Baroreflex sensitivity and heart rate variability in conscious rats with myocardial infarction

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Baroreflex sensitivity and heart rate variability (HRV) were studied in conscious rats with myocardial infarction. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2240–H2247, 1997.—The baroreflex sensitivity (BRS) and the heart rate variability (HRV) were studied in conscious rats after myocardial infarction (MI; induced by coronary artery ligation) and after sham operation (SH). BRS was determined by linear regression of R-R interval vs. arterial pressure changes induced by nitroprusside or methoxamine (intravenous bolus). HRV was calculated from 3-min electrocardiogram recordings. Left ventricular end-diastolic pressure and plasma atrial natriuretic peptide were increased after MI; plasma norepinephrine and basal heart rate (HR) remained unchanged. At 3 and 28 days after MI, BRS was reduced as indicated by decreased reflex bradycardia (RB) (MI, 0.66 ± 0.13 and 0.78 ± 0.07 ms/mmHg; SH, 1.27 ± 0.16 and 1.48 ± 0.14 ms/mmHg, respectively; P < 0.05 MI vs. SH). At 56 days after MI, BRS was normalized, RB was unaffected by atropine 3 and 28 days after MI but reduced in all other groups. The increase of basal HR by atropine 3 and 28 days after MI was less than in all other groups. HRV (SD of mean R-R interval, coefficient of variance, low- and high-frequency power; studied at 28 and 56 days) was similar in all groups. It is concluded that BRS is transiently depressed in rats with left ventricular dysfunction after MI probably due to a reduced reflex vagal activity. Even though basal HR and HRV are unchanged after MI, a temporary attenuation of tonic vagal activity is unmasked after autonomic blockade.

A previous myocardial infarction (MI) is one of the most common causes of left ventricular dysfunction in humans. Approximately 50% of all deaths after MI have been classified as sudden deaths, the majority of which are caused by ventricular tachyarrhythmias (16). Evidence has been provided that both the baroreflex control of heart rate (“baroreflex sensitivity”; BRS) (3, 27) and the variability of the R-R interval (“heart rate variability”; HRV) during sinus rhythm (16, 20) may be impaired after MI and may identify subgroups of patients with a high susceptibility to malignant ventricular arrhythmias (13, 19). However, available data concerning both evidence and time course of reduction of both BRS and HRV are varying between studies. This may be due to inhomogeneous study populations (e.g., in respect to infarct localization or degree of left ventricular dysfunction) and/or differences in pharmacological and invasive therapy. Therefore, it is desirable to further characterize a small animal model. Experimentally induced chronic ligation of a coronary artery in rats has been shown to provide a MI model that has the advantage of highly reproducible infarct size and localization (24) and thus allows the study of a homogeneous group of experimental animals with chronic left ventricular dysfunction. To date, both BRS and HRV have been studied only at a single time point after MI in rats with pronounced left ventricular dysfunction. A reduced reflex tachycardia but preserved overall BRS (10) and an impaired HRV (30) were observed after MI. It remains unclear whether alterations of BRS and HRV are present only temporarily and whether they are dependent on post-MI hemodynamic changes. It was the aim of the present study to investigate the time course of possible alterations of both reflex and tonic control of heart rate (HR) in rats with MI and only moderately impaired left ventricular function.

Both BRS (7, 12) and HRV (2, 4) are considered as measures of autonomic nervous system activity. Because it is difficult to assess sympathetic and especially vagal tone from direct neural recordings in conscious rats, it was of interest whether the methods used to assess BRS and HRV in this study are suitable to estimate both sympathetic and vagal tone.

METHODS

Coronary artery ligation. All experiments of this investigation were approved by the federal authority and conform to the “Guide for the Care and Use of Laboratory Animals” [Department of Health and Human Resources Publication No. (NIH) 85–23, Revised 1985]. A transmural anterior MI was produced in modification of a method previously described (24). Adult male Sprague-Dawley rats (Charles River Wiga, Kisslegg, Germany; 230–275 g) were anesthetized with chloral hydrate (400 mg/kg ip, Riedel de Haen, Seelze, Germany). An oral endotracheal tube was inserted, and mechanical ventilation with room air was instituted. A left-sided thoracotomy was performed, and the proximal left anterior descending coronary artery (LAD) was ligated in situ. Each MI was confirmed by three criteria: 1) inspection immediately after LAD ligation in situ (paleness of left anterior ventricular myocardium); 2) inspection of the heart in situ after thoracotomy at the end of the interventions (groups studied 3 days after ligation, paleness; 28 and 56 days, signs of left ventricular fibrosis); and 3) macroscopic signs of chronic MI at autopsy. All rats with ligation fulfilled these criteria and therefore made up the MI groups. Rats appointed to the sham-operated (SH) groups were subjected to the same procedure except the LAD was not ligated. We did not measure infarct size in the present study. However, according to previous observations in our laboratory, and as described by others (11, 24), an increase of left ventricular...
end-diastolic pressure (LVEDP) to values of 10–15 mmHg reflects an infarct size of –30–45%. The perioperative mortality was 45% in MI rats and ~1% in SH rats.

Experimental protocol. Different groups of rats were studied 3, 28, and 56 days after SH and MI. On the day before measurements, each rat was anesthetized with chloral hydrate (400 mg/kg ip) to allow for electrocardiogram (ECG) and catheterization of the left femoral artery and vein with heparinized polyethylene tubes (0.58- and 0.4-mm ID, respectively; overall length 50 mm). The free ends of the catheters were subcutaneously led to the back of the neck, exteriorized, and protected using a jacket with a steel tether (Harvard Apparatus, Kent, UK). On the following day (i.e., 3, 28, and 56 days after SH and MI), all experiments were carried out in conscious rats under standardized conditions to avoid possible influences of circadian variation. Via the arterial catheter, 1.5-ml blood samples were withdrawn and replaced by an equal volume of heparinized donor rat blood. The arterial catheter was then connected to a pressure transducer (P231 ID, Gould, Cleveland, OH). The pressure signal was amplified by a manometer (Hugo Sachs, March-Hug-stetten, Germany) and recorded on a two-channel ink writer (Brush 220, Gould). Mean arterial pressure (MAP) was calculated as diastolic pressure plus one-third of the difference between diastolic and systolic pressures. Both R-R interval and HR were derived by beat-to-beat analysis of the peak systolic pressure signal with a HR coupler (Biotachometer; Hugo Sachs).

Pharmacological interventions. The influence of β-adrenoceptor inhibition on basal HR was studied 3, 28, and 56 days after SH and MI. A bolus intravenous injection of 0.5 mg/kg metoprolol (0.1-metoprolol; Sigma, Munich, Germany; dissolved in 1 ml/kg saline) was administered (followed by 200-µl saline flush) to conscious rats. The effect of muscarnic receptor inhibition with 0.5 mg/kg atropine (atropine methyl bromide; Sigma) on basal HR was studied in the same manner in a separate series of rats. The doses of metoprolol and atropine chosen evoked immediate changes of basal HR that remained constant over at least 90 min, but did not affect basal MAP.

Determination of baroreflex sensitivity. To study BRS, intra venous bolus injections of the vasodilator nitroprusside (sodium nitroprusside; Sigma) in femoral vessels (0.2–2.0 µg/kg; each in 100 µl of 0.9% saline followed by 200 µl of 0.9% saline flush to flush the catheter) were administered with constant monitoring of MAP and R-R interval. Thereafter, four increasing doses (2.0–20.0 µg/kg) of the α1-adrenoceptor agonist methoxamine (methoxamine hydrochloride; Sigma) were injected in the same manner. Subsequent injections of nitroprusside or methoxamine were only performed when MAP and R-R interval had returned to baseline. The doses of nitroprusside and methoxamine were chosen according to the results of previous experiments (unpublished data) so that the linear range of the relationship between MAP and R-R interval (12, 29) was covered, i.e., changes of basal MAP up to ±25 mmHg. After examination of BRS, each rat was anesthetized with chloral hydrate (100 mg/kg iv) with subsequent tracheotom y and cannulation of the trachea for mechanical ventilation. A transverse thoracotomy was then instituted, and the heart was visually inspected. The left ventricle was directly cannulated at the apex (1.1-mm ID; Venofix S, Braun, Melsungen, Germany) for measurement of LVEDP. The pressure signal was recorded as described above. The influence of metoprolol and atropine on BRS was additionally studied, as described above.

For each injection of either nitroprusside or methoxamine, the peak change of MAP and the maximum of the thereby induced change of beat-to-beat measured R-R interval were determined. With these data pairs, linear regression analysis was performed for each rat. Only animals with a correlation coefficient R >0.8 and a P value <0.05 were included into the study. The BRS was expressed as the slope of the individual regression line.

Measurements of HRV. In the studies of HRV, two electrodes for chronic recording of apex-base lead ECG were implanted subcutaneously under anesthesia (chloral hydrate, 400 mg/kg ip). The electrodes were led to the back of the neck, exteriorized, and inserted into a custom-made plug. For ECG recordings, the plug was connected to a rotating swivel, allowing the conscious rat to remain undisturbed. After an accommodation period of 3 min, the ECG was recorded for 3 min under standardized conditions (see above) by modification of a method previously described (28). The signals (0.05–2,500 Hz) were amplified using a one-channel ECG amplifier (Schiller, Bahr, Switzerland) and digitized with a time resolution of 0.1 ms (10-kHz sampling frequency) and 12-bit amplitude resolution by means of a data acquisition board (DT 2812, Data Translation, Marlboro, MA) and a laptop computer (T 3200, Toshiba, Tokyo, Japan). On a 486-DX50 computer (DSM, Munich, Germany), R-wave recognition and R-R interval tachogram calculation and analysis were carried out. The A/D board of the R spike served as a reference point for the temporal location of the R wave. Tachograms were checked visually for misdetections and ectopic beats, which were interpolated linearly by taking the mean value of the preceding and following R-R interval. Tachograms containing >800 beats were excluded from processing because of the averaging requirements of the spectral analysis (see below). In the time domain, the mean interval between normal beats ("R-R interval"), its standard deviation, and the coefficient of variance [CV; 100 x standard deviation of mean N-N interval (SDNN)/mean R-R interval] were calculated. For analysis in the frequency domain, the tachogram was divided into segments of 256 intervals overlapping each other by half. After removal of the linear trend and application of the Hanning window, each segment was padded with 256 zeros, submitted to a fast Fourier transform, and magnitude-squared for calculation of the power spectrum according to the periodogram method. The power spectra of all segments were averaged to reduce the variance of fast Fourier transform as spectral estimator. Moreover, the obtained average power spectrum was smoothed using a three-point sliding rectangular window. According to previous HRV studies in rats (4), two regions of interest were defined: low-frequency (LF; >0.5 Hz, <0.8 Hz) and high-frequency bands (HF; >0.8 Hz up to Nyquist frequency, determined by the mean R-R interval of the tachogram, generally <4.5 Hz), expressed as percent of total spectral power. From each rat, ECG signals were recorded both 28 and 56 days after SH and MI. In an additional series of control rats, a venous catheter was inserted as described above. Three days after surgery, ECG signals were recorded in conscious rats both before and 10 min after intravenous injection of atropine (0.5 mg/kg).

Determination of plasma concentrations of atrial natriuretic peptide and norepinephrine. For determination of atrial natriuretic peptide (ANP), blood samples were immediately cooled, stabilized by addition of K2-EDTA (final concentration 1 mg/ml), and centrifuged at 4,000 revolutions/min for 10 min. The plasma was stored at −20°C until analysis. The plasma samples were extracted as previously described (14). The ANP was determined by radioimmunoassay using a polyclonal antiserum (Peninsula Laboratories, Heidelberg, Germany). Norepinephrine was radioenzymatically assayed (9).

Statistics. Results are expressed as means ± SE. Differences between separate groups were tested by analysis of
Results

Baseline characteristics. At time of study, neither respiratory distress nor ascites, peripheral edema, or pleural effusion was noted in any animal. Total body weight was not different between respective SH and MI rats studied in different groups 3, 28 and 56 days postoperatively (Table 1). However, total heart weight-to-body weight index was increased after MI as compared with controls (Table 1). The basal HR was similar in all groups, whereas body weight-to-body weight ratio was not different from SH rats (Table 1). The total wet lung weight-to-body weight index was increased after MI as compared with controls (Table 1). The basal MAP was lower 28 and 56 days after MI as without major change over time (Table 1). However, basal MAP was lower 28 and 56 days after MI as compared with SH rats (Table 1). The basal HR was similar in all groups, whereas body weight-to-body weight ratio was not different from SH rats (Table 1). The total wet lung weight-to-body weight ratio was not different between SH and MI groups (Table 1). The MAP was not significantly affected by either drug in any group (data not shown).

Pharmacological inhibition of the autonomic nervous system. The reduction of basal HR induced by metoprolol (0.5 mg/kg iv bolus) was similar between the respective SH and MI groups 3, 28, and 56 days postoperatively (Table 2). In contrast, the increase of basal HR after application of atropine (0.5 mg/kg iv bolus) was markedly less pronounced in the 3- and 28-day MI groups, as compared with SH controls (Table 2). However, this difference in atropine-induced HR changes between SH and MI was no longer observed 56 days after operation, thus representing a normalization in MI rats. The MAP was not significantly affected by either drug in any group (data not shown).

BRS. The average MAP and R-R interval changes induced by nitroprusside and methoxamine are shown in Fig. 1, A–C. With the individual data pairs, BRS was analyzed for effects of either nitroprusside (causing reflex tachycardia) or methoxamine (causing reflex bradycardia). Reflex tachycardia was not different between any SH and MI group (Table 3; Fig. 2). In contrast, reflex bradycardia was significantly reduced in both the 3- and the 28-day groups as compared with controls (Table 3; Fig. 3). However, reflex bradycardia was normalized 56 days after MI (Table 3; Fig. 3). To examine the relationship between BRS and parameters of left ventricular dysfunction, linear regression analysis was performed with these data. There was no significant correlation between reflex bradycardia and either total heart weight-to-body weight index, basal HR, basal MAP, LVEDP, plasma norepinephrine, or ANP at any of the three time points after MI (data not shown).

Table 2. Effects of metoprolol and atropine on basal heart rate

<table>
<thead>
<tr>
<th>Change of Basal Heart Rate, beats/min</th>
<th>Metoprolol</th>
<th>Atropine</th>
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<tbody>
<tr>
<td>Sham operation</td>
<td></td>
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<tr>
<td>3 Days</td>
<td>−32.0±9.3 (6)</td>
<td>+51.6±14.5 (5)</td>
</tr>
<tr>
<td>28 Days</td>
<td>−37.0±10.9 (5)</td>
<td>+61.2±10.2 (6)</td>
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<tr>
<td>56 Days</td>
<td>−42.0±10.3 (5)</td>
<td>+51.9±9.1 (8)</td>
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<tr>
<td>Myocardial infarction</td>
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<tr>
<td>3 Days</td>
<td>−27.2±6.2 (6)</td>
<td>+17.0±4.3* (7)</td>
</tr>
<tr>
<td>28 Days</td>
<td>−41.5±5.3 (10)</td>
<td>+25.6±5.6* (12)</td>
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<tr>
<td>56 Days</td>
<td>−58.0±6.4 (5)</td>
<td>+66.3±8.3 (6)</td>
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</table>

Values are means ± SE of number of rats given in parentheses. Effects of metoprolol (0.5 mg/kg iv bolus) and atropine (0.5 mg/kg iv bolus) on basal heart rate at respective postoperative day are shown. *P < 0.05 vs. sham-operated group (treated with atropine) of same time point.

Additional experiments were carried out in subgroups treated with either metoprolol or atropine as mentioned above. In animals pretreated with metoprolol, reflex tachycardia was studied that was attenuated both in SH and MI rats as compared with controls without pretreatment at 3, 28, and 56 days (Table 3; Fig. 2). In animals pretreated with atropine, reflex bradycardia was investigated that was markedly reduced in the SH groups, as compared with controls without atropine (Table 3; Fig. 3). In contrast, 3 and 28 days after MI, no additional attenuation of reflex bradycardia by atropine was found as compared with MI controls without pretreatment. However, in the 56-day MI group, atropine impaired reflex bradycardia similarly as in SH rats (Table 3; Fig. 3).

HRV. To validate the effect of muscarinic receptor inhibition on HRV, ECG signals were recorded both
before and 10 min after intravenous injection of atropine (0.5 mg/kg) in control rats without thoracotomy (n = 6). In addition, a mean reduction of the R-R interval of \(-29 \pm 5\) ms (P < 0.05 between control and treatment) and a decrease of SDNN of \(-2.3 \pm 0.9\) ms (P < 0.05) in the time domain was induced by atropine. In the frequency domain, LF and HF power was markedly reduced (LF, \(-1.4 \pm 0.4\%\); HF, \(-9.9 \pm 2.9\%\), i.e., differences in percent of total spectral power; P < 0.05 both).

Furthermore, HRV was determined in seven SH and seven MI rats both at 28 and 56 days. There were no significant differences between the respective SH and MI group in any of the HRV parameters determined both in the time (mean R-R interval, SDNN, CV) and frequency domain (LF and HF power, LF/HF) (Table 4).

**DISCUSSION**

Severity of left ventricular dysfunction. In the present study, several parameters were determined to assess the severity of left ventricular dysfunction after MI. In all MI groups, the LVEDP, one of the most sensitive criteria of decreased left ventricular function (24), was approximately doubled as compared with SH groups. An increase of LVEDP to values of 10–15 mmHg reflects an infarct size of 30–45% (11, 24). The interindividual variance of LVEDP was very low in all SH controls as well as within each MI group, thus indicating not only a high reproducibility of the technique used for measurement of LVEDP, but also a high homogeneity with respect to left ventricular dysfunction within MI groups. In agreement with previous investigations, several other characteristics of moderately impaired left ventricular systolic function were observed; the plasma concentrations of ANP were approximately threefold higher after MI as compared with SH controls (14). Moreover, the total heart weight-to-body weight index was increased in all MI groups. However, there was no evidence of severe heart failure, since the plasma levels of norepinephrine were not elevated, the lung weight-to-body weight index and total body weight remained unchanged, and neither pleural effusion nor ascites was observed. Furthermore, basal HR remained unchanged, and only a slight decrease in MAP was found 4 and 8 wk after MI. In summary, these observations clearly demonstrate the induction of moderate left ventricular dysfunction associated with compensated heart failure early after MI without major changes over the first 8 wk of the post-MI period.

Effect of pharmacological autonomic inhibition on basal heart rate. Even though basal HR remained unchanged in MI rats, a latent imbalance of autonomic control of HR might occur in these rats. Therefore, the

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**Fig. 1.** Average changes (Δ) of mean arterial pressure (MAP) and R-R interval induced by bolus injections of nitroprusside or methoxamine (each in 4 increasing doses) 3 (A), 28 (B), and 56 days (C) after sham operation and after myocardial infarction. Bold dashed lines were calculated by linear regression of average R-R interval vs. MAP (separately for nitroprusside and for methoxamine). Values are means ± SE.
effect of pharmacological inhibition of β-adrenoceptors (by metoprolol) and muscarinic acetylcholine receptors (by atropine) on basal HR was investigated. The dose of either drug was chosen so that MAP remained unchanged to avoid interferences with the baroreflex component of HR control. The decrease of basal HR by metoprolol was not different between MI and SH rats, which may indicate that the tonic sympathetic influence on HR is preserved in this model. In contrast, a reduced increase of basal HR by atropine was observed 3 and 28 days after MI. However, the effect of atropine on basal HR was normalized by the 56th day of the post-MI period. The fact that atropine had a reduced effect on basal HR 3 and 28 days after MI even though these rats had no change in basal HR and no difference in the effect of metoprolol on basal HR might be explained by a change of intrinsic HR. However, the intrinsic HR was not evaluated (by combined administra-

### Table 3. Baroreflex sensitivity and effects of metoprolol and atropine

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<thead>
<tr>
<th></th>
<th>BRS (Reflex Tachycardia), ms/mmHg</th>
<th>BRS (Reflex Bradycardia), ms/mmHg</th>
<th>BRS (Reflex Tachycardia): Effect of Metoprolol, ms/mmHg</th>
<th>BRS (Reflex Bradycardia): Effect of Atropine, ms/mmHg</th>
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<tr>
<td><strong>Sham operation</strong></td>
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<tr>
<td>3 Days</td>
<td>1.33 ± 0.19</td>
<td>1.27 ± 0.16 (7)</td>
<td>0.66 ± 0.08† (6)</td>
<td>0.64 ± 0.13† (5)</td>
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<td>28 Days</td>
<td>1.37 ± 0.19</td>
<td>1.48 ± 0.14 (8)</td>
<td>0.78 ± 0.09† (5)</td>
<td>0.58 ± 0.04† (6)</td>
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<td>56 Days</td>
<td>1.16 ± 0.15</td>
<td>1.47 ± 0.22 (7)</td>
<td>0.54 ± 0.14† (5)</td>
<td>0.50 ± 0.14† (8)</td>
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<td><strong>Myocardial infarction</strong></td>
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<tr>
<td>3 Days</td>
<td>1.12 ± 0.14</td>
<td>0.66 ± 0.13* (9)</td>
<td>0.64 ± 0.08† (6)</td>
<td>0.55 ± 0.08 (7)</td>
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<tr>
<td>28 Days</td>
<td>1.29 ± 0.12</td>
<td>0.78 ± 0.07* (15)</td>
<td>0.92 ± 0.10† (10)</td>
<td>0.63 ± 0.07 (12)</td>
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<tr>
<td>56 Days</td>
<td>1.24 ± 0.12</td>
<td>1.40 ± 0.19 (10)</td>
<td>0.78 ± 0.08† (5)</td>
<td>0.43 ± 0.07 (8)</td>
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</table>

Values are means ± SE of number of rats given in parentheses. BRS, baroreflex sensitivity; reflex tachycardia (induced by nitroprusside) and reflex bradycardia (induced by methoxamine) at respective postoperative day. Effects of metoprolol (0.5 mg/kg iv bolus) on reflex tachycardia and of atropine (0.5 mg/kg iv bolus) on reflex bradycardia are shown. *P < 0.05 vs. sham-operated group without pretreatment of same time point. †P < 0.05 vs. corresponding group without pretreatment of same time point (see also Figs. 2 and 3).

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**Fig. 2.** Baroreflex sensitivity (BRS; reflex tachycardia, induced by nitroprusside) and effect of pretreatment with metoprolol (0.5 mg/kg iv bolus) 3, 28, and 56 days after sham operation (A) and myocardial infarction (B). BRS is expressed as slope of regression line of individual data pairs of R-R interval vs. MAP. Values are means ± SE. †P < 0.05 vs. corresponding group without pretreatment of same time point. There were no significant differences between any sham operation and myocardial infarction group without pretreatment. For data, see Table 3.

**Fig. 3.** BRS (reflex bradycardia, induced by methoxamine) and effect of pretreatment with atropine (0.5 mg/kg iv bolus) 3, 28, and 56 days after sham operation (A) and myocardial infarction (B). BRS is expressed as slope of regression line of individual data pairs of R-R interval vs. MAP. Values are means ± SE. *P < 0.05 vs. sham-operated group without pretreatment of same time point. †P < 0.05 vs. corresponding group without pretreatment of same time point. For data, see Table 3.
control of HR in MI rats, BRS and HRV were assessed. To further analyze autonomic post-MI attenuation of tonic vagal activity is uncovered. In summary, these data indicate that a transient attenuation of metoprolol and atropine) in the present study. The basal HR is much higher than in humans. Parallel those in humans even though for a given MAP decreases in reflex bradycardia, whereas reflex tachycardia was not different between MI and SH groups. Likewise, the recovery of BRS observed 56 days after MI was due to a normalized reflex bradycardia.

In agreement with the present results, clinical studies have indicated that BRS is only temporarily depressed after acute MI and that BRS depression is due to impaired reflex bradycardia. In a study in post-MI patients without pronounced impairment of left ventricular function, BRS was found to be reduced 2 days after MI (22). In this study, BRS recovered within 10 days, whereas in other studies with greater variation in ventricular function, BRS was still diminished 7–10 days (13) and even 4 wk (19) after MI, respectively. Schwartz et al. (27) reported a decreased BRS 18 days after MI, which was followed by a normalization after 3 mo and no further change after 13 mo. These discrepancies might be explained by inhomogeneous study populations with, e.g., different medication and/or variation in infarct localization and extension. Taken together, it may be concluded that in humans BRS is depressed early after MI and recovers within the first months of the post-MI period. As indicated by the present investigation, a similar time course of BRS changes occurs in rats after MI. However, despite the well-documented similarity of post-MI ventricular remodeling between humans and rats (23), it has to be emphasized that chronic coronary artery ligation is principally different from MI in humans because the latter is usually preceded by arteriosclerosis and development of collaterals. It is remarkable that BRS alterations in rats parallel those in humans even though for a given MAP the basal HR is much higher than in humans.

With the data of the present study, a complete sigmoid curve of the relation of R-R interval vs. MAP, as previously described (15), cannot be generated. The aim was to measure primarily the linear portion of the R-R interval-MAP relationship. The pilot experiments revealed that doses of nitroprusside and methoxamine producing MAP changes up to ±25 mmHg provide linearity of the baroreflex curve calculated by linear regression analysis of the individual data pairs. Because larger MAP changes relating to the sigmoid part of the curve were not included, the operational point of the R-R interval cannot be identified. Despite similar levels of basal HR in all groups, a post-MI shift of the lower plateau HR may have occurred. As compared with the SH controls, the data pairs of the baroreflex curve may lie on the plateau portions and not on the linear center portion of the curve (rather than 28 days after MI; see Fig. 1, A and B). Therefore, it cannot be excluded that the decrease in reflex bradycardia observed 3 and 28 days after MI does not represent BRS depression alone but also changes in the range of the baroreflex, even though there were no significant differences in maximum changes of MAP induced by nitroprusside and methoxamine between the MI and SH groups. Not only HR but also basal MAP and stroke volume may influence BRS. However, basal MAP was reduced only 28 and 56 days after MI, but BRS was altered similarly 3 and 28 days after MI. No correlation was found between BRS data and basal MAP in any group, nor between BRS and basal HR. If smaller stroke volumes were present in the MI groups, the differences in BRS between SH and MI rats may have been even underestimated, because in animals with smaller stroke volumes, a bigger reflex change in R-R interval for a given change in MAP would be necessary (25). Stroke volume was not determined in the present study; however, there was no correlation between BRS data and either LVEDP or plasma ANP, thus indicating that BRS depression in rats was not a direct consequence of left ventricular dysfunction and emphasizing the value of BRS as an independent variable.

The temporal reduction of BRS in MI rats could be due to either an impaired reflex vagal activity or an increased sympathetic tone or both. Even though the pacemaker cells in the sinus node are influenced by both parasympathetic and sympathetic nerves, the beat-to-beat regulation of HR is primarily controlled by the vagus because of its very fast signal transduction.

### Table 4. Parameters of heart rate variability

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<th>n</th>
<th>R-R Interval, ms</th>
<th>SDNN, ms</th>
<th>CV, %</th>
<th>LF, %</th>
<th>HF, %</th>
<th>LF/HF, %</th>
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<tr>
<td>28 Days</td>
<td>7</td>
<td>152 ± 6</td>
<td>6.9 ± 0.8</td>
<td>4.5 ± 0.5</td>
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<td>26.5 ± 6.2</td>
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<tr>
<td>56 Days</td>
<td>7</td>
<td>146 ± 4</td>
<td>7.4 ± 1.2</td>
<td>5.1 ± 0.8</td>
<td>6.0 ± 1.5</td>
<td>22.4 ± 3.7</td>
<td>30.9 ± 7.5</td>
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<tr>
<td>Myocardial infarction</td>
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<tr>
<td>28 Days</td>
<td>7</td>
<td>154 ± 7</td>
<td>6.7 ± 1.4</td>
<td>4.2 ± 0.6</td>
<td>3.9 ± 1.1</td>
<td>20.2 ± 5.3</td>
<td>19.3 ± 4.2</td>
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<tr>
<td>56 Days</td>
<td>7</td>
<td>154 ± 5</td>
<td>5.7 ± 1.0</td>
<td>3.8 ± 0.7</td>
<td>3.9 ± 0.6</td>
<td>21.4 ± 3.5</td>
<td>22.1 ± 2.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. 28, 56 Days, days after sham operation and coronary artery ligation, respectively; SDNN, standard deviation of mean N-N interval; CV, coefficient of variance (100 × SDNN/mean R-R interval); LF, low-frequency power (<0.5 Hz < 0.8 Hz); HF, high-frequency power (<0.8 Hz) (both expressed as percentages of total spectral power). There were no significant differences between myocardial infarction and sham operation rats at respective time points.
Therefore, it has been postulated that the autonomic imbalance underlying the post-MI changes of instantaneous baroreflex control of HR is primarily due to a reduction of reflex vagal activity. In the present study, the depression of reflex bradycardia was not further augmented by inhibition of muscarinic receptors with atropine 3 and 28 days after MI. In contrast, atropine markedly reduced reflex bradycardia both in SH control rats and in the 56-day post-MI group to values similar to those observed 3 and 28 days after MI without pretreatment. These data provide further evidence for an impaired reflex vagal activity after MI.

Because reflex tachycardia was not altered in MI rats and could be inhibited by pretreatment with metoprolol to a similar extent in all MI and SH groups, it may be concluded that the sympathetic influence on BRS is unchanged in MI rats. However, because we measured BRS during acute changes of MAP, but not also after prolonged ramp infusions of vasoactive drugs, the sympathetic influence on BRS may have been evaluated only in part (5, 7). Impaired reflex tachycardia has been observed in more severe states of heart failure in rats 42 days after MI; however, overall BRS was found to be preserved in that study (10).

The present data do not allow us to discriminate further whether the afferent, the central integrating, or the efferent portion of the baroreflex arch is primarily involved in the transient BRS depression after MI. Moreover, the role of cardiac vs. arterial baroreceptors in this model needs further investigation. From previous BRS studies in heart failure dogs subjected to rapid ventricular pacing it may be concluded that the depressed baroreflex is related to a multifocal dysfunction at the level of arterial and/or cardiac baroreceptors and of the central nervous integration (6, 31). Even though the pathophysiology of this model of heart failure may be different, functional changes may be similarly responsible for BRS depression after MI. An immediate reduction of activity of cardiac vagal efferents in response to blood pressure rises during a 1-h coronary artery ligation has been described in cats (5). The present observations in rats revealing an attenuation of reflex bradycardia as soon as 3 days after MI may support the hypothesis of an early and therefore functionally reduced reflex vagal activity. An increase of cardiac afferent sympathetic nerve discharge has been previously shown to inhibit efferent vagal nerve activity directed to the sinoatrial node (26). Infarction-induced partial autonomic denervation within the ventricle may similarly enhance sympathetic nerve traffic (21, 33). It remains unclear whether the recovery of BRS in rats 8 wk after MI may be explained by a normalization of cardiac afferent sympathetic traffic (e.g., reinnervation during remodeling) (33) or by a functionally compensating mechanism (e.g., adaptation of central reflex integration over time).

HRV. In contrast to the depression of BRS, no significant changes of HRV parameters were observed, thus underlining the independence (13, 18) of the two methods used to assess autonomic HR control. The analysis of beat-to-beat HRV has been previously shown to provide an estimation of autonomic control of HR in humans (16, 20, 28) as well as in dogs (1) and rats (2, 17). In the time domain, a reduction of SDNN is considered to reflect a decrease in vagal tone (13). In the frequency domain, an impaired vagal tone has been attributed to a reduced power of the HF band, whereas the LF band is thought to be modulated by both sympathetic and vagal tone (2, 17). The very-low-frequency band was not analyzed in the present study because it is less suitable to detect sympathetic or vagal activity (4). Because R-R intervals in rats are much smaller than in humans and dogs, we developed a novel data analysis software to allow for adequate resolution of ECG signals. To validate whether the resolution of the method used is sufficient to evaluate vagal tone, the effect of atropine on HRV was examined in healthy control rats. Both SDNN and LF and HF power were reduced by atropine. However, both 28 and 56 days after MI, all HRV parameters determined in the time and frequency domain were unchanged. As previously described for HRV in rats, a relatively high interindividual variance of HRV parameters in the frequency but not in the time domain (17) was found. Because a transient post-MI reduction of resting vagal tone could be unmasked after autonomic blockade (see above) but could not be detected by the assessment of HRV without autonomic blockade, it may be concluded that HRV in rats is less dependent on the absolute vagal tone than on relative influences of vagal and sympathetic tone. Furthermore, both the threshold and the time course of infarction-induced changes in HRV may be different from the other estimates of vagal activity. In more severe states of heart failure in rats, HRV (studied in the time domain only) was found to be reduced 56 days after MI (30). In dogs, a reduction of HRV was observed at 5 but not at 10–30 days after MI (1); however, these HRV parameters remained decreased in a subgroup of MI dogs susceptible to ventricular fibrillation during the whole observation period. Clinical trials revealed HRV depression in patients 2 wk after MI with recovery 6 mo later (20). In summary, the present data indicate a normal HRV in rats with moderate left ventricular dysfunction 28 and 56 days after MI when analyzed as a whole group. The study design did not allow us to differentiate in regard to subgroups at increased risk for mortality. Moreover, the effects of atropine and β-adrenoceptor antagonists on HRV in MI rats have not yet been characterized. Likewise, it remains to be determined whether arterial blood pressure variability is altered in this model.

Conclusions. The present study indicates that BRS is transiently depressed in conscious rats with moderate left ventricular dysfunction after MI, whereas HRV remains unchanged. The BRS changes do not appear as a direct consequence of the hemodynamic alterations after MI. The methods used allow the evaluation of vagal activity and discrimination between its tonic and reflex components. It is concluded that MI rats exhibit temporarily reduced vagal activity; however, the attenuation of its tonic component is unmasked only after autonomic blockade. Because of the discrepancies in
HRV data between rats and humans described above, MI rats may have only a limited value as a model for studies of tonic vagal control of HR. However, both the time course and the pathophysiological pattern of BRS depression and recovery in rats parallel the BRS changes observed in humans after MI. There is evidence of a causal relationship between reduction of BRS and impaired hemodynamic tolerability of both general cardiovascular stress and sustained ventricular tachycardia (8, 18, 31). Therefore, therapeutic interventions improving BRS after MI are desirable. The MI rat may be considered as a small animal model to further investigate both pathophysiological mechanisms and pharmacological modulation of reflex control of heart rate.

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REFERENCES


