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Research Article

RESEARCH ARTICLE

Blunted autonomic reactivity to pharmacological panic challenge under long-term escitalopram treatment in healthy men

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Abstract

Background: Central serotonergic pathways influence brain areas involved in vagal cardiovascular regulation and, thereby, influence sympathetic efferent activity. Selective serotonin reuptake inhibitors (SSRIs) affect multiple serotonergic pathways, including central autonomic pathways. However, only a few studies have assessed SSRI-mediated effects on autonomic reactivity in healthy individuals using heart rate variability (HRV).

Methods: The present study assessed the influence of long-term treatment with escitalopram (ESC) on autonomic reactivity to an intravenous application of 50 µg cholecystokinin tetrapeptide (CCK-4) in 30 healthy young men using a double-blind, placebo (PLA)-controlled, randomized, within-subject cross-over design. Main outcome measures were time- and frequency-domain HRV parameters, assessed at both baseline and immediately after CCK-4 application.

Results: Results showed substantial effects for the treatment × CCK-4 challenge interaction with respect to heart rate ($p < 0.001$; ${}_p\eta^2 = 0.499$), SDNN ($p < 0.001$; ${}_p\eta^2 = 576$), RMSSD ($p = 0.015$; ${}_p\eta^2 = 194$), NN50% ($p = 0.008$; ${}_p\eta^2 = 0.224$), and LF% ($p = 0.014$; ${}_p\eta^2 = 0.196$), and moderate effects with respect HF% ($p = 0.099$; ${}_p\eta^2 = 0.094$), with PLA subjects showing a higher increase in HR and SDNN and a higher decrease in RMSSD, NN50, LF and HF than subjects in the ESC condition. Thus, ESC treatment significantly blunted the autonomic reactivity to CCK-4. Secondary analysis indicated no effect of the 5-HTTLPR polymorphism on CCK-4-induced autonomic response.

Conclusions: Our results support findings suggesting an effect of SSRI treatment on autonomic regulation and provide evidence that ESC treatment is associated with blunted autonomic reactivity in healthy men.

Keywords: 5-HTTLPR, autonomic nervous system, CCK-4, cholecystokinin tetrapeptide, escitalopram, heart rate variability, panic, serotonin-transporter-linked polymorphic region, SSRI

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Introduction

The autonomic nervous system (ANS) regulates a plethora of physiological reactions and contributes to self-regulation and adaptability of the organism in order to meet the metabolic demands of ongoing physical, emotional, and cognitive challenges (Thayer and Lane, 2000; Critchley, 2005). On the other hand, autonomic imbalance is associated with decreased dynamic adaptability of the organism and increased morbidity and mortality (Thayer and Lane, 2000; Thayer and Sternberg, 2006).

Central autonomic control underlies the task- and division-specific influence of the brainstem and other cerebral and cerebellar structures of the central autonomic network (Beissner et al., 2013). The activity and functional connectivity of these brain regions is partly influenced by serotonergic signaling (Strawn et al., 2012; Fisher and Hariri, 2013). Central serotonin (5-hydroxytryptamine [5-HT]) transmission is thus not only involved in the modulation of emotional and cognitive behavior, but also in autonomic regulation (Ramage, 2001; Jordan, 2005; Youn et al., 2013). Consequently, altered serotonin regulation in the central nervous system (CNS) is associated with autonomic dysregulation, while reduced CNS serotonergic activity is linked to elevated autonomic responsiveness to stressors (Audero et al., 2008; Hildreth et al., 2008; Cummings et al., 2011).

The serotonergic activity of the CNS can be pharmacologically modulated. Selective serotonin reuptake inhibitors (SSRIs) have emerged as a major therapeutic advance in psychopharmacology and are currently the most frequently prescribed and best characterized therapeutic compounds affecting multiple central projection pathways in the topography of serotonin function (Vaswani et al., 2003; Sghendo and Mifsud, 2012). Binding of these drugs to the presynaptic serotonin transporter leads to its negative allosteric modulation, which effectively inhibits its ability to reuptake serotonin from the synaptic cleft (Goodnick and Goldstein, 1998). However, the acute SSRI action is associated with rather modest synaptic serotonin increases due to negative feedback loops through somatodendritic 5-HT_{1A} autoreceptors. However, chronic SSRI administration leads to desensitization of 5-HT_{1A} autoreceptors and downregulation of the negative feedback inhibition and, thus, results in increased serotonin release at postsynaptic heteroreceptor sites (Sghendo and Mifsud, 2012; Walker, 2013).

Since serotonergic pathways influence brain areas involved in vagal cardiovascular regulation and thereby modulate sympathetic efferent activity (Ramage, 2001; Jordan, 2005; Youn et al., 2013), SSRI administration affecting serotonergic transmission may also modulate autonomic reactivity. For example, there is an FDA safety warning on the SSRI Celexa (citalopram hydrobromide) for causing pathological cardiovascular symptoms at high doses (FDA, 2012; Castro et al., 2013). These symptoms can be mimicked in mice by strong activation of postsynaptic 5-HT_{1A} receptors to which serotonin has a high affinity (Youn et al., 2013). On the other hand, SSRIs demonstrate the safest cardiovascular profile of all antidepressants and are considered the first choice of drugs in cardiovascular-risk patients (Roose and Miyazaki, 2005; Hamer et al., 2011; Chittaranjan et al., 2013; Hare et al., 2014).

Nevertheless, relatively few studies have assessed the acute and long-term effects of SSRIs on autonomic function. Most of those studies have investigated autonomic effects in either psychiatric patients or patients with cardiovascular disease. In healthy adults, the few studies suggest absent clinically-significant effects on baseline autonomic measures through single-dose (Ahrens et al., 2007), short-term (Penttila et al., 2001; Siepmann et al., 2003; Chappell et al., 2013), or long-term treatment with SSRIs (Pohl et al., 2003). Only two studies investigated the effects of acute SSRI treatment on autonomic stress reactivity in healthy adults, reporting beneficial effects of a single dose of escitalopram (ESC) through attenuated autonomic responses to social and physiological stress tasks in healthy females (Hanson et al., 2013; Kemp et al., 2014). However, to date, no study has assessed the effects of long-term SSRI treatment on ANS reactivity in healthy individuals using autonomic measures in the clinical dose range.

Thus, the main objective of our study was to assess the effects of long-term SSRI treatment on autonomic reactivity to a pharmacological panic challenge in healthy individuals. One of the best-established non-invasive methods to assess parasympathetic activity is the analysis of heart rate variability (HRV; Camm et al., 1996; Reyes del Paso et al., 2013). HRV results from heart rate (HR) oscillations within its physiological range (beat-to-beat variability), controlled by parasympathetic and sympathetic modulation of intrinsic cardiac pacemakers (Akselrod et al., 1981) and reflects the capacity of the organism for regulated physical and emotional responses. Higher HRV implicates parasympathetic dominance favoring energy conservation, while low HRV suggests attenuated cardiac parasympathetic modulation (Reyes del Paso et al., 2013). Low HRV is associated with higher overall mortality, specifically heart mortality, and is considered a valid marker of heart disease (Thayer and Sternberg, 2006; Thayer et al., 2012). Psychiatric research has repeatedly used HRV to investigate physiological alterations in psychiatric disorders (Gorman and Sloan, 2000; Kemp and Quintana, 2013), suggesting an association between psychopathology and reduced parasympathetic activity (Thayer and Sternberg, 2006; Kemp et al., 2010).

We therefore investigated autonomic reactivity measured by HRV to the pharmacological panic challenge by the cholecystokinin tetrapeptide (CCK-4) in a homogenous group of young, healthy men. Intravenous administration of CCK-4 reliably reproduces consistent, dose-dependent, short-lasting anxiety paroxysms via CCK B receptors in the CNS and constitutes a well-established model to investigate autonomic and neuroendocrine panic reactions in healthy volunteers (Bradwejn et al., 1991; Eser et al., 2007; Kellner, 2011). Based on previous findings (Golding et al., 2002; Hanson et al., 2013; Kemp et al., 2014), we hypothesized that chronic SSRI treatment would lower the autonomic reactivity elicited by CCK-4. To determine differences potentially resulting from altered neuroautonomic control depending on the serotonin transporter gene-linked polymorphic region (5-HTTLPR) genotype, as previously reported by assessing only the non-medicated subjects of our sample (Agorastos et al., 2014), we additionally analyzed differences between short/short (s/s) and long/long (l/l) carriers of the 5-HTTLPR genotype.

Methods and Materials

Subjects

We collected data from 30 healthy young Caucasian male study volunteers (15 subjects with the s/s genotype of the 5-HTTLPR and 15 randomly-chosen eligible l/l genotype subjects from the screening sample), who participated in an experimental panic provocation study approved by the Ethics Committee of the Hamburg Medical Board. Screening procedure and study protocol have been described in detail elsewhere (Kellner et al., 2009; Hinkelmann, Dragoi, et al., 2010). Participant selection and attrition across the experiment, based on specific exclusion criteria, are provided in Figure 1. Exclusion criteria were: presence or history of any physical and Axes I and II mental co-morbidities, history of sporadic

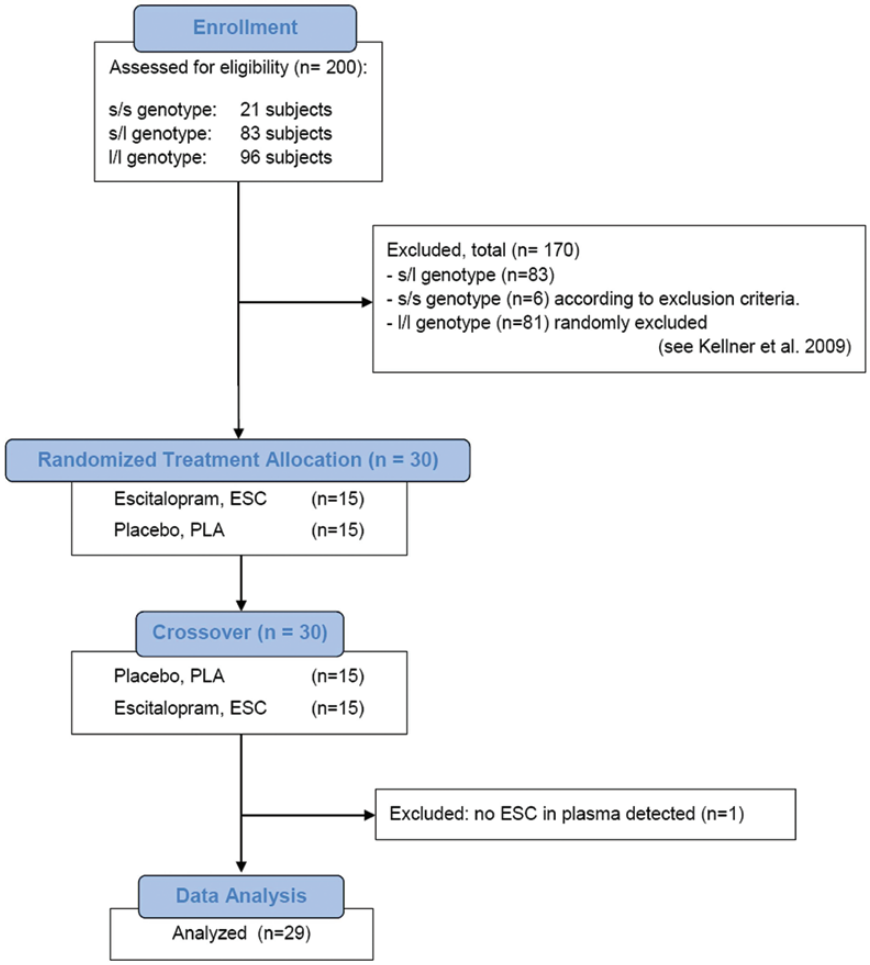

Figure 1. CONSORT Flow Diagram displaying the progress of all participants through the trial.

panic attacks, family history of Axis I mental disorders, frequent usage of any illicit or prescribed drugs or over-the-counter medications, current use of any medication, consumption of more than four cigarettes per day, drinking of more than four cups of coffee a day and more than 100 g of alcohol per week, current adverse life events, night shifts or transcontinental flights across more than four time zones during the past four weeks, abnormal physical and neurological examinations, basic blood laboratory test values deviating from the normal range (including thyroid function tests, transaminases, electrolytes, CO_2 anion gap, fasting glucose, basic blood and coagulation tests, blood lipids, hemoglobin A1c, C-reactive protein, creatinine, folic acid, and vitamin B12), positive urine toxicology screen, and pathological chest x-ray or initial electrocardiogram (ECG). Current or lifetime psychiatric disorders were excluded using the Structured Clinical Interview for the DSM-IV, Axes I and II, assessed by a trained physician. All other exclusion criteria were assessed in a clinical interview setting through study questionnaires. After full oral and written explanation of the purpose and procedures of the investigation, written informed consent was obtained from each subject.

Procedures

Panic challenges were performed after 42 days of daily treatment with 10 mg escitalopram in a double-blind, placebo (PLA)-controlled, randomized, within-subject, crossover design with a wash-out phase of at least three weeks in between pre-treatment periods. To check for compliance of intake of study medication, ESC was measured in plasma specimens taken the day after the study had been finished, as described in Greiner et al. (2007).

Venous blood samples were obtained for DNA extraction, and the 5-HTTLPR genotype was determined as described before (Maron et al., 2004; Kellner et al., 2009). After standardized lunch at 12:00 hours, subjects were studied from 13:00 to 17:00 hours, exclusively in a supine position in a soundproof experimental room. CCK-4 (Clinalfa) was stored at -80°C and freshly prepared for each injection. A total of 50 µg of CCK-4 were dissolved in 10 ml sterile saline. At 15:00 hours, subjects received 50 µg CCK-4 as an intravenous bolus injection within 20 s. Subjects were closely monitored after the injection of CCK-4. ECG recordings were obtained throughout using a 5-lead holter recording system (Schiller medilog® AR12, Schiller Medizintechnik GmbH). Data were recorded at a 4096 Hz sampling rate in 16-bit resolution and were stored digitally on the recorder. ECG recording was performed by specially-trained study staff. ECG analyses were performed using specific software (Schiller medilog® DARWIN, Schiller Medizintechnik GmbH). Adverse side effects were assessed through a German version of the Udvalg for Kliniske Undersogelser (UKU) side effects rating scale (Lingjaerde et al., 1987).

ECG Analysis

Data from two 5-min segments (baseline and immediately after CCK-4 application) were used to determine differences between

treatment conditions. Recording of CCK-4–mediated effects was initiated 45–60 s after the bolus injection at 15:01 hours, while baseline recording started 60 min before CCK-4 injection at 14:00 hours. Instantaneous HR was calculated on the basis of the RR interval. HRV in the time domain was calculated by taking the standard deviation of the N-N intervals (SDNN), by calculating the root-mean-square of subsequent interval differences (RMSSD), and by the percentage of the number of pairs of adjacent NN intervals that differ in length by >50 ms (NN50%). HRV in the frequency domain was calculated by analysis of two frequency components [low frequency (LF): 0.04–0.15 Hz; high frequency (HF): 0.15–0.4 Hz]. Results are presented as the percentage of each frequency component from the total power and the LF/HF ratio. The assessed HRV variables have been selected according to the guidelines for short-term recordings of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Camm et al., 1996), which are also supported by new studies (Meyer and Stiedl, 2003; Jarrin et al., 2012). We used the percentage of the total power of each frequency component to compare the fractional energy (Camm et al., 1996), rather than the absolute energy per frequency band.

Statistical Analyses

Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity, and homoscedasticity. Parameters with skewed distribution, i.e., the LF/HF ratio, were $\log_{10}$ transformed for further parametric analysis. All data are given in mean values (standard error of the mean). CCK-4-induced changes have been calculated as $\Delta_M = M_{CCK\text{-}4} - M_{baseline}$. Differences between treatment conditions were tested for significance by analyses of variance (ANOVAs). Additionally, mixed between-within subjects ANOVAs were used to assess the impact of CCK-4 application (baseline vs. CCK-4), CCK-4 × treatment, and then, additionally, 5-HTTLPR genotype (s/s vs. l/l; CCK-4 × treatment × genotype) on HR dynamics and the differences within groups (PLA vs. ESC). An error probability of $p < 0.05$ was accepted as statistically significant. Effect size is reported as eta squared ($\eta^2 = 0.01$: small effect size, $\eta^2 = 0.06$: medium effect size, $\eta^2 = 0.14$: large effect size). To correct for potentially inflated type I error because of multiple comparisons, we used the false discovery rate approach (Benjamini and Hochberg, 1995), as in our previous studies (Agorastos et al., 2013, 2014). Following a previously reported procedure (Verhoeven et al., 2005) p-values were corrected by the minimum positive false discovery rate with a threshold set at 5%. Statistical analyses were conducted using the Statistical Package for Social Sciences Version 20 (SPSS).

Results

All 30 volunteers completed the study. Enrolled subjects had a median age of 27 years (range 19–36) and BMI of 21.9 kg/m^2 (range 18.0–27.1) and there were no differences in these measures between the ESC and PLA conditions (data not shown). In one subject, no ESC could be detected in plasma after 42 days of verum treatment. Therefore, this subject was excluded from further analyses. Mean plasma ESC concentration was 15.5 ng/ml on day 42, and mean plasma desmethyl-ESC level was 6.6 ng/ml. No active drug was detected in any subject during PLA intake. Side effects, as per UKU ratings, did not differ significantly between treatments.

CCK-4 Challenge x Treatment Interaction Effects

Mixed ANOVAs were conducted to assess the impact of treatment (ESC vs. PLA) on dependent variables across the two time periods (baseline, post-CCK-4). The results showed substantial effects for the treatment × CCK-4 challenge interaction with respect to HR (Wilks $\lambda = 0.501$; $F_{(1,28)} = 27.853$; $p < 0.001$, $_p\eta^2 = 0.499$), SDNN (Wilks $\lambda = 0.424$; $F_{(1,28)} = 38.051$; $p < 0.001$; $_p\eta^2 = 576$), RMSSD (Wilks $\lambda = 0.806$; $F_{(1,28)} = 6.723$; $p = 0.015$, $_p\eta^2 = 194$), NN50% (Wilks $\lambda = 0.776$; $F_{(1,28)} = 8.066$; $p = 0.008$, $_p\eta^2 = 0.224$), and LF% (Wilks $\lambda = 0.804$; $F_{(1,28)} = 6.845$; $p = 0.014$, $_p\eta^2 = 0.196$) and moderate, but not statistically significant, effects with respect to HF% (Wilks $\lambda = 0.906$; $F_{(1,28)} = 2.908$; $p = 0.099$, $_p\eta^2 = 0.094$). Subjects showed an significantly higher increase in HR and SDNN and a higher decrease in RMSSD, NN50, and LF in the PLA than in the ESC condition (Figure 2; Table 1).

Main Effects of CCK-4 Challenge

When investigating the effect of CCK-4 challenge in the total population, repeated-measures ANOVAs indicated significant to highly significant differences between baseline and post-CCK-4 measures with very large effect sizes in all assessed measures, leading to higher HR (Wilks $\lambda = 0.453$; $F_{(1,58)} = 69.081$; $p < 0.001$; $_p\eta^2 = 0.548$), SDNN (Wilks $\lambda = 0.247$; $F_{(1,58)} = 173.605$; $p < 0.001$; $_p\eta^2 = 0.753$), and LF/HF_{log} (Wilks $\lambda = 0.760$; $F_{(1,58)} = 17.976$; $p < 0.001$; $_p\eta^2 = 0.240$), and lower RMSSD (Wilks $\lambda = 0.697$; $F_{(1,58)} = 24.808$; $p < 0.001$; $_p\eta^2 = 0.303$), NN50% (Wilks $\lambda = 0.651$; $F_{(1,58)} = 30.578$; $p < 0.001$; $_p\eta^2 = 0.349$), LF% (Wilks $\lambda = 0.328$; $F_{(1,58)} = 116.745$; $p < 0.001$; $_p\eta^2 = 0.672$), and HF% (Wilks $\lambda = 0.349$; $F_{(1,58)} = 106.489$; $p < 0.001$; $_p\eta^2 = 0.651$) values (Figure 2; Table 1).

Treatment Differences

Baseline

A group comparison revealed only differences with moderate effect sizes but no statistical significance between the two groups with respect to baseline HR measures (Figure 2; Table 2).

CCK-4 Challenge

Post-CCK-4-injection HR measures indicated statistically significant differences with large effect sizes between the two treatment conditions (Figure 2; Table 2). Subjects in the ESC condition showed overall significantly lower HR increases (Wilks $\lambda = 0.409$, $F_{(1,28)} = 40.450$, $p < 0.001$, $_p\eta^2 = 0.591$) and SDNN (Wilks $\lambda = 0.391$, $F_{(1,28)} = 43.642$; $p < 0.001$, $_p\eta^2 = 0.609$), and higher NN50% (Wilks $\lambda = 0.900$, $F_{(1,28)} = 3.119$; $p = 0.088$, $_p\eta^2 = 0.100$), LF% (Wilks $\lambda = 0.565$, $F_{(1,28)} = 21.537$; $p < 0.001$, $_p\eta^2 = 0.435$), and HF% (Wilks $\lambda = 0.528$, $F_{(1,28)} = 25.026$; $p < 0.001$, $_p\eta^2 = 0.472$) values.

Differences Depending on the 5-HTTLPR Genotype

Since we previously reported significantly lower autonomic reactivity to CCK-4 in s/s versus l/l 5-HTTLPR carriers without treatment (Agorastos et al., 2014) we also conducted an additional analysis to investigate the genotype effects. Mixed models using repeated-measures ANOVA were used to assess the impact of the 5-HTTLPR genotype, i.e., s/s vs. l/l, on HRV measures, including both treatment conditions (CCK-4 × treatment × genotype). There was no statistically significant effect of genotype and no significant interaction between genotype and treatment condition (data not shown). When subjects in the ESC condition were analyzed separately, s/s carriers displayed reduced autonomic reactivity in comparison to the l/l carriers, with a similar trend to our previous findings (Agorastos et al., 2014). However, these differences were not statistically significant.

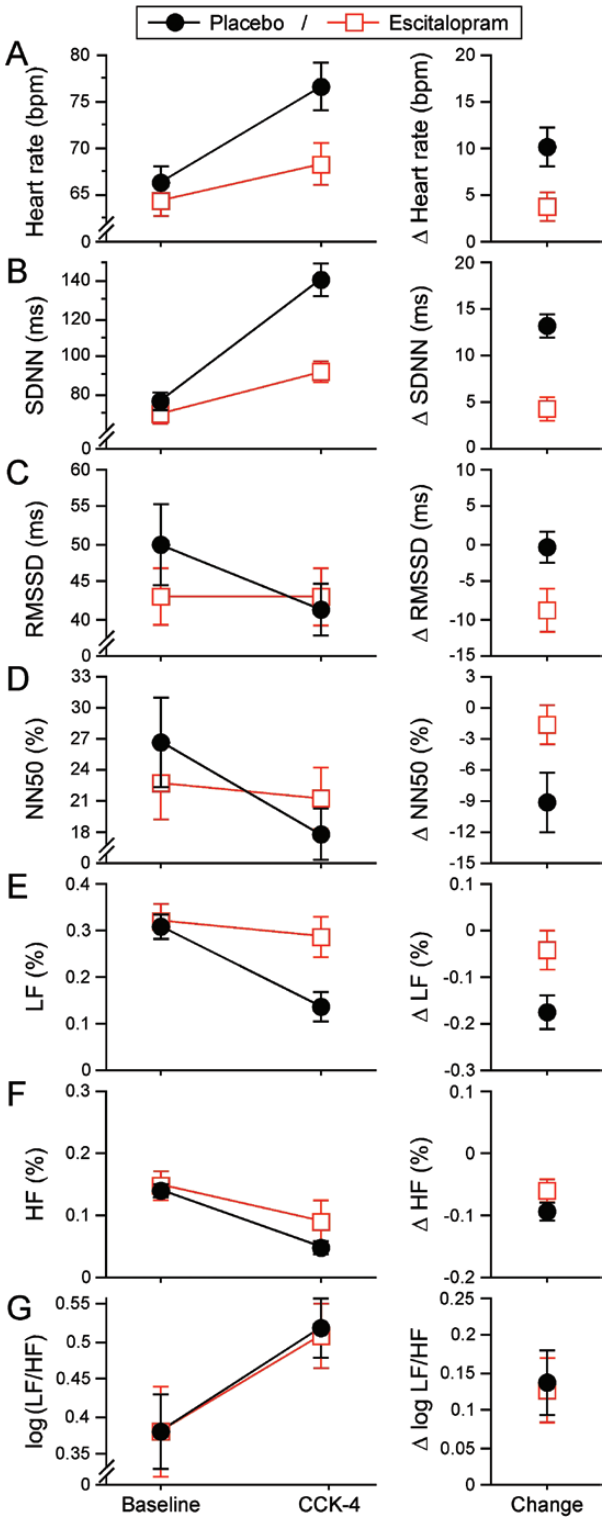

Figure 2. Effects of placebo and long-term escitalopram treatment on a range of cardiovascular time domain (A–D) and frequency domain measures (E–G). Values are presented as means ± standard error of the mean. Measures were determined at baseline and after pharmacological panic challenge by cholecystokinin tetrapeptide (CCK-4; left panels). CCK-4-induced changes (right panels) have been calculated as $\Delta_M = M_{post\ CCK\text{-}4} - M_{baseline}$. SDNN: standard deviation of the N-N intervals; RMSSD: root mean square of subsequent differences; NN50%: percentage of the number of pairs of adjacent NN intervals that differ in length by >50 ms; LF: low frequency 0.04–0.15 Hz; HF: high frequency 0.15–0.4 Hz; LF% and HF%: percentage of each frequency component from the total power.

Discussion

This study assessed the effects of long-term SSRI treatment on acute CCK-4–mediated effects on HRV in healthy men, using the most selective SSRI available (Burke, 2002; Sanchez et al., 2003) as a potential modulator of autonomic control. Although not the first study to examine the impact of SSRI treatment on stress responsiveness, to the best of our knowledge, our study is the first one analyzing autonomic responses following long-term SSRI treatment related to pharmacological panic challenge by CCK-4 based on HR measures in healthy young men.

The main findings of this study include: (1) no statistically significant evidence for ESC-associated effects on baseline vagal activity; (2) attenuated cardiac vagal modulation in both treatment conditions by CCK-4; (3) significantly lower vagal tone and increased autonomic reactivity upon CCK-4 challenge in the PLA vs. ESC group; and (4) no 5-HTTLPR genotype effect on HRV measures and their changes after CCK-4 application over both treatment conditions. Jointly, these findings indicate blunted autonomic reactivity to CCK-4 in the ESC treatment condition, in comparison to PLA.

CCK-4 application has been repeatedly shown to lead to a robust increase of HR and systolic blood pressure (Benkelfat et al., 1995; Jerabek et al., 1999; Eser et al., 2007). However, so far only two previous studies from our group reported autonomic effects of CCK-4 on HRV (Wiedemann et al., 2001; Agorastos et al., 2014). Thus, our findings of enhanced sympathetic and/or attenuated vagal cardiac modulation of HRV by CCK-4 in both PLA and ESC treatment conditions replicate and extend our previous findings.

The present study investigated resting autonomic measures as a function of central serotonergic activity in both treatment conditions. HRV effects of long-term SSRI administration have been investigated in various categories of patients. In depression, in contrast to other antidepressant categories (e.g., tricyclic antidepressants, serotonin-norepinephrine reuptake inhibitors; Siepmann et al., 2007; Kemp et al., 2010; Chang et al., 2012), SSRI treatment has been reported not to have a significant impact on resting HRV (Rechlin, 1994; Straneva-Meuse et al., 2004; Koschke et al., 2009; Kemp et al., 2010, 2011, 2014; Brunoni et al., 2013; Hanson et al., 2013), although contradictory results have also been reported (Licht et al., 2010). In patients with panic disorder or post-traumatic stress disorder, SSRI treatment has been mainly associated with positive effects on HRV measures, i.e., reduced sympathetic activity/normalized ANS activity and baroreflex response (Tucker et al., 1997; Cohen et al., 2000). SSRI treatment in patients with cardiovascular diseases has been also associated with an improvement of HRV indices independent of the improvement of depressive symptoms, suggesting a clear benefit for the prognosis of the cardiovascular disease (Gorman and Sloan, 2000; Leftheriotis et al., 2010; Mazza et al., 2010; Pizzi et al., 2011). However, only a few studies have investigated single-dose or long-term SSRI treatment effects on HRV or other autonomic measures in healthy adults at rest. These studies suggest no clinically significant autonomic SSRI effects in either males or females in resting states (Penttila et al., 2001; Pohl et al., 2003; Siepmann et al., 2003; Ahrens et al., 2007; Chappell et al., 2013; Hanson et al., 2013; Kemp et al., 2014). Thus, our results in males support these previous studies.

Our study also assessed the modulating effect of long-term ESC treatment on autonomic reactivity in response to a pharmacological panic challenge in healthy subjects. To date, effects of SSRI treatment on autonomic reactivity have been only sparsely investigated, predominantly in psychiatric patients using

Table 1. Effects of CCK-4 Challenge and Treatment x CCK-4 Challenge on Heart Dynamics

		rmANOVA		Mixed ANOVA	
		CCK-4 challenge		Treatment x CCK-4 challenge	
Domain	Measure	p	${}_p\eta^2$	p	${}_p\eta^2$
Time	HR (bpm)	**<0.001**	**0.548**$^{++}$	**<0.001**	**0.499**$^{++}$
	SDNN (ms)	**<0.001**	**0.753**$^{++}$	**<0.001**	**0.576**$^{++}$
	RMSSD (ms)	**<0.001**	**0.303**$^{++}$	**0.015**	**0.194**$^{++}$
	NN50%	**<0.001**	**0.349**$^{++}$	**0.008**	**0.224**$^{++}$
Frequency	LF%	**<0.001**	**0.672**$^{++}$	**0.014**	**0.196**$^{++}$
	HF%	**<0.001**	**0.651**$^{++}$	0.099	0.094^{+}
	LF/HF_{log}	**<0.001**	**0.240**$^{++}$	0.888	0.001

Repeated-measures ANOVAs and between- and within-subjects ANOVAs were used to assess the impact of the cholecystokinin tetrapeptide (CCK-4) challenge and the differences within treatment groups (placebo [PLA] vs. escitalopram [ESC]). HR: heart rate; SDNN: standard deviation of the N-N intervals; RMSSD: root mean square of subsequent differences; NN50%: percentage of the number of pairs of adjacent NN intervals that differ in length by >50 ms; LF: low frequency 0.04–0.15 Hz; HF: high frequency 0.15–0.4 Hz; LF% and HF%: percentage of each frequency component from the total power. *P*-values denoting statistically significant differences are shown in bold. False discovery rate analysis revealed no potential type I errors.
$^{+}$moderate effect size; $^{++}$large effect size.

Table 2. Differences Between Treatment Conditions in HRV Measures

		PLA vs. ESC				Baseline vs. CCK-4			
		Baseline		CCK-4		PLA		ESC	
Domain	Measure	p	${}_p\eta^2$	p	${}_p\eta^2$	p	${}_p\eta^2$	P	${}_p\eta^2$
Time	HR (bpm)	0.129	0.080	**<0.001**	**0.591**$^{++}$	**<0.001**	**0.541**$^{++}$	**0.007**	**0.233**$^{++}$
	SDNN (ms)	0.119	0.085	**<0.001**	**0.609**$^{++}$	**<0.001**	**0.780**$^{++}$	**<0.001**	**0.438**$^{++}$
	RMSSD (ms)	**0.044**§	**0.137**$^{+}$	0.483	0.018	**0.007**	**0.230**$^{++}$	0.886	0.001
	NN50%	0.072	**0.111**$^{+}$	0.088	**0.100**$^{+}$	**0.002**	**0.301**$^{++}$	0.369	0.029
Frequency	LF%	0.690	0.006	**<0.001**	**0.435**$^{++}$	**<0.001**	**0.591**$^{++}$	0.372	0.029
	HF%	0.557	0.012	**<0.001**	**0.472**$^{++}$	**<0.001**	**0.670**$^{++}$	**0.001**	**0.307**$^{++}$
	LF/HF_{log}	0.930	0.000	0.930	0.000	0.003	**0.271**$^{++}$	**0.001**	**0.318**$^{++}$

HR: heart rate; SDNN: standard deviation of the N-N intervals; RMSSD: root mean square of subsequent differences; NN50%: percentage of the number of pairs of adjacent NN intervals that differ in length by >50 ms; LF: low frequency 0.04–0.15 Hz; HF: high frequency 0.15–0.4 Hz; LF% and HF%: percentage of each frequency component from the total power; PLA: placebo; ESC: escitalopram; CCK-4: cholecystokinin tetrapeptide. *P*-values denoting statistically significant differences or a trend are shown in bold.
$^{+}$moderate effect size; $^{++}$large effect size; §potential type I error based on false discovery rate analysis.

various stressor models. Effects of SSRI treatment on autonomic reactivity have been reported in patients with panic disorder and post-traumatic stress disorder, suggesting a positive effect of SSRIs on HRV reactivity to physiological (orthostatic) or psychological challenges, towards normalizing autonomic indices (Tucker et al., 1997, 2000).

In healthy male and female individuals, Takata et al. (2002) reported decreased orthostatic baroreflex sensitivity after long-term paroxetine treatment, while Golding et al. (2002) observed decreased autonomic reactivity to a psychological stressor, which is also supported by our results. However, both studies used only HR and blood pressure measures to assess autonomic activity. Two recent studies investigated ESC treatment effects on autonomic stress reactivity in healthy females, reporting attenuated autonomic responses in social and physiological stress tasks (Hanson et al., 2013; Kemp et al., 2014). Both studies, however, only investigated effects of a single-dose of ESC. Besides a previous report from our group (Kellner et al., 2009) on the same sample group, only one additional study investigated long-term effects of ESC on CCK-4 challenge in both male and female healthy volunteers (Toru et al., 2013). In contrast to results in patients with panic disorder (Shlik et al., 1997; van Megen et al., 1997), no inhibitory effect of ESC was observed upon panic symptoms elicited by CCK-4 in healthy subjects. However, neither study reported any ESC effects on autonomic measures. Thus, this study is the first one to report long-term effects of the SSRI ESC on ANS reactivity to a pharmacological panic challenge by CCK-4 in healthy subjects, indicating reduced autonomic reactivity following long-term treatment by ESC in comparison to the PLA control in healthy subjects.

The CCK-4-induced increase in HR, together with a decrease in SDNN, RMSSD, NN50%, LF%, and HF%, is interpreted as increased sympathetic and/or attenuated parasympathetic (vagal) cardiac modulation, consistent with previous claims (Camm et al., 1996; Lombardi and Stein, 2011). The initial reactivity hypothesis proposed that enhanced cardiovascular autonomic reactivity is associated with increased cardiovascular risk as a mediator of psychosocial and behavioral risk factors (Phillips, 2011; Phillips and Hughes, 2011). Following this hypothesis, our data suggest that the reduced autonomic reactivity to the CCK-4 challenge observed in the ESC group may represent a marker of reduced cardiovascular risk, as postulated in a prior study (Brummett et al., 2011). This is also supported by the statistically absent differences in baseline HR between the two treatment conditions, as resting HR is considered an independent predictor of cardiovascular risk (Fox

et al., 2007). SSRI treatment has shown beneficial cardiovascular effects in cardiovascular disease, which are associated with reduced autonomic reactivity. Similarly, advantageous cardiovascular effects of SSRIs leading to normalization of autonomic measures have been also reported in psychiatric disorders (see above), which have been associated with increased (Monk et al., 2001; Blechert et al., 2010; Felmingham et al., 2012) or reduced (Kikuchi et al., 2009; McTeague et al., 2010; Shinba, 2013) autonomic reactivity. In healthy females, comparable moderating effects on HR and HRV have been observed by acute SSRI treatment and regular high-intensity exercise to a physical stress challenge (Hanson et al., 2013). In contrast, robust evidence has been provided that reduced cardiovascular reactivity and slower recovery are associated with overall cardiovascular risk (Heponiemi et al., 2007; Salomon et al., 2009), which should be discussed here. Unfortunately, our study did not assess data on cardiac recovery after the stress challenge, which is also a major indicator of cardiac reactivity. Thus, on the basis of the currently used measures we cannot unambiguously resolve whether the reduced stress responsiveness reflects a beneficial ('anxiolytic-like') or a maladaptive state ('hypo-responsiveness'). Nonlinear measures (Meyer and Stiedl, 2003) may provide for an unambiguous interpretation of physiological versus pathological change, but cannot be applied with short ECG recordings as used here.

The existence of functional subpopulations of serotonergic neurons acting at numerous sites of the CNS and the evidence for their tight control by stress hormones (Chaouloff, 1993; Johnson et al., 2004) suggest a complex interplay of central serotonergic activity with ANS and cardiac stress responsiveness. SSRI treatment may, thus, both influence supra-ordinate mechanisms (e.g., the central autonomic network) and/or affect hormone secretion patterns (Shores et al., 2001; Agelink et al., 2004). With respect to cardiovascular disease patients specifically, additional pleiotropic SSRI effects may also be responsible for the beneficial effects reported (Escolar et al., 2005; Paraskevaidis et al., 2006). Our study also investigated the potential contribution of the 5-HTTLPR polymorphism on PLA versus ESC treatment towards affecting CCK-4–mediated HR responses. Prior studies have reported a moderating role of 5-HTTLPR polymorphisms on the brain-heart interaction (Kauppila et al., 2013; Mueller et al., 2013). However, to date, our prior study assessing 5-HTTLPR genotype effects exclusively in healthy subjects receiving PLA was the first one investigating the association of 5-HTTLPR with ANS function using HRV in healthy individuals. We reported enhanced sympathetic and/or diminished baseline cardiac vagal activity and blunted autonomic reactivity in s/s versus l/l carriers without drug treatment (Agorastos et al., 2014). Our current analyses revealed no overall effect of the 5-HTTLPR polymorphism on autonomic responses to CCK-4 challenge. When ESC condition was investigated separately, analyses suggested a similar 5-HTTLPR polymorphism effect as to our previous study on PLA condition, but without statistical significance.

Limitations

Some limitations have to be taken into account for the presented study. To avoid putative confounding effects of the menstrual cycle phase on response to CCK-4 (Le Melledo et al., 1999) and to avoid problems associated with contraception, only male subjects were studied. Thus, long-term HRV effects still need to be investigated in women, as gender differences cannot be ruled out. Similarly, our results should be replicated in individuals of older age, since age-related changes in 5-HT transmission and SSRI effects have been reported (Olivier et al., 2011; Kemp et al., 2014). Effects of SSRIs after longer treatment (>4 weeks, as commonly used in psychiatric practice) still need to be investigated.

Since ESC is considered the most selective SSRI (Burke, 2002; Sanchez et al., 2003), our results may not be generalizable to other substances of the SSRI group. Despite sharing the same principal mechanism of action, recent reviews investigating SSRIs effects in healthy persons suggest various inconsistencies dues to differences in their pharmacodynamic and pharmacokinetic profiles (Goodnick and Goldstein, 1998; Knorr and Kessing, 2010). In addition, several long-term SSRI effects (e.g., neuroplastic and neurotrophic changes, effects on gene expression, anti-inflammatory properties) have been postulated, opposing the initially-assumed simplistic, monoaminergic pharmacological properties of these drugs (Kroeze et al., 2012; Walker, 2013), so that different pathways of action may be responsible for the observed effects. Another limitation is the use of one standardized dosage of ESC only. However, the ESC plasma levels measured were within the range recommended for treatment of affective disorders.

Furthermore, as disturbed sleep is also associated with autonomic alterations (Nielsen et al., 2010), it is important to note that we did not determine sleep quality in our initial assessments and can herewith not exclude sleep quality–related bias, although we did not encounter complaints about altered sleep patterns. In order to rule out conditioning effects of repeated CCK-4 administration (Hinkelmann, Yassouridis, et al., 2010), we controlled for the randomized treatment order, which did not affect our results. In addition, our study did not include subjects with the s/l genotype. Since some studies suggest a nonlinear gene-dose effect of the 5-HTTLPR (Neumeister et al., 2006), inclusion of s/l subjects in further studies with a considerably larger sample size is necessary to precisely characterize genotype differences. Regarding functional variants of the long allele, posterior analyses of our DNA specimens revealed that all long alleles in this study were of the LA subtype (Lesch, personal communication).

Finally, it is important to note that across all parameters investigated, no subject had a cardiovascular history and deviating laboratory or physical tests. We particularly accounted for several laboratory markers (e.g., fasting glucose, hemoglobin A1c levels, cholesterol/lipoproteins, pro-inflammatory cytokines, acute-phase proteins) and certain lifestyle habits (e.g., drug, alcohol, or tobacco intake) that have been shown to be associated with ANS dysregulation, altering cardiovascular measures, including HRV (Thayer and Sternberg, 2006; Dinas et al., 2013).

Conclusions

Studies about differential long-term effects of SSRIs on HRV reactivity using pharmacological stress challenges have not been reported so far in healthy subjects. By assessing autonomic responses to a defined pharmacological panic challenge, we provide the first data that chronic SSRI treatment is associated with reduced autonomic reactivity in healthy subjects. Our study supports an important role of central serotonergic activity in the modulation of the magnitude of acute cardiovascular responsiveness, which may affect susceptibility to stress-related disorders. Future studies are needed to replicate this finding and to further explore the functional contribution of effects through and regulation of different receptor subtypes of the serotonergic system to mechanistically understand the role of SSRI on ANS function in both health and disease.

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Dr Kellner designed the study and wrote the protocol. Drs Demiralay and Muhtz collected the data. Dr Agorastos managed the literature searches. Drs Agorastos and Stiedl had access to the raw data and performed all statistical analyses and data interpretation. Drs Agorastos and Demiralay wrote the first draft of the paper. Drs Kellner, Wiedemann, and Stiedl revised the draft for important intellectual content. All authors have contributed to, read, and approved the final version of the manuscript.

Statement of Interest

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