Cardiovascular alterations and autonomic imbalance in an experimental model of depression

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Grippo, Angela J., Julia A. Moffitt, and Alan Kim Johnson. Cardiovascular alterations and autonomic imbalance in an experimental model of depression. Am J Physiol Regulatory Integrative Comp Physiol 282: R1333–R1341, 2002.—Depressed patients with and without a history of cardiovascular pathology display signs, such as elevated heart rate, decreased heart rate variability, and increased physiological reactivity to environmental stressors, which may indicate a predisposition to cardiovascular disease. The specific physiological mechanisms associating depression with such altered cardiovascular parameters are presently unclear. The current study investigated cardiovascular regulation in the chronic mild stress rodent model of depression and examined the specific autonomic nervous system mechanisms underlying the responses. Sprague-Dawley rats exposed to a series of mild, unpredictable stressors over 4 wk displayed anhedonia (an essential feature of human depression), along with elevated resting heart rate, decreased heart rate variability, and exaggerated pressor and heart rate responses to air jet stress. Results obtained from experiments studying autonomic blockade suggest that cardiovascular alterations in the chronic mild stress model are mediated by elevated sympathetic tone to the heart. The present findings have implications for the study of pathophysiological links between affective disorders and cardiovascular disease.

autonomic blockade; cardiovascular disease; chronic mild stress; heart rate variability

MAJOR DEPRESSIVE DISORDER (clinical depression) (1) is a debilitating psychological condition that affects an individual’s mental and physical health. Although the psychological aspects of depression (e.g., affect, negative temperament, and cognitive deficits) have been studied extensively, the physiological and pathophysiological consequences of this disorder are not well understood. Depression is an independent risk factor for coronary artery disease (3). Previous research demonstrated that depression predisposes an individual to myocardial infarction, sudden death, thrombosis, and arrhythmias (23). This association exists in patients with currently diagnosed coronary artery disease as well as individuals with no prior history of heart disease, but for whom the physiological pathology is imminent.

Although the prevalence of depression in the general population is 2–9% (1), its prevalence among postmyocardial infarction patients is estimated to be 45% (31). Major depression doubles the risk that patients with newly diagnosed coronary artery disease will experience an adverse cardiovascular event (e.g., myocardial infarction and sudden death) within 12 mo (6). Its impact on the pathogenesis of subsequent cardiovascular disease is equivalent to that of a history of previous myocardial infarction or smoking (5, 12). Depressed patients with no history of heart disease are also at risk for cardiovascular pathology. Approximately 50% of patients who are depressed at the time cardiovascular disease is initially diagnosed have a prior history of depression (8).

Patients with depressive disorder display some functional cardiovascular characteristics similar to those observed in heart disease. For example, medically well, but psychologically depressed, patients often exhibit increased resting heart rate (HR) (17). In a group of individuals with cardiovascular disease, HR was found to be greater in depressed patients, compared with those with no evidence of psychological disorders (7). Elevated resting HR is related to sudden death, myocardial ischemia, and cardiac failure, and it is also linked to cardiovascular risk factors such as hypertension, elevated blood glucose, and increased body mass index (11, 26). Depression is also associated with reduced HR variability. Fluctuations in the intervals between heartbeats are mediated by autonomic inputs, with HR variability reflecting the interaction between sympathetic and parasympathetic influences on the cardiac pacemaker (34). Changes in heart period are negatively correlated with severity of depression; cardiac patients with more severe depression display lower HR variability scores than those with less severe depression (16). Decreased HR variability is found in patients with coronary artery disease, and it predicts arrhythmic complications (25) and long-term survival (15) following myocardial infarction.

Studies with human populations demonstrate a link between depression and coronary artery disease, but they have not progressed beyond correlational analy-
METHODS

Animals. Twenty-two male Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 300–400 g, were used for the experimental procedures. Rats were allowed 1 wk to acclimate to the surroundings before beginning any experiment.

Animals were housed in individual plastic cages with bedding. Food (Purina Rat Chow 5012) and tap water were available ad libitum for the duration of the experiments unless otherwise noted. Sucrose solution (1%) was available ad libitum for 1 wk preceding the experimental procedures to allow for adaptation to the taste of the sucrose. The temperature was maintained at 22 ± 2°C. The light cycle was held at 12:12 h with lights on at 0600, unless otherwise noted. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Iowa Animal Care and Use Committee.

Sucrose preference tests. Sucrose preference tests, similar to those described by Muscat and Willner (22), were employed to operationally define anhedonia. Specifically, anhedonia was defined as a reduction in sucrose preference relative to a control group and baseline values. A sucrose preference test consisted of first removing the food and water from each rat’s cage for a period of 20 h. Water and 1% sucrose were then placed on the cages in preweighed glass bottles, and animals were allowed to consume the fluids for a period of 1 h. The bottles were then removed and weighed. Two baseline preference tests were performed, separated by at least 5 days, and the results were averaged. Preference tests were conducted weekly throughout the CMS period.

CMS. After two baseline sucrose preference tests, animals were randomly separated into two groups, CMS (n = 12) and control (n = 10). The CMS procedure was a variation of methods described by Solberg et al. (32), and it was designed to maximize the unpredictable nature of the stressors. The CMS group was exposed to the following stressors in random order: continuous overnight illumination, 40° cage tilt along the vertical axis, paired housing, soiled cage (300 ml water spilled into bedding), restraint in a small cage (equipped with breathing holes), exposure to an empty water bottle immediately following a period of acute water deprivation, stroboscopic illumination (300 flashes/min), and white noise. Details of the CMS procedure, including time and length of activities, are presented in Table 1. The CMS procedure was carried out for a total of 4 wk. Control animals were left undisturbed in the home cages with the exception of general handling (i.e., regular cage cleaning and measuring body weight), which was matched to that of the CMS group. Immediately following the CMS period, all animals were instrumented with femoral arterial and venous catheters for the recording of arterial blood pressure and administration of drugs.

Catheter surgery. Surgical procedures for the implantation of catheters were conducted while the animals were under halothane anesthesia, with the use of aseptic surgical techniques. Polyethylene (PE-10 fused to PE-50) catheters were inserted into the aorta and abdominal vena cava via the left femoral artery and vein for measurement of arterial pressure and administration of pharmacological agents, respectively. Catheters were tunneled subcutaneously and exteriorized at the dorsal cervical region. They were filled with heparinized saline (200 U/ml) and capped with airtight plugs when not in use. Animals were given butorphanol (3 mg/kg sc) for postoperative analgesia. After immediate recovery from anesthesia, animals were returned to their cages for an additional 72 h before the collection of cardiovascular data began.
Arterial pressure recordings. Direct mean arterial pressure (MAP) was recorded in unrestrained, unanesthetized rats (CMS and control groups). Animals were removed from their home cages and placed in the testing cages with no access to food or water. Catheters were connected to a pressure transducer (Maxxim Medical, Athens, TX) coupled to a multichannel recorder through a custom-designed amplifier (University of Iowa, Iowa City, IA). The analog input was converted into a digital signal using a PowerLab data-acquisition system (ADInstruments, Mountain View, CA). This program permits sampling of hemodynamic data directly onto a computer. MAP was derived electronically using a low-pass filter. HR was determined by measuring the number of heartbeats triggered from the arterial pressure pulse. Hemodynamic parameters were monitored for 30–60 min to ensure stabilization of MAP and HR. After stabilization, these baseline parameters were continuously recorded for 10 min.

HR variability recordings. A 10-min period of stable arterial pressure was recorded to evaluate the variations in heart period. The systolic pulse recording was statistically analyzed by taking the standard deviation of all normal-to-normal (N-N) intervals from the systolic pulse waveform, from a 5-min segment of data in each individual rat (standard deviation of N-N interval (SDNN) index, as described by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (35)). A group mean SDNN index was calculated from these individual values.

Cardiac autonomic blockade. The study of HR was performed on a randomly selected subset of animals (n = 5 CMS and n = 6 control) under conditions of pharmacological autonomic blockade, using procedures similar to Perlini et al. (28). HR and HR variability responses were measured under the following conditions over a 2-day period: 1) alone (without autonomic blockade), 2) during β-adrenergic receptor blockade with propranolol (2 mg/kg iv), 3) during muscarinic cholinergic receptor blockade with methylatropine (1 mg/kg iv), and 4) during complete autonomic blockade with propranolol + methylatropine. The order of drug administration was counterbalanced across all animals so that each animal received either propranolol or methylatropine alone on the first day, and it received the opposite drug alone on the second day. No more than two air jet tests were performed on the same day. Animals were returned to their home cages between the first and second day of testing.

The protocol for delivering the air was similar to that described by Stauss et al. (33). A flexible hose (0.7-cm internal diameter) connected to a cylinder of compressed room air was directed at the top of the rat’s head from a distance of ~5 cm. Air pressure was maintained at 20 psi, an intensity that was strong enough to part the fur on the rat’s head. The compressor was located in a separate room so as to minimize noise. The air jet stimulus was directed at the rat for a period not exceeding 3 min, during which time the resultant changes in MAP and HR were recorded. Animals were allowed at least 20 min between air jet presentations and before any further experimental manipulations took place. Care was taken not to include data in the analysis during animal movement.

Data analysis. Values are presented as means ± SE for the indicated experiments. One-hour sucrose preference tests were conducted before commencing the protocol (baseline) and weekly throughout the CMS period. Water and sucrose bottles were weighed to determine the amount of fluid consumed (in grams). Data from the preference tests were analyzed using mixed-design ANOVAs (1 factor for independent groups and 1 repeated-measures factor) and Student’s t-tests where appropriate. Body weight was statistically compared using a mixed-design ANOVA. Baseline resting hemodynamic parameters were analyzed using independent Student’s t-tests. The absolute responses in MAP and HR to autonomic blockade and air jet stress were analyzed with mixed-design ANOVAs, whereas changes from baseline were compared using independent Student’s t-tests. A Bonferroni correction was used for any multiple comparisons. A probability value of P < 0.05 was considered to be statistically significant.

RESULTS

Sucrose preference tests. Figure 1 displays the fluid intake during the sucrose preference tests used to define anhedonia in the CMS and control groups at...
baseline and throughout the CMS period. Figure 1A presents absolute water and sucrose intake in the two groups. Mixed-design ANOVAs were performed on water and sucrose intake separately, across time (baseline through 4 wk CMS). No significant differences in water intake were found at any point during the protocol. An ANOVA performed on sucrose intake yielded a main effect of time \( F(4, 70) = 388.19 \) but no main effect of group. No differences in sucrose intake between the two groups at baseline. After 4 wk of CMS, the preference for sucrose was reduced in the CMS group compared with control animals and baseline values.

To determine whether the CMS procedure significantly affected the body weight of animals in the present experiments, an ANOVA was performed on the body weights of both groups across time (baseline through 4 wk CMS). No main effect of group was found. Table 2 presents the body weights of both groups throughout the protocol. There were no differences in body weight between CMS and control groups at baseline or throughout the CMS protocol.

**Baseline resting hemodynamic parameters.** Baseline resting MAP and HR were examined in the CMS and control groups following 4 wk of the CMS procedure. Resting MAP was \( 125 \pm 3 \) mmHg in the CMS group and \( 124 \pm 3 \) mmHg in the control group. These values were not significantly different. Resting HR was \( 382 \pm 7 \) beats/min in the CMS group and \( 364 \pm 8 \) beats/min in the control group. HR was significantly elevated in the CMS group. All statistical tests hereafter that report values relative to baseline are compared with these initial hemodynamic parameters.

**HR variability.** The SDNN index (35) was used as an indicator of HR variability. All N-N intervals were calculated (in milliseconds) during a 5-min period while the animal was resting. The standard deviation of all N-N intervals was computed for each animal. These standard deviations were then averaged across all animals in a group. The baseline resting SDNN index for control was \( 7.5 \pm 0.9 \) ms vs. \( 5.3 \pm 0.8 \) ms for CMS. Compared with controls, the CMS group exhibited significantly reduced HR variability.

**Cardiac autonomic blockade.** The alterations in resting HR following selective and complete autonomic blockade were analyzed in five CMS and six control rats using a mixed-design ANOVA for \( \beta \)-adrenergic receptor blockade with propranolol, muscarinic cholinergic receptor blockade with methylatropine, and complete autonomic blockade with both agents. Figure 2 displays resting HR in CMS and control groups under

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**Table 2. Body weight in CMS and control groups at baseline and during weeks 1–4 of the CMS protocol**

<table>
<thead>
<tr>
<th>Body Weight, g</th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMS</td>
<td>316 ± 2</td>
<td>353 ± 2</td>
<td>374 ± 3</td>
<td>390 ± 1</td>
<td>407 ± 1</td>
</tr>
<tr>
<td>Control</td>
<td>306 ± 3</td>
<td>350 ± 1</td>
<td>376 ± 4</td>
<td>395 ± 4</td>
<td>408 ± 3</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. As expected, a main effect of time \( F(4, 70) = 388.19 \) but no main effect of group was found. No differences in body weight between CMS and control groups were found at baseline or at any point during the CMS period.
suggesting a trend toward reduced parasympathetic baseline resting HR, discussed above in Baseline resting HR is shown in Fig. 2 and complete autonomic blockade. The absolute resting-adrenergic receptor, muscarinic cholinergic receptor, /H9252 groups. to selective and complete autonomic blockade in CMS and control Fig. 2. A: mean (±SE) absolute resting heart rate (HR) in response to selective and complete autonomic blockade in CMS and control groups. B: mean (±SE) changes in resting HR (from baseline) following selective and complete autonomic blockade in CMS and control groups. Baseline HR values were 382 ± 7 and 364 ± 8 beats/min (bpm) in CMS and control groups, respectively. A main effect of drug treatment was found [P(3,40) = 34.85]. The CMS group displayed a greater bradycardia in response to propranolol administration, compared with the control group [t(9) = 1.85]. *P < 0.05 vs. control.

Changes in resting HR (Fig. 2B) were analyzed following β-adrenergic receptor, muscarinic cholinergic receptor, and complete autonomic blockade. The absolute resting HR is shown in Fig. 2A, and change in HR (from baseline resting HR, discussed above in Baseline resting hemodynamic parameters) is shown in Fig. 2B. A main effect of drug treatment was found; however, the main effect of group and the interaction effect were not significant.

Changes in resting HR (Fig. 2B) were analyzed following β-adrenergic receptor, muscarinic cholinergic receptor, and complete autonomic blockade, and they were compared with the groups’ baseline resting HR values (reported above in Baseline resting hemodynamic parameters). Compared with control animals, the CMS rats showed a greater bradycardia following β-adrenergic receptor blockade with propranolol (−52 ± 11 vs. −24 ± 9 beats/min for CMS and control, respectively), indicating greater sympathetic influence in this group. After cholinergic receptor blockade with methylatropine, the tachycardia was attenuated in the CMS animals, compared with control rats (+56 ± 3 vs. +70 ± 11 beats/min for CMS and control, respectively), suggesting a trend toward reduced parasympathetic tone in the CMS group. The intrinsic HR (under total autonomic blockade with propranolol and methylatropine) was not significantly different between CMS and control groups.

Figure 3 displays the mean SDNN index in CMS and control groups during rest (baseline) and under β-adrenergic receptor blockade with propranolol. The absolute HR variability response (SDNN index) was analyzed with a mixed-design ANOVA following β-adrenergic and cholinergic receptor blockade. No significant main effects or interactions were found. Further analyses suggested that HR variability was slightly elevated in both groups during β-adrenergic receptor blockade with propranolol and that the statistically significant difference between the groups disappeared (relative to baseline resting HR variability). Blockade of muscarinic cholinergic receptors with methylatropine did not affect the HR variability of either group.

Air jet stress. MAP and HR responses during air jet stress were analyzed in five CMS and five control animals using a mixed-design ANOVA for air jet alone, air jet + β-adrenergic receptor blockade with propranolol, air jet + cholinergic receptor blockade with methylatropine, and air jet + complete autonomic blockade with both agents. MAP and HR changes were compared with the groups’ respective baseline values (reported above in Baseline resting hemodynamic parameters) using Student’s t-tests.

Figure 4 displays the pressor and HR responses to air jet stress in CMS and control groups. Absolute pressor and change in blood pressure (from baseline) are shown in Fig. 4A, whereas absolute HR and change in HR (from baseline) are shown in Fig. 4B. The ANOVA for MAP yielded significant main effects of drug and group but no significant interaction effect. Compared with control rats, the CMS animals showed

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a greater pressor response to air jet stress alone. The ANOVA for HR yielded significant main effects of drug and group but no significant interaction effect. Compared with control rats, the CMS animals showed a greater tachycardic response to air jet stress alone.

Figure 5 presents the HR responses to air jet stress under pharmacological autonomic blockade in CMS and control groups. Data are represented as absolute responses and changes from baseline. For mean arterial pressure (MAP), main effects of drug treatment [F(4,40) = 8.27] and group [F(1,40) = 5.55] were found. For HR, main effects of drug treatment [F(4,40) = 19.37] and group [F(1,40) = 5.87] were observed. The CMS group displayed exaggerated pressor [t(8) = 1.84] and HR [t(6) = 3.26] responses to air jet stress, relative to the control group. *P < 0.05 vs. control.

**DISCUSSION**

The purpose of the present investigation was to determine the effects of exposure to CMS on behavioral and physiological responses in rats. To investigate these effects, we measured hedonic function, basal MAP and HR, an index of HR variability, HR responses to pharmacological autonomic blockade, and cardiovascular responses to air jet stress alone and under autonomic blockade. Animals exposed to CMS for 4 wk displayed anhedonia as indicated by reduced sucrose preference, relative to control and baseline values. Importantly, the CMS group showed many altered cardio-
vascular responses compared with the control group. Rats exposed to CMS exhibited resting tachycardia with no evidence of altered blood pressure. These animals displayed reduced HR variability as determined by the SDNN index. The CMS group also showed greater pressor and tachycardic responses to air jet stress than the control group. The responses to autonomic blockade suggest that the cardiovascular alterations in the CMS model are primarily due to an underlying elevation of cardiac sympathetic tone. Exposure to chronic stress, therefore, has both psychological (behavioral) effects and physiological consequences involving elevated sympathetic tone to the heart.

In the present study, animals exposed to CMS exhibited anhedonia, which is an essential component of human depression. The reduced sucrose preference seen in the CMS group is consistent with previous research. The examination of sucrose preference, rather than absolute sucrose intake, is a sensitive and specific measure of anhedonia. A generalized decrement in fluid intake can be ruled out in the present study by the finding that the CMS procedure altered sucrose consumption, but it left water intake unaffected in the CMS group. Our data also indicate that the anhedonia is not secondary to a loss in body weight, as both groups gained weight at the same rate during the protocol (Table 2).

Resting hemodynamic parameters were altered in animals exposed to CMS, similar to signs observed in human depression. We found resting tachycardia and decreased HR variability in the CMS group. Elevated resting HR and reduced HR variability are observed in depressed individuals and depressed patients with established heart disease. Data from the present study also indicate that exposure to an environmental stressor (air jet stress) elevates blood pressure and HR in both the CMS and control groups. Research suggests that air jet stress leads to increases in MAP, HR, and renal and mesenteric vascular resistance, as well as decreases in hindquarter vascular resistance. In the present study, animals exposed to CMS showed exaggerated pressor and tachycardic responses to air jet stress, compared with controls. Depressed human patients display increased plasma norepinephrine concentrations and elevated components in the hypothalamic-pituitary-adrenal system (e.g., cortisol, and adrenocorticotropic hormone) in response to stressors and orthostatic challenges, suggesting a hyperreactive physiological stress response. The similarities between the animal model and the human condition of depression contribute additional evidence supporting the face validity of CMS as a model of this psychological disorder.

In addition to further characterizing behavioral and physiological signs associated with the CMS model, the present study provides insight into an underlying influence of cardiovascular regulation in depression. Animals exposed to CMS displayed significantly elevated sympathetic tone to the heart, compared with controls. When β-adrenergic receptors were blocked with propranolol, the bradycardic response (relative to baseline resting HR) was greater in the CMS group than the control group. There are several possible explanations for this effect. Norepinephrine acts on the heart through β-adrenergic receptors to increase both HR and contractility. Therefore, elevated resting HR may derive from increased levels of neuronal norepinephrine release or target organ hypersensitivity to catecholamines. Moreover, depressive disorder may involve altered epinephrine and norepinephrine release from the adrenal medulla, which can ultimately lead to increased cardiac output and peripheral resistance. The mediating components in the altered HR response may also be located within the central nervous system.

Autonomic blockade also affected the HR variability in animals exposed to CMS. Blockade of β-adrenergic receptors with propranolol abolished the difference in HR variability between the CMS and control groups. This suggests that the variations in heart period may be influenced by sympathetic nervous system inputs to the heart in depression. Although it is possible that neural or humoral factors are mediating the sympathetic nervous system effects on HR variability in the CMS group, it is noteworthy that propranolol administration increased the statistical variability in both groups of rats (Fig. 3). A specific interpretation of the increased variance of HR variability (which, by definition, is itself a measure of variation) requires further investigation.

Similar to the resting HR responses, the increased tachycardia to air jet stress in the CMS group also appears to be mediated by the sympathetic nervous system. The CMS group displayed a greater tachycardia to air jet stress alone compared with controls (as shown in Fig. 4B). However, in contrast to this finding and the resting HR difference between the two groups following β-adrenergic receptor blockade, both groups exhibited similar HR responses to air jet stress under β-adrenergic receptor blockade. These data suggest that the exaggerated HR response to air jet stress in the CMS model of depression is sympathetically mediated.

Although the CMS group displayed exaggerated pressor responses to air jet stress alone compared with the control group, the reason for this effect cannot be determined by the selective autonomic blockade. Neither propranolol nor methylatropine affected the pressor responses to air jet stress of CMS or control in the present experiments. It is possible that neurohumoral factors, such as angiotensin or vasopressin, are altered in the CMS model of depression. However, because no baseline blood pressure differences were observed between the two groups in the present study, it is not very likely that a generalized humoral alteration is responsible for the exaggerated pressor responses to air jet stress in the CMS group. Alternatively, circulating epinephrine may influence the cardiovascular responses to air jet stress by affecting HR, cardiac output, or peripheral resistance. Cardiac output and total peripheral resistance are the major determinants of systemic arterial pressure.

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The present study is one of the first to characterize altered cardiovascular responses in the CMS model of depression. The current findings implicate elevated sympathetic tone as a peripheral mechanism underlying the impaired cardiovascular regulation in the CMS model. These data complement the finding that rats with bilateral removal of the olfactory bulbs, which is an animal model of depression used for the screening of antidepressant drugs, display altered sympathoexcitatory reflexes (19). The research approaches used in these studies may provide useful clinical information regarding pathophysiological mechanisms underlying depressive disorder and coronary artery disease.

Further studies investigating the specific association between anhedonia and cardiovascular pathology are necessary to determine whether these conditions can be physiologically linked via autonomic or other mechanisms. The CMS model, similar to other animal models of psychological dysfunction, involves the use of stressors to induce depressive signs. Indeed, the effects of environmental stressors on the cardiovascular system have been studied extensively (30). Therefore, it will be useful to examine the specific time course of the behavioral and cardiovascular effects of CMS. It may also be interesting to examine the effects of behavioral and pharmacological treatments on the relationship between anhedonia and cardiovascular pathology as well as associated central nervous system mechanisms.

The current investigation highlights a need for further research to elucidate the specific mechanisms that underlie depression and cardiovascular pathology. Although traditional treatments for depression may be effective for many psychiatric patients, they may not reduce the risk of cardiovascular morbidity and mortality unless the underlying pathophysiological mechanisms are also altered. Depression may have residual psychological and physiological effects that do not normalize following its successful treatment (29). The study of pathophysiological mediators underlying depression and cardiovascular dysfunction in animal models may lead to enhanced understanding of causal and/or common mechanisms and the development of more comprehensive treatments for patients with depression and cardiovascular disease.

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