Effect of chronic volume overload on baroreflex control of heart rate and sympathetic nerve activity

ROLAND WILLENBROCK, HARALD STAUSS, MICHAELA SCHEUERMANN, KARL JOSEF OSTERZIEL, THOMAS UNGER, AND RAINER DIETZ
Franz-Volhard-Klinik, Virchow-Klinikum, and the Max-Delbrück-Centrum für Molekulare Medizin, Institut für Physiologie der Charité, Humboldt-Universität, 13125 Berlin; and Institut für Pharmakologie, Christian-Albrechts Universität, 24105 Kiel, Germany

Willenbrock, Roland, Harald Stauss, Michaela Scheuermann, Karl Josef Osterziel, Thomas Unger, and Rainer Dietz. Effect of chronic volume overload on baroreflex control of heart rate and sympathetic nerve activity. Am. J. Physiol. 273 (Heart Circ. Physiol. 42); H2580–H2585, 1997.—Baroreceptor-heart rate reflex sensitivity is decreased in congestive heart failure. The reflex control of heart rate and sympathetic nerve activity in rats with chronic volume overload, an established model for moderate heart failure, is still unknown. Therefore, we investigated the regulation of humoral and neuronal sympathetic activity and the baroreflex control of heart rate and sympathetic nerve activity in conscious, unrestrained rats with aortocaval shunt. Rats with aortocaval shunts had larger hearts (388 ± 11 vs. 277 ± 4 mg/100 g body wt), elevated central venous pressures (14 ± 4 vs. 4 ± 3 mmHg), and higher atrial natriuretic peptide plasma levels (87 ± 16 vs. 25 ± 3 pmol/l) than controls but had similar systemic blood pressure and heart rate values. Plasma epinephrine (0.63 ± 0.16 vs. 0.21 ± 0.08 pmol/l, P < 0.05) and norepinephrine concentrations (0.27 ± 0.03 vs. 0.16 ± 0.02 pmol/l, P < 0.05) were elevated in shunted rats compared with controls. Nitroprusside-induced hypotension led to a significantly greater increase in efferent splanchic sympathetic nerve activity in shunted rats than in controls (0.9 ± 0.1 vs. 2.6 ± 0.6 µV, P < 0.05), whereas the heart rate responses were not different between the groups. These results indicate that the regulation of the autonomic nervous system is altered in chronically volume-overloaded rats. The arterial baroreflex control of efferent splanchic sympathetic nerve activity was dissociated from the control of heart rate. Therefore, analysis of the activation of sympathetic nervous system assessed by direct measurements of efferent sympathetic nerve activity appears to be more sensitive for the detection of altered autonomic nervous system function than the analysis of baroreflex control of heart rate.

CONCLUSION

A decrease of the arterial baroreflex sensitivity could be demonstrated by several groups in human heart failure (16) and in experimental heart failure induced by rapid atrial pacing in dogs (7, 38). In rats with heart failure induced by myocardial infarction, both the arterial baroreflex and efferent renal sympathetic nerve activity were attenuated (9). In rabbits with pacing-induced heart failure, increased sympathetic activity, decreased vagal tone, and impaired baroreflex sensitivity were observed simultaneously (23).

Regulation of the autonomous nervous system during the early stages of heart failure is particularly interesting, because a depressed baroreflex function may affect survival. Hohnloser et al. (19) and Osterziel et al. (27) showed previously that altered autonomous nervous activity as assessed by baroreflex sensitivity and plasma norepinephrine concentrations is a predictor of survival in patients with heart failure and after myocardial infarction. The same observations could be made in rabbits with experimental myocardial infarction where baroreflex sensitivity correctly identified the survivors (18).

Whether baroreflex sensitivity and efferent sympathetic nerve activity are already disturbed before overt heart failure develops is still unknown. To our knowledge, no direct measurements of efferent sympathetic nerve activity and its regulation by the arterial baroreflex had previously been performed in chronically volume-overloaded rats.

MATERIALS AND METHODS

Animals. Male Wistar rats (230–250 g, Thomae, Biberach, Germany) were fed normal rat chow and allowed free access to tap water. The animals were kept on a 12-h light-dark cycle. All experiments were performed between 7 and 12 AM. The studies were approved by the local authorities and were performed according to the “Guiding Principles for Research Involving Animals and Human Beings” corresponding to American Physiological Society guidelines. The studies were performed with seven to eight animals in each group.

Shunt operation. The aortocaval shunt was induced under ether anesthesia by a modified method developed by García and Diebold (15). Briefly, a laparotomy was performed and the aorta was punctured with a 1.2-mm disposable needle (Braun Melsungen, Melsungen, Germany) distal to the renal

ABNORMALITY OF THE ARTERIAL BARORECEPTOR-HEART RATE REFLEX IN HEART FAILURE has been known for almost three decades (11). The baroreflex arc includes the baroreceptors (carotid artery and aorta) as part of the afferent limb, the central processing (nucleus tractus solitarii), and the efferent pathways (for review see Ref. 31). In healthy subjects, the arterial baroreflex is regulated by a tonically excited vagal afferent activity and a tonically inhibited sympathetic tone (for review see Ref. 34). In heart failure, the main abnormality of the baroreflex is located at the afferent arc, specifically at the carotid sinus baroreceptors that show diminished threshold and peak discharge when the blood pressure is lowered. The most sensitive part of the baroreflex is the baroreceptor unloading where abnormal regulation could first be observed after a blood pressure decrease with nitroprusside in conscious, paced dogs (5). These results could be confirmed in heart failure patients when the disturbed baroreflex regulation was most pronounced during baroreceptor unloading (12).

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The blood was placed in prechilled heparinized tubes, and conscious, chronically instrumented animals in a quiet room. Urine measurements were taken from the carotid artery of control animals were treated identically, and the vessels were dissected free of surrounding tissue, clamped, and glued, but no puncture of the vessels was performed. Experiments were carried out 30 days after shunt operation.

Determination of catecholamines and atrial natriuretic peptide (ANP). Blood samples for epinephrine and norepinephrine measurements were taken from the carotid artery and the vena cava. After the vessels were temporarily clamped, the plasma was kept at 2°C and pepstatin (3 µM). The blood was centrifuged at 4°C at 1000 g for 10 min, 4°C). For determination of catecholamines (30), 1 ml of HCl was added to 1 ml of plasma. After centrifugation, 100-µl aliquots of the supernatants were stored at 80°C until determination by the radioenzymatic method (6). Blood samples for ANP (500 µl) were withdrawn from the carotid artery in Na-EDTA preloaded (final concentration 7 mM) and prechilled tubes. Degradation of ANP was prevented with phenylmethylsulfonyl fluoride (final concentration 10 µM) and pepstatin (3 µM). The blood was centrifuged at 4°C at 2000 g for 10 min immediately after withdrawal, and the plasma was kept at 80°C until extraction with C18 Sep-Pak columns following a previously described protocol (17). Samples were then measured by radioimmunoassay (17), which was performed with ANP-antibodies kindly provided by Dr. J. Gutkowska, Montreal, Canada.

Chronic instrumentation for measurements of baroreflex and sympathetic nerve activity. Three days before the experiment, the rats were anesthetized (chloral hydrate 400 mg/kg) and instrumented with catheters placed in the carotid artery and in the superior vena cava. The arterial catheter consisted of a polyethylene (PE)-10 catheter (outer diameter 0.61 mm), which was inserted into the common carotid artery and sealed to a PE-50 catheter to minimize resistance. The catheters were exteriorized at the nape of the neck for recording of blood pressure and heart rate and for administration of drugs. During implantation of the carotid artery catheters, care was taken not to touch the vagus nerve or the area of the carotid bifurcation. After arterial and venous catheters were implanted, the area of the left splanchnic nerve was exposed retroperitoneally through a flank incision during the same session. The splanchnic nerve branch between the celiac ganglion and the suprarenal complex was dissected free of fat and connective tissue for a length of 10 mm. The nerve was placed on a thin, bipolar stainless steel electrode and insulated with a small amount of silicon rubber gel (Wacker Silgel 604 A, Munich, Germany) as described previously (33). The wires of the electrodes were tunneled under the skin and exteriorized at the neck.

To verify the postganglionic efferent nature of the splanchnic nerve signals, bolus injections of the ganglionic blocker hexamethonium (10 mg/kg) were given at the end of the experiments. All rats included in the study demonstrated a dramatic decrease in nerve activity by this procedure. Measurements of sympathetic nerve activity and baroreflex sensitivity. Three days after the catheters and nerve electrodes were implanted, recordings were performed in conscious, unrestrained animals. After the animals were allowed an acclimatization period of 30 min, we measured basal sympathetic nerve activity, heart rate, and blood pressure, and we assessed the baroreflex sensitivity by lowering blood pressure with sodium nitroprusside (20 µg/100 µl infusion over 30 s). In preliminary experiments we demonstrated that this dose similarly decreased blood pressure between 30 and 35 mmHg in control and shunted rats.

Signal recording. On the day of the recordings, catheters were attached to a Statham pressure transducer (P23 XL), which was connected to a Gould pressure processor 2600 for signal amplification. Heart rate was derived from the arterial blood pressure signal. The signals were analog-to-digital converted and recorded with a commercially available PC-based recording and analyzing system (MEGA, Stauss and Weidner, Ettlingen, Germany). The sampling rate for all signals was 40 Hz. Splanchnic nerve activity was amplified with a differential preamplifier (bandwidth 30 Hz-10 kHz), rectified, and further amplified (range of amplification: 1 × 10^4 to 3 × 10^5) and integrated with a second-order, 20-Hz, low-pass filter (29).

Hemodynamic measurements. The assessment of cardiac function was performed in a different set of experiments in anesthetized rats (chloral hydrate 400 mg/kg). Left ventricular end-diastolic pressure and maximal pressure development over time (dp/dt max) were measured by cannulating the right carotid artery and advancing the catheter (PE-50) into the left ventricle. The left ventricular pressure was registered with a Statham transducer (P23 XL) and a Gould AMP 4600 amplifier, and dp/dt max was obtained from a Gould differentiator (G4615).

Statistical analysis. Differences between groups were evaluated with the unpaired Student’s t-test. The significance level was set at P < 0.05. All data are expressed as means ± SE.

RESULTS

Hemodynamic evaluation of aortocaval shunt model. Rats with an aortocaval shunt developed significant hypertrophy of all heart chambers after 30 days (Table 1). Hemodynamic parameters demonstrated an increase of central venous pressures from 4 ± 3 to 14 ± 4 mmHg (P < 0.05) and normal left ventricular contractility (dp/dt max). Systolic and diastolic blood pressure as well as heart rate were unchanged. Plasma levels of ANP increased more than threefold in chronic volume-overloaded rats (25 ± 3 to 87 ± 16 pmol/l, P < 0.01).

<p>| Table 1. Body weight, heart weight, and hemodynamic parameters |</p>
<table>
<thead>
<tr>
<th>Control</th>
<th>Shunt</th>
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<tbody>
<tr>
<td>BW, g</td>
<td>336 ± 4</td>
</tr>
<tr>
<td>RA, mg</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>LA, mg</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>RV, mg</td>
<td>173 ± 7</td>
</tr>
<tr>
<td>LV, mg</td>
<td>515 ± 17</td>
</tr>
<tr>
<td>HW/BW, mg/100 g BW</td>
<td>277 ± 4</td>
</tr>
<tr>
<td>CVP, mmHg</td>
<td>4 ± 3</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>142 ± 12</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>92 ± 9</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>348 ± 22</td>
</tr>
<tr>
<td>dp/dt max, mmHg/s</td>
<td>5,159 ± 425</td>
</tr>
<tr>
<td>ANP, pmol/l</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>SNA, µμl</td>
<td>8.1 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 shunt and 7 control rats. BW, body weight; RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; HW/BW, relative heart weight to body weight; CVP, central venous pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; dp/dt, first derivative of left ventricular pressure; ANP, atrial natriuretic peptide; SNA, sympathetic nerve activity. Experiments were performed 30 days after shunt induction. *P < 0.05, †P < 0.01 vs. control.
Plasma and cardiac norepinephrine concentrations. As shown in Fig. 1A, plasma epinephrine concentrations in rats with aortocaval shunt increased about threefold and plasma norepinephrine increased by ~70% (P < 0.05). Cardiac norepinephrine concentrations decreased significantly in both atria, whereas no significant difference was found in the ventricles (Fig. 1B). We also calculated the cardiac norepinephrine content (based on the cardiac weights, Table 1) and we did not obtain a lower norepinephrine content in any of the heart chambers (data not shown).

Baroreflex control of heart rate. Whereas heart rate and blood pressure in conscious control and volume-overloaded rats were not different under baseline conditions (Table 1), we analyzed the response to baroreceptor unloading by lowering arterial blood pressure with sodium nitroprusside. The original recording of a control and a shunted rat is shown in Fig. 2 and demonstrates the decrease of blood pressure and the parallel increase in heart rate. The decrease in mean arterial blood pressure was ~30 mmHg in both groups (29.3 ± 1.3 in controls, 30.6 ± 3.5 mmHg in shunted rats, Fig. 3A) and induced a similar maximal increase in heart rate, as shown in Fig. 3B. The slope for the shortening of the interbeat interval is shown for all animals in Fig. 4A. In control rats, the slope (Δinterbeat interval/Δblood pressure) was −0.604 ± 0.065 ms/mmHg and was not different from chronically volume-overloaded rats (slope = −0.576 ± 0.089 ms/mmHg).

Regulation of sympathetic nerve activity. We analyzed whether arterial baroreflex control of sympathetic nerve activity was affected by chronic volume overload. During baseline conditions, no difference in sympathetic nerve activity between sham-operated controls and volume-overloaded rats was observed (controls: 8.1 ± 1.1 µV, shunted rats: 7.6 ± 1.8 µV, Table 1). The original recordings in Fig. 2 show the change in sympathetic nerve activity for a control and a shunted rat in parallel with the blood pressure and heart rate recordings. Decrease of arterial blood pressure increased the sympathetic nerve activity three times more in volume-overloaded rats than in sham-operated controls (0.8 ± 0.2 vs. 2.5 ± 0.5 µV, P < 0.05, Fig. 3C). Similarly, the slopes for the increase of sympathetic nerve activity in shunted rats were significantly steeper in chronically volume-overloaded rats (m = 0.090 ± 0.022) than in controls (m = 0.026 ± 0.006, P < 0.05, Fig. 4B), indicating a stronger activation of sympathetic outflow to the periphery in response to baroreceptor unloading.

DISCUSSION

Impaired baroreceptor-heart rate reflex sensitivity has previously been described in human and experimental heart failure (7, 9, 16). However, no data are available on the baroreflex sensitivity of sympathetic nerve activity in aortocaval-shunted rats, an established model of chronic volume overload. Decreases in arterial blood pressure induced significantly higher increases in sympathetic activity in volume-overloaded rats than in sham-operated controls, indicating that
The baroreflex control of sympathetic nerve activity is modified in rats with chronic volume overload. The baroreflex control of heart rate, however, was not affected by an aortocaval shunt. Thus, alterations in baroreflex control of sympathetic nerve activity could be dissociated from modulations of the baroreflex control of heart rate.

The dissociation between baroreflex control of efferent sympathetic activity and of heart rate may be explained by an unchanged afferent portion and a modified efferent portion of the baroreflex arc. Although it was not measured in this study, downregulation of cardiac adrenergic β-receptors is a well-known characteristic of heart failure and cardiac hypertrophy (for review see Refs. 3 and 20). In rats with aortic insufficiency, decreased baroreflex sensitivity could be linked to β-adrenergic receptor downregulation (35). Downregulation of cardiac β-adrenergic receptors might indeed explain the dissociation between the baroreflex control of efferent sympathetic nerve activity and heart rate and may be a reason why an increased sympathetic response to baroreceptor unloading was not translated into a stronger cardiac acceleration in chronically volume-overloaded rats.

To assess the degree of systemic and cardiac sympathetic activation, we measured plasma and tissue catecholamine concentrations. In this model, norepinephrine concentrations were decreased in both atria, which developed hypertrophy earlier and to a relatively greater extent than the ventricles. In plasma of volume-overloaded rats, an increase in concentrations of epinephrine and norepinephrine could be observed. This elevation of plasma catecholamines suggests an early sympathetic activation in chronic volume-overloaded rats. It may, however, be puzzling that basal sympathetic nerve activity was unchanged, while plasma catecholamines were increased. This observation might be explained by a differential control of sympathetic outflow to various organ systems (1) such as the splanchnic region or the heart. In addition one has to keep in mind that measurements of plasma catecholamines are of limited value for the assessment of peripheral sympathetic outflow (14). Nevertheless, sympathetic nerve activity showed a greater response to baroreceptor unloading in shunted rats than in control animals. In an earlier study, surgically induced aortocaval shunt led to congestive heart failure in dogs and impaired the baroreceptor-heart rate reflex (25). However, no data of the blood pressure-induced regulation of efferent sympathetic nerve activity in chronically volume-overloaded rats were available up to now.

The aortocaval shunt model that we used induced cardiac hypertrophy and elevated central venous pressures along with neurohumoral activation but did not affect resting cardiac contractility (dP/dt max). This indicates that cardiac function was not severely impaired at this stage. The shunt-induced model of chronic volume overload has been described to induce heart failure depending on the shunt size and duration (21, 22, 26). In our study, we did not show evidence for heart failure but rather concentrated on early changes occurring during chronic volume overload in the absence of overt heart failure. It should be mentioned that the results obtained in our study cannot automatically be transferred to early stages of human heart failure because species differences and a different pattern of progression of heart failure could limit the generalization of the results.

To the best of our knowledge, this is the first report describing that baroreflex control of efferent sympathetic activity is impaired even before baroreflex control of heart rate is affected. It is important to note that our experiments were performed in conscious and unrestrained rats. Anesthesia is well known to modify the regulation of the autonomic nervous system function and of the baroreflex control of both heart rate and efferent sympathetic nerve activity (10).

Several mechanisms could have contributed to the early alterations of baroreflex control in this model. The aortocaval shunt model is characterized by an activated ANP system (40, 41). We measured a threefold increase of plasma concentrations of ANP in aorto-

Fig. 3. Changes (maximal values) in mean arterial blood pressure (ΔMBP, A), heart rate (ΔHR, B), and splanchnic sympathetic nerve activity (ΔSpNA, C) after infusion of sodium nitroprusside in control and shunted rats. All parameters in both groups were significantly different after infusion compared with values before infusion. Data are presented as means ± SE, n = 7–8, *P < 0.05 vs. Δ in controls.
caval-shunted rats. ANP has been suggested to modulate baroreflex function (for review see Ref. 37). More than 30 years ago, long before the discovery of ANP, acute volume expansion, the classical mechanism to release large amounts of ANP, had been described to reduce baroreflex sensitivity in dogs (36). Later studies confirmed that acute volume expansion decreased heart rate and inhibited sympathetic activity in newborn lambs (32) and in anesthetized dogs (39). Intravenous administration of ANP decreased baroreceptor sensitivity (4), augmented the parasympathetic effects in anesthetized rats (2), and inhibited sympathetic activity in humans (13). Even though high doses of ANP were employed in these studies compared with the relatively small increase we observed in volume-overloaded rats, a stimulation of the ANP system and its effect on the baroreflex may have contributed to the early changes in baroreceptor reflex regulation that we observed in our study.

The angiotensin system has been proposed to contribute to the impaired baroreflex control of heart rate in heart failure. We and others (8, 24, 28) demonstrated that the inhibition of the angiotensin-converting enzyme or of the angiotensin II type 1 receptor (AT₁-receptor) improved the baroreflex control. In the model of chronic volume overload, a moderate activation of the renin-angiotensin-aldosterone system occurs (41), and it is conceivable that this mechanism might contribute to the early depression of baroreflex control of sympathetic nerve activity, which we report in this study. Thus, an influence of humoral activation of the renin-angiotensin-aldosterone system and of ANP might contribute to the impaired baroreflex control of sympathetic nerve activity.

In summary, we demonstrated alterations of baroreceptor control of sympathetic nervous system function in chronically overloaded rats. Because the baroreflex control of efferent sympathetic activity is dissociated from the control of heart rate, these alterations can only be detected if direct sympathetic nerve activity recordings are performed and if the cardiovascular system is stimulated by procedures such as baroreflex unloading. Analysis of the arterial baroreflex control of heart rate alone might not be sensitive enough to detect early changes in autonomic nervous system function.

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Address for reprint requests: R. Willenbrock, Franz-Volhard-Klinik, Wiltbergstr. 50, 13125 Berlin, Germany.

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