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Reduced serotonin synthesis and regional cerebral blood flow after anxiolytic treatment of social anxiety disorder

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Abstract
Social anxiety disorder (SAD) is associated with increased fear-related neural activity in the amygdala and we recently found enhanced serotonin synthesis rate in the same region. Anxiolytic agents like selective serotonin re-uptake inhibitors (SSRIs) and neurokinin-1 receptor (NK1R) antagonists reduce amygdala activity and may attenuate serotonin formation according to animal studies. Here, we examined the effects of SSRI pharmacotherapy, NK1R antagonism, and placebo on serotonin synthesis rate in relation to neural activity, measured as regional cerebral blood flow (rCBF), and symptom improvement in SAD. Eighteen SAD patients were randomized to receive daily double-blind treatment for six weeks either with the SSRI citalopram (n=6; 40 mg), the NK1R antagonist GR205171 (n=6; 5 mg; 4 weeks following 2 weeks of placebo), or placebo (n=6). Serotonin synthesis rate at rest and rCBF during stressful public speaking were assessed, before and after treatment, using positron emission tomography with the tracers [^{11}C]5-hydroxytryptophan and [^{15}O]water respectively. The Liebowitz Social Anxiety Scale (LSAS-SR) indexed symptom severity. All groups exhibited attenuated amygdala serotonin synthesis rate after treatment, which was associated with

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reduced amygdala rCBF during public speaking and accompanied by symptom improvement. These results are consistent with the notion that serotonin in the amygdala exerts an anxiogenic influence and, conversely, that anxiolysis is achieved through decreased serotonin formation in the amygdala.

1. Introduction

Social anxiety disorder (SAD) is one of the most common anxiety disorders, characterized by excessive fear of being negatively evaluated by others (American Psychiatric Association, 2000). SAD and other anxiety conditions are associated with exaggerated anxiety-related responses in the amygdala (Brühl et al., 2014; Etkin and Wager, 2007), a central node in the brain's fear circuit (Shin and Liberzon, 2010) modulated by serotonin (Åhs et al., 2015; Asan et al., 2013; Fisher et al., 2006; Rhodes et al., 2007).

First-line pharmacological treatment for anxiety disorders include selective serotonin reuptake inhibitors (SSRIs) (Murrough et al., 2015). Although the biological target of SSRIs is a well-characterized inhibition of serotonin reuptake, the anxiolytic mechanism of action remains to be explained. Suggested mediators include increased levels of extracellular serotonin and adaptation of pre- and postsynaptic receptors (Nutt et al., 1999; Zangrossi and Graeff, 2014), but altered serotonin synthesis may also play a part (Esler et al., 2007; Honig et al., 2009). Moreover, blood sampling from the internal jugular vein has suggested that initially elevated brain serotonin turnover in patients with panic disorder and major depressive disorder is attenuated by SSRI treatment (Barton et al., 2008; Esler et al., 2007). Furthermore, animal studies indicate that chronic SSRI administration reduces serotonin synthesis (Honig et al., 2009), and that anxiolysis is associated with reduced serotonin levels and release (Näslund et al., 2015; Rex and Fink, 2011). In a positron emission tomography (PET) study with the radiotracer [^{11}C]5-hydroxytryptophan ([^{11}C]5-HTP) probing the second enzymatic step in serotonin synthesis, we recently showed that SAD is associated with increased serotonin formation in several brain regions including the amygdala where the synthesis rate correlated positively with symptom severity (Frick et al., 2015a). The finding of increased serotonin formation was recently corroborated in an independent cohort of SAD patients (Furmark et al., 2016). Collectively, these findings suggest that serotonin synthesis in the amygdala contributes to anxiety, and consequently that reduction of serotonin synthesis in this brain region is involved in the anxiolytic action of SSRIs.

The brain serotonergic system is co-expressed and interacts with the substance P/neurokinin-1 system (Frick et al., 2015c; Gobbi and Blier, 2005; Sergeyev et al., 1999). Patients with SAD have enhanced serotonin synthesis (Frick et al., 2015a; Furmark et al., 2016) and reduced serotonin 1A receptor levels (Lanzenberger et al., 2007) as well as increased levels of neurokinin-1 receptors (NK1R) (Frick et al., 2015b) in the amygdala. Also we and others have previously shown that SSRIs (Furmark et al., 2005, 2002; Phan et al., 2013), NK1R antagonists (Furmark et al., 2005), and pill placebo (Faria et al., 2012) exert their anxiolytic actions in SAD through a common neural pathway involving reduced activity in this brain region. However, it is not known if there are shared neurochemical mechanisms underlying the anxiolytic effects, like reduced serotonin synthesis.

Here we used PET with [^{11}C]5-HTP and [^{15}O]water in a randomized controlled trial to study serotonin synthesis rate and neural activity (regional cerebral blood flow; rCBF) during stressful public speaking before and after treatment of SAD with the SSRI citalopram, the NK1R antagonist GR205171, and placebo. Based on the positive association between serotonin formation in the amygdala and SAD symptom severity (Frick et al., 2015a), we hypothesized that attenuated serotonin synthesis rate in the amygdala would be an anxiolytic mechanism shared across treatments with an SSRI, an NK1R antagonist, and placebo. Furthermore, because serotonergic modulation of amygdala activity (Fisher et al., 2006; Rhodes et al., 2007) has been suggested, we expected an association between attenuated serotonin synthesis rate and diminished anxiety-related neural activity (rCBF) after treatment.

2. Experimental procedures

2.1. Participants

The current sample is a subset of the patients in a previously published PET rCBF treatment study (Furmark et al., 2005) who were additionally scanned with [^{11}C]5-HTP PET before and after the treatment. Treatment-related changes (i.e., reductions) in rCBF and social anxiety symptoms were reported in the initial study. Baseline [^{11}C]5-HTP data have been reported in Frick et al. (2015a), but the [^{11}C]5-HTP data after treatment has not previously been published.

Eighteen SAD patients (mean $\pm$ SD age 32.6 $\pm$ 8.2 years, 9 men), recruited through newspaper advertising, participated. Main exclusion criteria were any other primary major psychiatric or neurologic disorder, somatic disease, ongoing or within 2 months discontinued psychological treatment or treatment with psychotropic medication, chronic use of prescribed medication, current drug or alcohol abuse/dependency, family history of cancer, previous PET examination, pregnancy, menopause, and left-handedness. Patients meeting the initial SAD screening criteria on the Social Phobia Screening Questionnaire (SPSQ) (Furmark et al., 1999), and who did not fulfill any exclusion criteria, subsequently underwent face-to-face interviews. The Structured Clinical Interview for the DSM-IV (SCID-I) (First et al., 1998) was administered to ascertain that they fulfilled the DSM-IV criteria for SAD (American Psychiatric Association, 2000) and the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998) was administered to exclude other serious psychiatric disorders. A medical examination was also performed. All patients had a primary SAD diagnosis. Two of the patients

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