.77).

mechanisms whereby the SSRIs act in social phobia (Kent et al 2002a) and other disorders.

Consistent with data previously reported by Kramer et al (1998) in depressed patients, the NK1 antagonist and SSRI reduced anxiety to a similar extent. The current PET data suggest that attenuation of MTL neural activity could be an important anxiolytic mechanism also in NK1-targeted pharmacotherapy. The anxiolytic effect of NK1 antagonists like GR205171 may be attributed to reduced SP neurotransmission or subsequently lowered levels of central SP, as suggested by animal studies (Hasenohrl et al 2000), resulting in a net inhibition of MTL neural activity. However, this could also be the result of an interaction with other neurotransmitters. For instance, SP may coexist in serotonergic neurons, thereby modifying their release and effects (Hasenohrl et al 2000). Drugs that act on serotonin neurotransmission can reduce levels of central SP, e.g., in the amygdala (Shirayama et al 1996). Animal studies indicate that the anxiolytic effect following genetic disruption of the NK1 receptor is paralleled by increased firing of serotonergic neurons in the dorsal raphe nucleus and desensitization of inhibitory serotonin-1A autoreceptors (Santarelli et al 2001). Recently, it was demonstrated that SP also might interact with the gamma-aminobutyric acid (GABA)ergic system (Ribeiro and De Lima 2002).

Besides the MTL, a few other regions showed altered activation with treatment. In the NK1 group, rCBF increased significantly in the left occipital cortex. Speculatively, this could be related to improved visual attention which otherwise appears to be shifted away from potentially threatening environmental cues in social phobia (Chen et al 2002). Consistently, a recent PET study noted deactivation of the visual cortex during symptom provocation in male patients with generalized social phobia (Van Ameringen et al 2004). Citalopram subjects showed rCBF diminution in the posterior cingulate cortex. This region, in particular the retrosplenial cortex, has been linked to episodic memory retrieval and is frequently activated in imaging studies of emotional processing (Maddock 1999). Activation of the left cerebellum was noted in placebo subjects, possibly reflecting alterations in motor activity or cognitive processes (Allen et al 1997).

The present study cannot determine whether true normalization of MTL activity occurred, because pretreatment rCBF values were not compared with nonfearful control subjects. We previously observed that the amygdalohippocampal response to a stressful speaking task was enhanced in untreated patients with social phobia relative to nonanxious control subjects (Tillfors et al 2001). Thus, treatment may normalize preexisting abnormalities in the MTL. However, at baseline, phobic subjects and control subjects differed also in widespread cortical regions (Tillfors et al 2001) that remained unaffected in the present but also in our previous (Furmark et al 2002) treatment study. Congruently, imaging studies of major depression suggest that treatments involve both normalization and other adaptive metabolic changes in the brain (Mayberg et al 2000).

Among the limitations, it should be noted that although a sample size of 12 subjects per group yields sufficient power to demonstrate rCBF changes (Andreasen et al 1996), larger sample sizes are generally required to verify differences between active treatment and placebo on behavioral measures. A clear differential response between drug treatment and placebo was found only on the STAI-S and CGI-I, whereas robust between-group differences were not observed on the LSAS-SR or the secondary measures. This could also be due to short treatment periods and limited scale sensitivity. Measures of brain activity may have greater sensitivity than behavioral measures when evaluating emotional reactions (Hariri et al 2002).

In this study, social anxiety reactions may differ from naturalistic settings, and the most severe cases of social phobia were perhaps not willing to participate, which could restrict the external validity. Another limitation is the lack of a control condition without which it cannot be ruled out that rCBF changes reflect nonspecific drug effects on cerebral

Table 4. Mean (SD) and Paired *t* Values for Secondary Clinical Outcome Measures Before and After Treatment

Measure		GR205171	Citalopram	Placebo
CGI-S	pre	4.8 (.9)	4.7 (1.1)	4.8 (.8)
	post	3.6 (.9)	3.6 (1.2)	4.1 (1.2)
	t(11)	4.84[d]	5.61[d]	3.45[b]
SPSQ	pre	33.6 (6.9)	28.2 (9.2)	32.3 (7.2)
	post	25.1 (6.3)	22.0 (9.4)	24.8 (7.3)
	t(11)	4.51[d]	3.43[b]	3.85[c]
SPS	pre	34.0 (10.5)	31.3 (13.5)	37.1 (17.2)
	post	22.8 (8.5)	22.5 (11.8)	29.3 (15.6)
	t(11)	5.91[d]	4.26[c]	2.37[a]
SIAS	pre	50.5 (11.8)	44.8 (16.0)	50.0 (11.4)
	post	38.9 (11.9)	35.3 (16.2)	42.2 (10.9)
	t(11)	9.21[d]	4.94[d]	2.1
GAF	pre	74.5 (12.1)	72.7 (12.7)	76.3 (11.5)
	post	86.2 (8.6)	81.5 (10.3)	82.9 (8.6)
	t(11)	3.76[c]	3.13[b]	2.1
Fear	pre	57.1 (27.4)	62.2 (28.8)	55.9 (26.9)
	post	33.9 (19.4)	50.0 (26.4)	39.4 (35.7)
	t(11)	4.15[c]	1.62	2.70[a]
Distress	pre	69.3 (22.5)	68.9 (25.4)	62.5 (28.8)
	post	46.7 (19.3)	52.1 (27.3)	52.1 (31.6)
	t(11)	3.38[b]	2.92[a]	2.05
HR	pre	82.5 (18.7)	85.2 (19.7)	87.9 (13.5)
	post	76.0 (18.7)	79.5 (15.7)	83.7 (15.2)
	t(11)	2.34[a]	1.91	1.42

GR205171 is a Neurokinin-1-antagonist.

CGI-S, Clinical Global Impression severity scale; SPSQ, Social Phobia Screening Questionnaire; SPS, Social Phobia Scale; SIAS, Social Interaction Anxiety Scale; GAF, Global Assessment of Functioning self-report; HR, Heart rate.

[a] $p < .05$.
[b] $p < .01$.
[c] $p < .005$.
[d] $p < .001$.

vascular functions. However, in both drug groups, the rCBF reduction was significant only in subjects showing large state anxiety reduction and not in those showing a small anxiety reduction, in spite of similar drug intake. This suggests that nonspecific vascular effects are unlikely to explain the rCBF changes observed in MTL during public speaking. Further, nonspecific vascular effects are not compatible with the fact that global blood flow did not change with treatment. It has previously been reported that chronic treatment with fluoxetine does not affect regional or global cerebral blood flow in healthy volunteers (Bonne et al 1999).

The trial reported here was experimental, and in clinical practice, longer treatment periods are generally required to obtain robust and enduring therapeutic effects. For the SSRIs, the anxiolytic effect is typically seen after 2 to 6 weeks (Bandelow and Stein 2004), but additional improvement may continue over a considerable time span (Blomhoff et al 2001). Future imaging studies could use more extended treatment periods and more assessment points and relate anxiety reduction not only to changes in rCBF but also to neurotransmitter/receptor functions such as serotonin synthesis and NK1 receptor occupancy. Neuroimaging techniques could also be used to study dose-response relationships, drug-psychotherapy combinations, and genetic influences on treatment outcome and symptom severity (Furmark et al 2004).

In conclusion, social anxiety was significantly alleviated after short-term treatment with either the NK1 receptor antagonist GR205171 or citalopram. Both drugs were superior to placebo in terms of response rate and reduction of public speaking state anxiety. Neurokinin-1 receptor blockade, as well as serotonin reuptake inhibition, was associated with reduced neural activity in the MTL, which has been ascribed a crucial role in the regulation of fear and anxiety.

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