Chronic selective serotonin reuptake inhibition modulates endothelial dysfunction and oxidative state in rat chronic mild stress model of depression

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1Department of Biomedicine, Aarhus University, Aarhus, Denmark; 2Department of General Physiology, Faculty of Biology, St. Petersburg State University, St. Petersburg, Russia; and 3Translational Neuropsychiatry Unit, Department of Clinical Medicine, Aarhus University, Risskov, Denmark

Submitted 13 August 2014; accepted in final form 7 August 2015

Matchkov VV, Kravtsova VV, Wiborg O, Aalkjaer C, Bouzinova EV. Chronic selective serotonin reuptake inhibition modulates endothelial dysfunction and oxidative state in rat chronic mild stress model of depression. Am J Physiol Regul Integr Comp Physiol 309: R814–R823, 2015. First published August 12, 2015; doi:10.1152/ajpregu.00337.2014.—Major depression is known to be associated with cardiovascular abnormalities, and oxidative stress has been suggested to play a role. We tested the hypothesis that antidepressant treatment reduces oxidative stress and endothelial dysfunctions in the chronic mild stress (CMS) model of depression in rats. Rats with >30% reduction in sucrose intake after 4 wk of CMS were defined in the study as CMS-susceptible and compared with unstressed controls. Sixteen CMS-susceptible and eight unstressed rats were treated during weeks 5 to 8 of the CMS protocol with escitalopram. Escitalopram-treated rats with >20% recovery in the sucrose consumption during the last 2 wk of treatment were defined as escitalopram responders. Rats that did not reach these criteria were defined as escitalopram nonresponders. In the open field test, escitalopram responders demonstrated anxiolytic effect of treatment. In mesenteric small arteries, escitalopram affected neither NO nor cyclooxygenase-1 (COX-1)-mediated vasodilatation. Escitalopram potentiated endothelium-dependent hyperpolarization-like response, which was suppressed in the vehicle-treated CMS-susceptible rats and reduced COX-2-dependent relaxation, which was elevated in the vehicle-treated CMS-susceptible rats. Escitalopram did not affect blood pressure and heart rate, which were elevated in the vehicle-treated CMS-susceptible rats. Oxidative stress markers were changed in association with CMS in liver, heart, and brain. Escitalopram normalized oxidative stress markers in the majority of tissues. This study demonstrates that the antidepressant effect of escitalopram is associated with partial improvement of endothelial function in small arteries affecting COX-2 and endothelium-dependent hyperpolarization-like pathways.

Cardiovascular diseases and major depressive disorder are the two most prevalent health problems in high-income countries (31). Their comorbidity is well recognized, but the mechanisms and critical factors underlying this comorbidity are unknown. Interactions between several factors common for both disorders are complex (17; 43). Thus, a dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis and activation of the renin-angiotensin-aldosterone system lead to changes associated with both depression and cardiovascular abnormalities.

Also dysfunction of the autonomic nervous system (sympathetic/parasympathetic) is known to be involved, as well as an activation of proinflammatory cytokines (46). Importantly, these factors can induce both depression and changes in structure and function of the heart and blood vessels.

Studies of reliable preclinical models are a useful approach in the studies of the factors influencing both depression and cardiovascular abnormalities. Several animal models of depression have been developed. The chronic mild stress (CMS) model involves exposing rodents to a period of unpredictable mild stressors and is considered a realistic model with a very high validity (70, 71). Previous studies with the CMS model have provided insight into potential behavioral and physiological changes associated with depression. The behavioral changes observed in this model are consistent with depression symptoms, e.g., reduced physical activity and an inability to experience pleasure from normally pleasurable stimuli—anhedonia, shown by a reduction in the consumption of sucrose solution (28, 39). An association between a hippocampal dysfunction and depression in the CMS rats has recently been shown, suggesting a mechanistic insight into the disorder (35). Oxidative stress is one of the suggested mechanisms behind comorbidity between cardiovascular abnormalities and major depression (65). Oxidative stress reflects an imbalance between production of reactive oxygen species (ROS) and the ability of the biological system to inactivate these free radicals. Normally, ROS are quickly cleared to prevent cell damage. However, under some pathological conditions, such as an inadequate intake of antioxidants, exposure to noxious chemicals or ultraviolet light, injury, and eccentric exercise, the endogenous antioxidant system is not able to remove excessive ROS (23). At high levels, ROS cause cellular impairment by damaging DNA, proteins, and lipids (23). Lipid peroxidation has been found to limit different aspects of cell function by decreasing the fluidity of the membrane and thus complicating the membrane transport functions. Such damages are obviously critical for the functioning of nearly all physiological systems. Lipid peroxidation is commonly quantified by measuring the accumulating by-products, e.g., malondialdehyde (MDA). MDA is a common general product of nonenzymatic peroxidation of polyunsaturated fatty and arachidonic acids. MDA may be increased in major depression (49) and in cardiovascular patients (15, 59). Moreover, MDA is considered not only to be a product of lipid peroxidation but also known to be harmful for cell functions (1). To keep potentially harmful oxidative stress at low levels, the body maintains multiple types of antioxidants, i.e., glutathione, vitamins C, A, and E, as well as enzymes such as catalase, superoxide dismutase, and various...
peroxidas. Glutathione is a major antioxidant in nearly all biological systems. Its formation is stimulated by oxidative stress. Glutathione can be a measure of the protective responses to ROS elevation, and its level has also been shown to be changed in several pathologies, including major depression (5, 22, 56) and cardiovascular diseases (11, 15).

The vascular endothelium is one of the known targets for oxidative damage (25, 67). The endothelium is essential for regulation of vascular resistance, and at least three endothelial-dependent relaxing pathways are present, i.e., nitric oxide (NO), prostanoids, and endothelium-dependent hyperpolarization (EDH). Although the mechanism of EDH is controversial, it has been shown to depend on the activation of endothelial small- and intermittent-conductance $Ca^{2+}$-activated $K^+$ channels ($SK_	ext{Ca}$ and $IK_	ext{Ca}$, respectively) following agonist- and shear stress-induced increase of endothelial calcium (24). Excessive ROS in the vascular wall have been suggested to affect all endothelium-dependent relaxing pathways, leading to critical changes in vascular function (67).

Some cardiovascular changes are reported for the CMS model, e.g., elevated heart rate and reduced heart rate variability associated with changes in the sympathetic/parasympathetic balance (26, 27, 29). Moreover, although anhedonia disappears following cessation of the stressors, the cardiovascular changes persist for some time, suggesting that they are firmly established (26, 28). Our recent findings demonstrate that resistance arteries from CMS-susceptible rats have increased neuronal monoamine reuptake, which compensates for stress-induced suppression of corticosterone-sensitive extra-neuronal reuptake (7). These changes were due to an increase in neuronal noradrenaline transporter expression and a decrease of corticosterone-sensitive extra-neuronal organic cation cotransporter 2. Furthermore, we have shown that the CMS susceptibility is also associated with reduced endothelium-dependent relaxation due to suppressed EDH-like pathway despite upregulation of the NO and cyclooxygenase 2 (COX-2)-dependent pathways in rat mesenteric resistance arteries (8). Importantly, these arteries are essential for the control of the blood pressure and tissue perfusion, but knowledge about their abnormalities in association with depression symptoms is limited (9). Since increased oxidative stress is common for both the endothelial dysfunction (67) and major depression disorder (46), we hypothesized that it might be one of the links between depression and cardiovascular diseases. Knowing that antidepressant treatment with fluoxetine and citalopram (both are selective serotonergic reuptake inhibitors, SSRI) reduces serum oxidative stress markers in patients (44), we hypothesized that behavioral responses to the treatment with SSRI is accompanied with reduction of oxidative stress and improvement of endothelial function. Therefore, in this study, we investigated the effects of SSRI treatment on behavior, oxidative stress, and endothelial-dependent relaxation of resistance arteries from CMS-susceptible rats.

**MATERIALS AND METHODS**

Male Wistar rats were purchased from Taconic AS (Ry, Denmark). Animal weight was about 200 g when adaptation to sucrose consumption was initiated, and it was ~350 g at the start of the stress protocol. The animals were singly housed, except when grouping was applied as a stressor. Food and water were available ad libitum, except when food and/or water deprivation was applied as a stressor. The standard 12:12-h light-dark cycle (light phase 0600–1800) was only changed in the course of the stress exposure (14, 33, 39). The weight of a susceptible of rats was obtained regularly throughout the study.

The entire course of study from arrival of rats to the animal facility through the CMS protocol and until in vitro measurements was in accordance with the standard procedures applied to this type of study in our laboratory (4, 6, 12, 39, 40, 55). The first 2 wk after arrival, rats were adapted to the animal facility. The following 2 wk, all rats were trained to drink palatable sucrose solution. Throughout the training period, the rats were exposed to the sucrose consumption test (SCT; see below) twice weekly. Afterward, SCTs were conducted once weekly until the end of the experiment. The baseline sucrose consumption was measured during 3 wk. Then rats were divided into two groups, where one group was exposed to CMS for 8 wk and the other remained unchallenged (3, 7, 8, 13, 14, 33, 34, 36, 41, 42, 57). The treatment with SSRI antidepressant escitalopram (5 mg·kg$^{-1}$·day$^{-1}$) ip started after 4 wk of CMS and continued during next 4 wk together with CMS protocol until the end of the experiment (4, 6, 12, 39, 40).

Experiments were conducted in accordance with the national guidelines for animal research and after permission from the Animal Experiments Inspectorate of the Danish Ministry of Food, Agriculture and Fisheries.

**SCT.** SCT measures the sucrose intake of individual rats during 1-h exposure to a bottle with 1.5% sucrose solution. To achieve a comparable level of thirst and hunger prior to the SCT, rats were deprived of food and water for 14 h (for review, see Ref. 70). The baseline sucrose intake was taken as an average intake from three consecutive SCTs prior to starting the CMS protocol. For each SCT during the experiment, the sucrose index (SI) was calculated as a ratio of the current intake into the baseline intake. The average SI for CMS during week 3 and 4 was used as a parameter to evaluate hedonic status—the core behavioral feature of depression (70). Rats with a SI less than 0.7 during this period were considered as CMS-susceptible, i.e., being anhedonic. This is a standard definition of anhedonia-like behavior in CMS rat studies (3, 4, 6–8, 12–14, 33, 34, 39–42, 55, 57). The anhedonic-like behavior correlates strongly with other depression markers (3, 12, 33).

**CMS protocol.** The applied CMS procedure was described in detail previously (33, 39, 57) and was repeated in accordance with the previous studies (3, 4, 6–8, 12–14, 34, 40–42, 55). Briefly, the chronic mild stress protocol with a 14-day cycle consisted of one period of intermittent illumination, strobescope, starvation, and cage deprivation, two periods of soiled cage, and no stress, and three periods of 45° box tilting. During grouping, rats were housed in pairs with different partners alternately serving as resident or intruder. All stressors lasted 10–14 h. The CMS protocol was applied during 8 wk.

The CMS paradigm originally involved a large group of rats whose hedonic state was analyzed after the first 4 wk of stress (3, 14, 33, 57). Rats exposed to CMS showed differential stress susceptibility with ~50% being anhedonic, while others were either resilient (no change in sucrose intake) or reduced their sucrose intake <30% (61, 70, 71).

**Escitalopram treatment.** During weeks 5 to 8 of CMS, 16 CMS-susceptible (having SI < 0.7 after 4 wk of CMS, i.e., anhedonia-like symptom) and 8 stress-unchallenged rats were treated with 5 mg·kg$^{-1}$·day$^{-1}$ ip of escitalopram (SSRI). Two additional groups (5 CMS-susceptible rats and 8 stress-unchallenged rats) were treated with an equal volume of vehicle (saline) and were used as controls for escitalopram treatment. CMS-susceptible rats with an increase in SCT of >20% and with an average SI >0.85 during the last 2 wk of treatment were defined as escitalopram responders. Escitalopram-treated rats that did not meet these criteria were defined as escitalopram nonresponders. Similar criteria for division of escitalopram-treated CMS rats into responders and nonresponders are used previously (4, 6, 12, 39, 40).
Activity test in the open field. During the last day of escitalopram treatment, rats were tested in a round 120-cm open field arena for 10 min in dim light condition. The behavior was recorded with Panasonic WV-CL93 CCTV camera and analyzed by the behavior analyzing software EthoVision ver. 9.0 (Noldus, Netherlands). During a 10-min session, the following parameters were calculated for each rat: total distance, time spent at periphery, the amount of central crossings, rearing, and the number of investigated holes.

Blood pressure measurements. Mean arterial blood pressure and heart rate were measured by determining the tail blood volume with a volume pressure recording sensor and an occlusion tail-cuff (CODA System, Kent Scientific, Torrington, CT). For tail-cuff measurements, in accordance with the manufacturer’s recommendation, rats were trained a minimum of three times before the interventions. Measurements were performed on rats restrained in a transparent animal nose cone holder, which provides unrestricted breathing and allows complete visibility to the researcher. Holders were placed on CODA Animal Warming Platform-5 in 5–10 min prior to the registration.

Tissue preparations. At the end of the CMS protocol, rats were anesthetized with isoflurane and decapitated. Brain, heart, liver, and mesenteric and tail arteries were snap frozen in liquid nitrogen and kept at −80°C until measurements of oxidative stress were made. A branch (internal diameter ~200 μm) of the mesenteric artery was dissected for myograph experiments.

Isometric force measurement. Third-order branches of mesenteric artery were dissected and cleaned of connective tissue in ice-cold salt solution (PSS) containing (in mM) 119 NaCl, 4.7 KCl, 1.18 KH2PO4, 1.17 MgSO4·7H2O, 25 NaHCO3, 1.6 CaCl2, 0.026 EDTA, and 5.5 glucose, gassed with 5% CO2 in air and adjusted to pH 7.4. The cleaned arterial segments were mounted in an isometric wire myograph (Danish Myo Technology), as described previously (52). The myograph chamber was heated to 37°C, while the PSS was constantly aerated with 5% CO2 in air. The artery diameter was set to a value where maximal active force is obtained (53). Force (in mN) was recorded with a Power Lab-Chart5 acquisition system (ADInstruments) and converted to wall tension (in mN/mm) by dividing the force with two times the vessel segment length.

Endothelial function was tested in the myograph using increasing concentrations of ACh. Arteries were preconstricted with 6 × 10−6 M noradrenaline (NA), and increasing concentrations of ACh were added in a cumulative manner after development of stable contraction to NA. Each ACh concentration was applied for 3 min. Relaxation was expressed as a percentage of the preconstricted level (0% relaxation) to passive tension (100% relaxation).

The ACh concentration-response curves were repeated four times in the presence of blockers of different components of the endotheli-um-dependent relaxation. All blockers were applied 15 min prior to preconstriction with NA. To assess the NO-dependent component of relaxation, the arteries were incubated with L-nitroarginine methyl ester (l-NAME). SC560 was added to block COX-1 activity. SC560 was used to block COX-2 activity. TRAM-34 and apamin were used to block SKCa and IKCa channels, respectively. All inhibitors were obtained from Sigma-Aldrich, (Brøndby, Denmark). NA, ACh, l-NAME, and apamin were dissolved in distilled water as 10−4, 10−3 and 10−4 M stock solutions, respectively. Stock solutions were aliquoted and stored at −20°C until use.

Oxidative stress evaluation. The total glutathione concentration in reduced and oxidized forms was measured with a glutathione assay kit (Sigma-Aldrich) in accordance with the standard protocol. Samples were first deproteinized with the 5% 5-sulfosalicylic acid solution. Glutathione content of the sample was measured using a kinetic assay in which catalytic amounts of glutathione cause a continuous reduction of 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB) to 5′-thio-2-nitrobenzoic acid (TNB). The oxidized glutathione is recycled by glutathione reductase and NADPH. The product, TNB, is assayed colorimetrically at 412 nm.

The level of MDA was measured by thiobarbituric acid (TBA)-reactive substances (TBARS) assay. MDA in the sample is reacted with TBA to generate the MDA-TBA adduct. The MDA-TBA adduct is quantified colorimetrically at 532 nm (58, 64).

Statistical analysis. Results are presented as means ± SE for all analyses, tables, and figures. A value of P < 0.05 was considered statistically significant.

Differences between groups were tested by t-test where appropriate. Results of SCT were analyzed by two-way ANOVA for effects of CMS and time, as well as for CMS and treatment followed by Tukey’s post hoc test. Results of the open field test and blood pressure measurements were analyzed by one-way ANOVA, and differences between the groups were tested by Tukey’s post hoc test. Differences in ACh concentration-response curves were analyzed by two-way ANOVA with group as the independent group factor and agonist as the repeated-measures factor. Differences in the effects of inhibitors were analyzed by one-way ANOVA with Tukey’s post hoc test for multiple comparisons.

Since the results from glutathione and MDA measurements were not normally distributed, the nonparametric Kruskal-Wallis (KWH) test was used to analyze the main effect of treatment and Dunn’s multiple pairwise comparison—to assess differences between groups. All statistical analysis was performed using XLSTAT2010 (Addinsoft, Paris, France) and GraphPad Prism 5 for Windows (GraphPad, San Diego, CA).

RESULTS

CMS reduced sucrose consumption, which can be recovered in a subpopulation of rats by SSRI. In accordance with our previous studies (4, 8, 12, 14, 39, 41, 57), exposure to CMS induced anhedonic-like behavior, in about one-third of the rat population. The CMS-susceptible rats reduced their sucrose intake by 36 ± 2% (n = 26) (Fig. 1). No significant change in sucrose intake was seen in stress-unchallenged rats treated with either vehicle or escitalopram (Fig. 1). The CMS-susceptible rats treated with vehicle maintained the lower sucrose intake compared with the stress-unchallenged groups (P < 0.001; Fig. 1). Treatment with escitalopram did not affect weight gain (67.1 ± 4.6 g) compared with vehicle treatment (57.8 ± 6.5 g, P = 0.356).

![Fig. 1. Effect of escitalopram treatment on sucrose consumption. The treatment of stress-unchallenged control rats with either saline (Con-Veh; n = 8) or escitalopram (Con-Esc; n = 8) was without significant effect on their sucrose intake. The chronic mild stress (CMS)-susceptible rats were divided into two groups and treated with either saline (CMS-Veh; n = 10) or escitalopram. The CMS-susceptible rats were subdivided into responders (Esc-R; n = 7) and nonresponders (Esc-NR; n = 9) in accordance with their response to escitalopram. Arrow indicates the beginning of escitalopram treatment. ***P < 0.001 by two-way ANOVA, ++P < 0.01 and +P < 0.05 by Tukey’s ANOVA post hoc test for Esc-R vs. Esc-NR and for Esc-R vs. CMS-Veh, respectively.](http://ajpregu.physiology.org/ by 10.22033.1 on October 30, 2017)
The CMS-susceptible rats responded differently to escitalopram treatment and were divided into two groups: responders and nonresponders. The SI of the responders was not different from the stress-unchallenged group at the end of the experiment (Fig. 1). Two-way ANOVA within the whole CMS-susceptible group treated with escitalopram \( [F(17,126) = 5.810; P < 0.0001] \) revealed a significant effect of treatment \( [F(2,10) = 16.239; P < 0.0001] \), time \( [F(5,10) = 4.775, P < 0.0001] \), and their interaction \( [F(7,10) = 4.074; P < 0.0001] \). Repeated-measures ANOVA demonstrates the effect of escitalopram treatment \( [F(2,21) = 6.32; P < 0.0001] \), time \( [F(5,105) = 6.952; P < 0.0001] \), and their interaction \( [F(15,105) = 5.938; P < 0.0001] \).

Escitalopram affects anxiety-related and exploratory activities. The CMS-susceptible rats demonstrated an increased locomotion in the open field test compared with the stress-unchallenged group (Fig. 2, inset). A detailed analysis of the open field activity within the CMS-susceptible group did not reveal any difference in locomotion between vehicle and escitalopram-treated groups \( [F(2,17) = 1.381; P = 0.278; \text{not shown}] \). However, escitalopram responders spent less time at the periphery of the arena (Fig. 2A) and had a higher number of central crossings (Fig. 2B), demonstrating the anxiolytic effect of the treatment; as well as activated exploratory behavior in form of rearing (Fig. 2C). Analysis of the number of holes investigated during the open field sessions did not reveal an effect of the treatment.

Escitalopram has no effect on blood pressure and heart rate in CMS-susceptible rats. No significant effect of escitalopram treatment was seen on mean arterial blood pressure, which was \( 109 \pm 5 \text{ mmHg} \), \( 103 \pm 5 \text{ mmHg} \), and \( 101 \pm 6 \text{ mmHg} \) in CMS-susceptible rats treated with vehicle \((n = 5)\), escitalopram responders \((n = 7)\), and escitalopram nonresponders \((n = 9)\), respectively. This was not significantly different from mean arterial blood pressure of stress-unchallenged rats \( 104 \pm 5 \text{ mmHg} \); \( n = 5 \) Importantly, CMS-susceptible rats had increased heart rate compared with the stress-unchallenged group, but escitalopram treatment did not affect heart rate (Fig. 3).

ACh induced relaxation in small-resistance arteries. Escitalopram treatment did not affect \( [F(2,204) = 1.139; P = 0.322] \) endothelial sensitivity to ACh (Fig. 4 and 5). There were no differences in the areas under ACh-relaxation curves under these conditions \( [F(2,18) = 0.124; P = 0.884] \). L-NAME pretreatment significantly reduced the ACh sensitivity in escitalopram responders \( [F(1,136) = 115.5; P < 0.001] \), escitalopram nonresponders \( [F(1,176) = 195.9; P < 0.001] \), and vehicle-treated groups \( [F(1,96) = 203.9; P < 0.001] \) (Figs. 4 and 5). The sensitivity to ACh after preincubation with L-NAME was significantly higher \( [F(2,204) = 6.548; P < 0.01] \) in escitalopram nonresponders compared with escitalopram responders and vehicle-treated CMS groups (Fig. 5). However, changes in the areas under the curve after the addition of L-NAME were the same for all three groups \( [F(2,18) = 0.714; P = 0.503] \) (Fig. 6).

Inhibition of COX-1 enzymatic activity with SC560 increased the ACh sensitivity in escitalopram responders \( [F(1,126) = 23.49; P < 0.001] \) but did not have any effect on ACh sensitivity in arteries from escitalopram nonresponders \( [F(1,1176) = 0.057; P = 0.81] \) and vehicle-treated CMS-susceptible rats \( [F(1,96) = 1.603; P = 0.21] \) (Fig. 5). Thus, two-way ANOVA revealed an effect of escitalopram treatment on ACh sensitivity under these conditions \( [F(2,204) = 36.40; 0.001, and P < 0.01, and P < 0.001, respectively, by Tukey’s ANOVA post hoc test.

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**Fig. 2.** Effect of escitalopram treatment on behavior in the open field test. **A:** time spent at periphery. **B:** central crossings. **C:** Rearing. CMS-veh, vehicle-treated CMS-susceptible rats \((n = 5)\); Esc-R, escitalopram responders \((n = 7)\), and Esc-NR, escitalopram nonresponders \((n = 9)\). Inset: difference in locomotion between stress-unchallenged (Con) and CMS-susceptible rats. *; **; ***P < 0.05, P < 0.01, and P < 0.001, respectively, by Tukey’s ANOVA post hoc test.

**Fig. 3.** Heart rate was significantly different between stress-unchallenged (Con) and CMS-susceptible (CMS) rats (left). Heart rate in the CMS-susceptible rats was not significantly affected by escitalopram treatment (right). The CMS-susceptible rats were treated with either vehicle \((n = 5)\) or escitalopram. Escitalopram responders (Esc-R; \( n = 7)\) and escitalopram nonresponders (Esc-NR; \( n = 9)\) were subdivided according to SCT (see Fig. 1). *P < 0.05 by Student’s t-test.
The effect of COX-1 inhibition on the areas under the curve was similar in all three groups of arteries \( F(2,17) = 2.305; P = 0.130; \) Fig. 6. Inhibition of COX-2 with NS398 produced different changes in ACh responses of vehicle-treated and escitalopram-treated rats revealing the effect of escitalopram treatment on this pathway \( F(2,194) = 64.10; P < 0.001; \) Fig. 5. These conditions revealed higher sensitivity to ACh in arteries from escitalopram-treated rats. COX-2 inhibition decreased the ACh sensitivity in arteries from vehicle-treated CMS-susceptible rats \( F(1,96) = 43.18; P < 0.0001 \) but increased ACh sensitivity in arteries from escitalopram responders \( F(1,116) = 6.47; P < 0.05 \) and was without effect on the arteries from escitalopram nonresponders \( F(1,176) = 2.707; P = 0.10 \).

Under these conditions, arteries from vehicle-treated CMS-susceptible rats relaxed significantly less to ACh \( F(2,17) = 3.777; P < 0.05 \) compared with arteries from escitalopram responders \( t = 2.744; P < 0.05; \) Fig. 6. The further addition of inhibitors of SKCa and IKCa channels, 100 \( \mu \)M TRAM-34, and 50 nM apamin completely abolished relaxation in all three groups of arteries (Fig. 4). Inhibition of the EDH-like component had a stronger effect \( F(2,17) = 3.677; P < 0.05 \) on relaxation of escitalopram responders compared with vehicle-treated CMS-susceptible rats \( t = 2.689; P < 0.05; \) Fig. 6). No significant difference in changes of relaxation after SKCa and IKCa channel inhibition was seen between vehicle-treated CMS-susceptible and escitalopram nonresponders \( t = 1.309 \).

Total glutathione and MDA concentrations. The total glutathione and MDA concentrations were measured in tissue samples collected from five groups of rats: stress-unchallenged, stress-unchallenged rats treated with escitalopram, and three groups exposed to CMS: treated with vehicle, escitalopram responders, and escitalopram nonresponders. Glutathione and MDA were measured in liver, heart, and brain, as well as in tail and mesenteric arteries.

CMS exposure was associated with a significant elevation of glutathione, in livers of vehicle-treated rats compared...
with stress-unchallenged rats (Fig. 7). Escitalopram treatment had no effect on the glutathione level in the stress-unchallenged group, but normalized glutathione level in the liver of both escitalopram responders and escitalopram nonresponders. These changes in glutathione level in the liver were not accompanied with any significant changes in MDA levels (Fig. 8).

Similarly, glutathione was increased in the hearts of CMS-susceptible rats compared with stress-unchallenged rats (Fig. 7). Although, the glutathione concentration was lower in rats treated with escitalopram, this was not significant. Thus, escitalopram responders and escitalopram nonresponders had higher glutathione levels in the heart compared with stress-unchallenged rats. Escitalopram treatment of stress-unchallenged rats elevated glutathione levels in the heart. Elevation of glutathione level in the hearts of CMS-susceptible rats treated with vehicle was accompanied with significant reduction in MDA (Fig. 8). MDA levels in escitalopram-treated and stress-unchallenged groups were similar.

Although CMS did not significantly modify glutathione levels in the brain, it did change after escitalopram treatment. Thus, escitalopram responders had significantly elevated glutathione, while escitalopram nonresponders had a reduced level of glutathione in the brain (Fig. 7). Nevertheless, CMS was associated with elevated MDA in the brain, and MDA was even higher in escitalopram responders but tended to normalize in the escitalopram nonresponders (Fig. 8). No significant change in glutathione and MDA levels in association with CMS and escitalopram treatment was found in mesenteric and tail arteries.

**DISCUSSION**

As with our previous studies (12, 13, 39), we found that only a subset of CMS-susceptible rats are escitalopram responders, as judged from their succrose intake and anxiety-related and exploratory activities. Our results also suggest that escitalopram treatment modifies the balance between different pathways of endothelium-dependent relaxation. Thus, COX-2-dependent and EDH-like pathways were modified by escitalopram, and the effects were most pronounced in escitalopram responders. We also found that oxidative stress markers were differently affected in escitalopram responders and nonresponders.

**Behavioral changes in escitalopram responders and nonresponders.** The response to treatment of CMS-susceptible rats with the SSRI antidepressant escitalopram was variable. Similar to previous reports (6, 12, 13, 39, 61), approximately half of CMS-susceptible rats in the current study were drug-resistant. Although drug resistance and treatment failures are frequent during antidepressant therapy (54, 62), the reason for this is unknown. Several factors important for antidepressant responsiveness/resistance have been suggested (16). In humans, these factors can be both acquired throughout life and be inherited in the form of mutations of key transporters and enzymes involved in the drug metabolism. In animal models, a variability in gene expression important for central and peripheral neurotransmission, HPA axis function, and drug metabolism have been suggested to be related to drug resistance (4, 6, 12, 13, 45). Thus, we have recently shown that responsiveness of CMS-susceptible rats to escitalopram is associated with reduced expression of the CYP2J10 in lateral habenula (13), which might be important for drug metabolism. Moreover, changes in several intracellular transduction pathways in the lateral habenula have been suggested to associate with responsiveness to escitalopram in CMS-susceptible rats (13). Importantly, most of the suggested changes in gene regulation are known to play an important role in the modulation of both neuronal activity and vascular function (13, 43), consistent with the possibility that drug resistance in terms of anhedonia and behavior may also be reflected in drug resistance in terms of vascular pathology (43). However, the current experimental protocol does not give us the possibility to distinguish between central and peripheral effects of escitalopram. But previous studies (13, 43) suggest a central action of the drug.

In accordance with previous results (30, 32, 33), CMS-susceptible rats showed an increased locomotor activity. Although the locomotor activity was not affected by escitalopram treatment, an anxiolytic-like effect and increased explorative behavior were observed in escitalopram responders (Fig. 2). These findings are in line with previous observations that both...
depression- and anxiety-like characteristics are reversed, at least in part, by antidepressant treatment in CMS rats (71).

Previous studies have shown that in rats exposed to CMS, an elevation of heart rate is associated with a significant reduction in cardiac output (27). In the current study, we found an elevated heart rate in CMS-susceptible rats compared with the unstressed group, but no difference in blood pressure. Escitalopram treatment did not affect heart rate and blood pressure in CMS-susceptible rats. These findings are consistent with a previous report showing that another SSRI, fluoxetine, failed to affect heart rate and cardiac output in rats exposed to CMS (27). Thus, escitalopram treatment does not seem to have a major effect on rat heart rate and blood pressure, which might suggest a lack of significant effect on total peripheral resistance, although this conclusion needs to be confirmed by measurements of cardiac output.

Escitalopram treatment changes the balance between different pathways of endothelial relaxation. We have recently demonstrated that CMS susceptibility is associated with reduced endothelium-dependent relaxation in rat mesenteric small arteries (8). A comprehensive analysis of factors involved in the relaxation revealed that this was associated with suppressed EDH-like response and accordingly a reduction in IKCa channel expression. Interestingly, endothelial dysfunction in Type 2 diabetic rats is also associated with reduced IKCa expression (10, 69), and this expression change was, at least in part, due to elevated oxidative stress of endothelial cells (72). Whether increased oxidative stress in depression-like state (5, 39, 45, 48) can affect IKCa is not known.

The suppression of EDH-like response in CMS-susceptible rats was, in part, compensated by upregulation of endothelial NO-synthase (eNOS) (8). Treatment with escitalopram did not normalize the endothelium NO signaling, but other pathways of endothelium-dependent relaxation were affected by the treatment (Fig. 6). Therefore, it seems that the depression-associated increase of the NO pathway is caused by mechanisms that are unaffected by SSRI treatment. We found, however, that escitalopram treatment significantly potentiated the EDH-like component of relaxation and that this effect was most pronounced in escitalopram responders. The observed changes are in contrast to a previous report showing that SSRI (fluoxetine) treatment in mice improves NO-dependent relaxation and suppresses EDH-like relaxation in the aorta (38). These contradictory findings could be a consequence of vessel size. In fact, the contribution of NO to endothelium-dependent relaxation decreases with vessel diameter, while EDH exhibits an inverse pattern contributing to a small extent in large arteries.

Changes in the balance between different pathways of endothelium-dependent relaxation also involve eicosanoid signaling. In accordance with our previous study (8), we have not found any changes in COX-1 signaling in CMS-susceptible rats treated with vehicle compared with unstressed rats. Surprisingly, COX-1 inhibition increased the ACh sensitivity of arteries from escitalopram-treated rats, suggesting that the COX-1 pathway is involved in the production of procontractile factors after escitalopram treatment. The synthesis and signaling pathways of eicosanoids are highly branched (66) and provide a high degree of flexibility for vascular responses to COX activation (18). Because in our previous (8) and current studies, the inhibition of COX-1 and COX-2 had opposite effects on the endothelium-dependent relaxation of the same artery, it is unlikely that the procontractile action of a COX-1 product is due to an unspecific action of overproduced prostacyclin on overexpressed thromboxane receptors, as it has previously been suggested for arteries from hypertensive rats (19). A possibility is that COX-1 may be involved in generation of the vasococontractor thromboxane A2 (37), but the role of other contractile prostaglandins, e.g., PGE2, PGH2, cannot be excluded (18).

Inhibition of the COX-2-dependent pathway suppressed the relaxation of arteries from vehicle-treated CMS-susceptible rats, suggesting that this is probably mediated via prostacyclin production (19). This is in accordance with our previous findings (8). We found here that escitalopram treatment significantly suppressed the effect of CMS on COX-2 signaling, minimizing its role in relaxation. Interestingly, while escitalopram treatment abolished the effect of COX-2 inhibition in escitalopram nonresponders, COX-2 inhibition further improved ACh relaxation in escitalopram responders. Changes in the balance of COX-1/COX-2 activities are often associated with the inflammatory state (60) and are known to be dependent on oxidative stress (18). COX-2 has been shown to be more sensitive to ROS than the constitutively expressed COX-1 (51, 63). Oxidative stress associated with depression symptoms (5, 39, 45, 48) could primarily stimulate COX-2 activity, and this activity will be normalized when oxidative stress is reduced because of antidepressant treatment. Thus, we suggest that oxidative stress may play an important role.

CMS and escitalopram treatment modulate oxidative stress markers in a tissue-specific manner. Meta-analysis indicates an inverse association between antioxidant enzymes and cardiovascular disease (20). At the same time, elevation of oxidative stress is a known feature of major depression (1). Accordingly, it has been shown that exposure to repeated and unpredictable stressors increases ROS production in the rat brain (21, 47, 48, 68), which, in turn, results in oxidative damage in the central nervous system. Moreover, studies investigating the oxidative stress profile found a reduction in antioxidant enzymes and elevation of the products of lipid peroxidation in the blood of patients with major depression (5, 44, 49, 56). We have tested an association between oxidative stress and CMS susceptibility by estimating the total concentration of the major antioxidant, glutathione, and a general product of peroxidation of membrane polyunsaturated fatty acids by ROS and MDA. Regula-
Perspectives and Significance

This study demonstrated that SSRI treatment partly normalized the endothelial dysfunction associated with depression-like symptoms in small resistant arteries. We suggest that CMS-induced endothelial dysfunction contributes to changes in blood perfusion of vital organs which, in turn, can contribute to the pathology and comorbidity between major depression and cardiovascular diseases. In accordance with the hypothesis of an association between depression symptoms and small artery function (9), escitalopram treatment partially normalized the balance between the different pathways of endothelium-dependent relaxation. Changes in EDH-like and COX-2-dependent relaxation correlated with the responsiveness of rats to escitalopram treatment. Further studies are needed to describe the mechanisms responsible for the association between depression-like symptoms and endothelial dysfunction. It will be important to find out whether the improved endothelial function after SSRI treatment is a result of or contributes to the antidepressive action of these drugs. The changes in oxidative stress markers were found to be well correlated with stress and antidepressive responsiveness, suggesting the importance of oxidative state for behavioral and cardiovascular changes.

ACKNOWLEDGMENTS

We thank Kim Henningsen and Stine Dhiin for help in running of the CMS model; Jørgen Andreasen, Jane Holbæk Rønn, and Viola Smed Larsen for excellent technical assistance in vitro experiments. We are especially thankful to David Farr for careful reading and proofing of the manuscript.

GRANTS

The study was supported by the Danish Heart Foundation, A. P. Møller and his wife C. Mc-Kinney-Møller Foundation for Medical Science Promotion, the Lundbeck Foundation, and the Saint-Petersburg State University Research Grant 1.0.133.2010.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: V.V.M. and E.V.B. conception and design of research; V.V.M., V.V.K., and E.V.B. performed experiments; V.V.M. and E.V.B. analyzed data; V.V.M. and E.V.B. interpreted results of experiments; V.V.M. and E.V.B. prepared figures; V.V.M. and E.V.B. drafted manuscript; V.V.M., O.W., C.A., and E.V.B. edited and revised manuscript; V.V.M., O.W., C.A., and E.V.B. approved final version of manuscript.

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