The bradycardic agent zatebradine enhances baroreflex sensitivity and heart rate variability in rats early after myocardial infarction

Carsten Krüger*, Vera Landerer, Christian Zugck, Heimo Ehmke, Wolfgang Kübler, Markus Haass

Department of Cardiology, University of Heidelberg, Bergheimer Strasse 58, D-69115 Heidelberg, Germany
Department of Physiology I, University of Heidelberg, Heidelberg, Germany

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Abstract

Objective: The bradycardic agent zatebradine (UL-FS 49) reduces heart rate without negative inotropic or proarrhythmic effects. The aim was to experimentally characterize the influence of zatebradine on arterial baroreflex sensitivity (BRS) and heart rate variability (HRV) which are generally considered as estimates of vagal activity and have prognostic value in patients after myocardial infarction (MI).

Methods: Conscious rats were studied 3 days after left coronary artery ligation or sham-operation (SH). BRS was determined by linear regression analysis of RR-interval and mean arterial pressure changes evoked by intravenous (i.v.) injections of methoxamine and nitroprusside. HRV at rest was calculated from high-resolution electrocardiogram-recordings.

Results: In MI-rats heart rate was similar to SH-rats, mean arterial pressure was lower and both BRS and HRV were markedly reduced. Zatebradine (0.5 mg/kg i.v.) reduced heart rate in MI-rats from 400±15 to 350±19 and in SH-rats from 390±19 to 324±6 beats/min without changing mean arterial pressure. Both BRS and HRV were restored in MI- and further increased in SH-rats by the drug. Effects of 0.05, 0.5 and 5 mg/kg zatebradine revealed a dose-dependency of heart rate reduction. The lowest dose enhanced reflex bradycardia despite little effect on heart rate and lack of effect on both reflex tachycardia and HRV.

Conclusions: Both BRS and HRV are reduced in rats early after MI, indicating a depressed reflex and tonic vagal activity. Treatment with zatebradine enhances both BRS and HRV. These data suggest that the drug has both peripheral and central effects, leading to an increase of vagal control of heart rate.

Keywords: Autonomic nervous system; Baroreflex; Bradycardia; Heart rate (variability); Infarction

1. Introduction

From large clinical studies on β-blocker treatment in patients after myocardial infarction (MI), evidence has been provided for a close relationship between the extent of heart rate (HR) lowering and reduction of mortality [1].

This can be explained in part by both an intrinsic effect of the HR reduction itself and a beneficial influence of the drug on the autonomic control of HR [1,2]. In patients after MI, the autonomic control of HR may be altered [3–6]. These patients have an increased incidence of ventricular tachyarrhythmias and a higher cardiac mortality than those patients with normal autonomic control of HR [7–9].

However, treatment with β-blockers in doses that markedly reduce HR may be problematic particularly in post-MI-patients with impaired left ventricular function or with obstructive lung disease. Therefore, other drugs such as the novel “(specific) bradycardic agents” may become an alternative or additive concept to achieve HR reduction. Zatebradine (UL-FS 49), a structure derivative of ver-

Abbreviations: ANOVA: analysis of variance; ANP: atrial natriuretic peptide; BRS: baroreflex sensitivity; CV: coefficient of variance; ECG: electrocardiogram; FFT: fast Fourier transform; HF: high frequency; HR: heart rate; HRV: heart rate variability; LF: low frequency; LVEDP: left ventricular end-diastolic pressure; MAP: mean arterial pressure; MI: myocardial infarction; RR-interval: interval between heart beats; NN-interval: interval between normal heart beats; SDNN: standard deviation of the mean interval between normal heart beats; SH: sham-operation

*Corresponding author. Tel.: +49-6221-568-611; fax: +49-6221-565-515.
E-mail address: carsten_krueger@med.uni-heidelberg.de (C. Krüger)

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apamil, is a bradycardic agent which inhibits the hyperpolarization-activated depolarizing cation inward current $I_h$ of the pacemaker cells of the sinuatrial node [10,11], without relevant effects on the L-type calcium inward current [12]. Zatebradine induces a reduction of HR without negative inotropic [13–15] or proarrhythmic properties [14,16,17].

Little is known about the effects of zatebradine on the autonomic control of HR. The autonomic control of HR can be evaluated by noninvasive methods. The arterial baroreflex sensitivity (BRS) is generally considered as an estimate of the reflex component and the HR variability (HRV) as a marker of the tonic component of autonomic control of HR [18–21]. The aim of this study was to characterize the influence of zatebradine on BRS and HRV under standardized conditions early after MI. The rat model of chronic coronary artery ligation was chosen as it allows study of a homogeneous population with left ventricular dysfunction [22]. Furthermore, evidence has been provided that MI-rats may show alterations of autonomic control of HR similar to those of post-MI-patients [23–26].

2. Methods

2.1. Coronary artery ligation

All experiments were approved by the federal authority and conform with the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health; NIH Publication No. 85-23, revised 1996). A transmural anterior MI was produced using a modification of a method previously described [22]. Adult male Sprague-Dawley rats (Charles River Wiga, Kiélegg, Germany; weight 230–275 g) were anesthetized with chloral hydrate and contractility ($Dp/Dt$) which was calculated by a differentiator (Hugo Sachs, March-Hugstetten, Germany). The cannula was connected to a pressure transducer (P 23 XL Spectramed Statham, Ohmeda, Madison, WI, USA). The pressure signal was amplified for measurement of left ventricular end-diastolic pressure (LVEDP) and contractility ($Dp/Dt$) which was calculated by a differentiator (Hugo Sachs, March-Hugstetten, Germany). In MI-animals, the infarct size was determined by in vitro staining of the sliced left ventricular myocardium with triphenyltetrazolium chloride [27]. The infarct size was calculated as the ratio of the scarred and the total circumference of myocardium.

2.2. Experimental protocol

All experiments were carried out on the third day after thoracotomy. Data for HRV analysis were obtained by means of 3-min ECG-recordings (except for the five SH-rats that received high-dose zatebradine: 4 min in order to obtain >700 beats). Then a 1.6 ml blood sample was taken for assays of atrial natriuretic peptide (ANP), norepinephrine and epinephrine, and replaced by an equal volume of donor rat blood, followed by the measurement of BRS. After collection of the baseline data, both the MI- and the SH-group were divided into three subgroups receiving different doses of zatebradine. An intravenous (i.v.)-bolus of zatebradine (Dr. Karl Thomae, Biberach/Riss, Germany; dissolved in 1 ml/kg saline; dose according to the respective group of rats) was given. After 30 min, blood sampling (for assays of norepinephrine and epinephrine), and measurement of HRV and BRS were repeated in the same manner. The drug-induced changes of parameters were compared to intraindividual pretreatment controls. Rats were anesthetized (100 mg/kg chloral hydrate i.v.), intubated and mechanically ventilated for thoracotomy. The left ventricle was cannulated at the apex (inner diameter: 1.1 mm; Venofix S, Braun, Melsungen, Germany). The cannula was connected to a pressure transducer (P 23 XL Spectramed Statham, Ohmeda, Madison, WI, USA). The pressure signal was amplified for measurement of left ventricular end-diastolic pressure (LVEDP) and contractility ($Dp/Dt$) which was calculated by a differentiator (Hugo Sachs, March-Hugstetten, Germany). In MI-animals, the infarct size was determined by in vitro staining of the sliced left ventricular myocardium with triphenyltetrazolium chloride [27]. The infarct size was calculated as the ratio of the scarred and the total circumference of myocardium.

2.3. Determination of BRS

To study BRS, arterial pressure was measured in the lower abdominal aorta via the femoral catheter connected to a transducer (see above). The signal was amplified and recorded on a computer with a custom-made software. Mean arterial pressure (MAP) was calculated from the integrals of pressure signals. From the intervals of systolic pressure peaks the RR-intervals were derived. An i.v.-bolus of the vasodilator nitroprusside (sodium nitroprusside; Sigma, Munich, Germany) (1.5 $\mu$g/kg in 100 $\mu$l 0.9% saline, followed by 200 $\mu$l 0.9% saline flush) was injected with constant monitoring of MAP and RR-inter-
val. Thereafter, 20 μg/kg of the α1-adrenoceptor agonist methoxamine (methoxamine hydrochloride; Sigma) were injected in the same manner. The doses of nitroprusside and methoxamine were chosen according to the results of previous experiments [26] so that the maximum changes of basal MAP were around ±20 mmHg. For each injection of nitroprusside or methoxamine, the transient changes in MAP and RR-interval were determined beat-to-beat. With these pairs of corresponding data from the first, i.e. linear, portion of the baroreflex loop [20,26,28], linear regression analysis was performed for each rat. Only data 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.

2.4. Measurements of HRV

The HRV was determined in conscious rats from ECG-recordings at rest, i.e., without application of vasoactive drugs, as previously described in detail [26]. Tachograms containing less than 700 beats were excluded from processing. In the time domain, the mean interval between normal beats (NN-interval), its standard deviation (SDNN), and the coefficient of variance (C.V.; 100× SDNN/mean NN-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 (FFT) and magnitude-squared for calculation of the power spectrum according to the periodogram-method. The power spectra of all segments were averaged in order to reduce the variance of FFT 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 [26,29,30], two regions of interest were defined: low-frequency (LF) (>0.5 Hz<0.8 Hz) and high-frequency (HF) (>0.8 Hz up to Nyquist-frequency, determined by the mean RR-interval of the tachogram, generally <4.5 Hz) bands.

2.5. Determination of plasma concentrations of ANP, epinephrine and norepinephrine

For determination of ANP, blood samples were cooled, stabilized by addition of K-EDTA (1 mg/ml) and centrifuged. The plasma was stored at −20°C until analysis. The plasma samples were extracted as described [31]. ANP was determined by radioimmunoassay, using a polyclonal antiserum (Peninsula Laboratories, Heidelberg, Germany). Norepinephrine and epinephrine were radioenzymatically assayed [32].

2.6. Statistics

Results are expressed as means±SEM. Differences between groups were tested by analysis of variance (ANOVA), or by the Mann–Whitney test where appropriate. Intraindividual drug-induced differences were tested by ANOVA for repeated measures, or by the Wilcoxon test where appropriate. Each ANOVA was followed by the Student–Newman–Keuls-test. Linear regression analysis was performed by the least squares method. A P-value <0.05 was considered significant.

3. Results

3.1. Baseline characteristics

Three days after thoracotomy, 18 rats with MI and 15 rats with SH were studied. The mean infarct size was 33±2% of the left ventricle. Neither respiratory distress, nor ascites, peripheral edema or pleural effusion were noted in any animal. In MI-rats, the total body weight was slightly lower, and the total heart-to-body weight ratio was increased, but the total wet lung-to-body weight ratio was not different from SH-rats (Table 1). The HR did not significantly differ between the MI- and the SH-group (Table 1), however, MAP was lower after MI (Table 1). As compared to SH-, in MI-rats left ventricular contractility was markedly reduced, and LVEDP was more than

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline characteristics; means±SEM</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sham-operation (n=15)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>252±2</td>
</tr>
<tr>
<td>Heart weight/body weight (mg/g)</td>
<td>3.5±0.1</td>
</tr>
<tr>
<td>Wet lung weight/body weight (mg/g)</td>
<td>5.8±0.2</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>391±8</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>112±3</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mmHg)</td>
<td>4.2±0.5</td>
</tr>
<tr>
<td>Left ventricular contractility (Δp/Δt) (mmHg/s)</td>
<td>8299±260</td>
</tr>
<tr>
<td>Plasma atrial natriuretic peptide (pmol/l)</td>
<td>19±4</td>
</tr>
<tr>
<td>Plasma norepinephrine (nmol/l)</td>
<td>1.9±0.2</td>
</tr>
<tr>
<td>Plasma epinephrine (nmol/l)</td>
<td>3.7±0.3</td>
</tr>
</tbody>
</table>

* P<0.05 vs. sham-operated group.
Table 2
Baroreflex sensitivity and heart rate variability after myocardial infarction; means±SEM

<table>
<thead>
<tr>
<th></th>
<th>Sham-operation (n=15)</th>
<th>Myocardial infarction (n=18)</th>
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<tbody>
<tr>
<td><strong>Baroreflex sensitivity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reflex bradycardia (ms/mmHg)</td>
<td>0.93±0.06</td>
<td>0.41±0.04*</td>
</tr>
<tr>
<td>Reflex tachycardia (ms/mmHg)</td>
<td>0.94±0.05</td>
<td>0.45±0.04*</td>
</tr>
<tr>
<td><strong>Heart rate variability</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time domain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation of the mean NN-interval (SDNN) (ms)</td>
<td>3.9±0.4</td>
<td>2.2±0.3*</td>
</tr>
<tr>
<td>Coefficient of variance (%)</td>
<td>2.5±0.2</td>
<td>1.5±0.2*</td>
</tr>
<tr>
<td>Frequency domain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total spectral power (ms²/Hz)</td>
<td>22.4±3.7</td>
<td>7.6±2.1*</td>
</tr>
<tr>
<td>High-frequency (HF) power (ms²/Hz)</td>
<td>6.8±1.3</td>
<td>2.0±0.6*</td>
</tr>
<tr>
<td>Low-frequency (LF) power (ms²/Hz)</td>
<td>1.2±0.3</td>
<td>0.3±0.1*</td>
</tr>
<tr>
<td>LF/HF-ratio</td>
<td>0.15±0.03</td>
<td>0.17±0.03</td>
</tr>
</tbody>
</table>

* P<0.05 vs. sham-operated group.

The BRS was markedly reduced in MI-rats as compared to SH-controls, as indicated by a decrease in both reflex bradycardia and tachycardia (Table 2). Furthermore, the HRV parameters SDNN and CV, as well as total, HF- and LF-spectral power were distinctly reduced after MI, while the LF/HF-ratio remained unchanged (Table 2).

3.2. Effects of zatebradine on hemodynamics and plasma catecholamines

Zatebradine reduced HR dose-dependently by maximal 30% approximately in both MI- and SH-rats (Fig. 1). MAP remained unaffected except for the highest dose in MI-rats where it slightly decreased (Table 3). Both left ventricular contractility and LVEDP were not significantly different between the three dose-groups of either SH- or MI-rats (Δp/Δt: SH, 0.05 mg/kg: 8320±411; 0.5 mg/kg: 8229±428; 5 mg/kg: 8381±762; MI, 0.05 mg/kg: 4762±459; 0.5 mg/kg: 5142±256; 5 mg/kg: 5333±241 mmHg/s) (LVEDP: SH, 0.05 mg/kg: 4.4±0.8; 0.5 mg/kg: 4.2±0.8; 5 mg/kg: 3.9±1.1; MI, 0.05 mg/kg: 12.8±1.4; 0.5 mg/kg: 10.9±1.5; 5 mg/kg: 11.3±2.9 mmHg) (SH: n=5; MI: n=6 each). The drug had no significant influence on the plasma concentrations of norepinephrine and epinephrine in any of the six groups (not shown).

Table 3
Effect of zatebradine on mean arterial pressure; means±SEM

<table>
<thead>
<tr>
<th></th>
<th>Mean arterial pressure (mmHg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sham-operation (n=5 each)</td>
</tr>
<tr>
<td>Control</td>
<td>118±5</td>
</tr>
<tr>
<td>0.05 mg/kg Zatebradine</td>
<td>115±6</td>
</tr>
<tr>
<td>Control</td>
<td>109±4</td>
</tr>
<tr>
<td>0.5 mg/kg Zatebradine</td>
<td>107±2</td>
</tr>
<tr>
<td>Control</td>
<td>108±5</td>
</tr>
<tr>
<td>5 mg/kg Zatebradine</td>
<td>102±4</td>
</tr>
</tbody>
</table>

* P<0.05 before vs. after treatment.
3.3. Effects of zatebradine on baroreflex sensitivity and heart rate variability

After administration of zatebradine BRS was markedly increased as compared to intraindividual pretreatment controls (Figs. 2, 3, 5 and 6). Treatment did not only restore BRS in all MI-groups, but also enhanced BRS in the SH-groups. Both reflex bradycardia and tachycardia were affected except for the lowest dose of zatebradine which enhanced only reflex bradycardia. The absolute values of the middle-dose-groups were significantly ($P<0.05$) higher than those of the low-dose-groups both in SH- and MI-rats (except for an insignificant difference of reflex bradycardia between the low- and middle-dose-SH-group). In contrast, the values of the high-dose-group were lower than those of the middle-dose-group only in MI-rats ($P<0.05$).

Similar to the effects on reflex tachycardia, HRV was enhanced by the two highest doses of zatebradine, as calculated in the time domain (Figs. 4 and 7; Table 4). This effect appeared to be rather independent on the prevailing HR as indicated by parallel changes of both SDNN and CV. The absolute values of the middle-dose-groups were significantly ($P<0.05$) higher than those of the low-dose-groups both in SH- and MI-rats. The values of the high-dose-group were lower than those of the middle-dose-group both in SH- and MI-rats ($P<0.05$; SDNN in SH-rats: nonsignificant).

In the frequency domain, HRV data showed a relatively high interindivdual variation. After treatment a statistically significant enhancement was observed in the total spectral power of SH-rats with 0.5 mg/kg and in MI-rats with 5 mg/kg zatebradine. All other data of spectral analysis of middle- and high-dose-groups were characterized by a tendency to an increase of total, HF- and LF-power, but without major changes in the LF/HF-ratio (Table 4).

4. Discussion

4.1. Evidence of moderate left ventricular dysfunction in MI-rats

In the present study several characteristics indicating moderately impaired left ventricular systolic function were observed in rats early after MI. The left ventricular contractility after MI was only half of that in controls, and the LVEDP was approximately three times higher. Furthermore the plasma concentrations of ANP, a marker of left ventricular dysfunction [31], were approximately doubled after MI, and the total heart-to-body weight ratio was increased [25,26]. However there was no evidence of advanced heart failure, as the plasma catecholamine concentrations were not elevated, the lung-to-body weight ratio remained unchanged, and neither pleural effusion nor ascites were observed [23,26].

4.2. BRS and HRV after MI

The BRS was markedly reduced after MI agreeing with previous investigations in conscious rats [23,26]. A decrease in BRS has been shown to indicate a depression of reflex vagal control of HR [18–20,26]. Furthermore, a decrease in HRV was found early after MI in the present study. In a previous investigation a normal HRV was observed later after MI (at 28 and 56 days) in rats with similarly moderate left ventricular dysfunction [26]. In rats with more severe heart failure, however, HRV was found to be reduced in the late stage after MI (at 56 days) [24], thus emphasizing the need to consider the extent of left ventricular dysfunction to allow for comparisons between studies. A reduction of HRV variables as observed in the present study is generally considered to reflect a depression of tonic vagal activity not only in humans [3,4,7], but also in rats [21,26,29] and in dogs [33]. The HRV analysis method used in the present study allows detection of a change in vagal tone [26]. Therefore, it may be concluded that not only reflex, but also tonic vagal control of HR is depressed early after MI in rats.

4.3. Effects of zatebradine on hemodynamics in MI-rats

The present data demonstrate the effects of a bradycardic agent in rats with MI. Treatment with zatebradine led to a dose-dependent HR reduction in both MI- and SH-rats. The study protocol did not allow direct comparison of left ventricular function estimates in rats before and after treatment. A distinct change of left ventricular function appears less probable since the drug had only little influence on MAP in conscious rats. Furthermore, left ventricular contractility and LVEDP were not different between the three dose-groups of both SH- and MI-rats. In other species, such as dogs [15,17] and rabbits [14], left ventricular function was preserved despite a pronounced HR reduction by zatebradine.

4.4. Effects of zatebradine on BRS and HRV

Treatment with zatebradine markedly enhanced BRS, thus augmenting BRS in MI-rats of the low-dose-group and eliminating the MI-induced BRS depression in the middle- and high-dose-groups. In the SH-groups treatment resulted in BRS values even higher than those measured intraindividually before treatment. The drug-induced increase of BRS included both reflex bradycardia and (in the middle- and high-dose-treated-groups) reflex tachycardia. These data partially contrast with the only previous study of the effects of zatebradine on BRS [34]. In that study BRS was found to be unchanged by the drug in healthy control rats as well as in rats with abdominal aortic constriction and reduced BRS. The discrepancy between
Fig. 2. Representative plots of RR-interval vs. mean arterial pressure showing the effects of zatebradine (0.5 mg/kg i.v.) on baroreflex sensitivity in a rat with sham-operation. Abbreviations: BRS=baroreflex sensitivity; r=correlation coefficient.
Fig. 3. Representative plots of RR-interval vs. mean arterial pressure showing the effects of zatebradine (0.5 mg/kg i.v.) on baroreflex sensitivity in a rat with myocardial infarction. Abbreviations: BRS=baroreflex sensitivity; r=correlation coefficient.
Fig. 4. Original recordings (1-min-sections of 3-min ECG-recordings) of RR-interval showing the effects of zatebradine (0.5 mg/kg i.v.) on heart rate variability in a rat with sham-operation (above; same rat as in Fig. 2) and with myocardial infarction (below; same rat as in Fig. 3). Abbreviations: SDNN=standard deviation of mean NN-interval.
the two investigations may be explained by methodological differences. In the previous study, experiments were carried out as early as 1.5 h after surgical implantation of the catheter, and vasoactive drugs and zatebradine were given intravenously. Furthermore, the influence of zatebradine on plasma catecholamines and HRV was not examined.

Not only BRS but also HRV was enhanced by zatebradine in the present study leading to marked increase of HRV in MI- and further augmentation in SH-rats. However, this was the case only in the middle- and high-dose-treated-groups suggesting that the threshold for zatebradine-related changes in BRS may be lower than for changes in HRV. The effects of the drug on HRV were more pronounced in the analysis of the time domain as compared to the analysis of the frequency domain. After treatment, a significant enhancement was observed in the total spectral power of SH-rats with 0.5 mg/kg and in MI-rats with 5 mg/kg zatebradine. All other data of spectral analysis of middle- and high-dose-groups were characterized by a tendency to an increase of total power, as well as of HF- and LF-power representing primarily vagal tone in rats [21,26,29,35]. However, these effects did not reach statistical significance due to a high inter-individual variation of spectral HRV data in rats [26,35]. The LF/HF-ratio was relatively low under all conditions, which may depend on the choice of the frequency bands [26,30,35]. In previous investigations using similar bands, a similarly low LF/HF-ratio was observed in rats [26,30]. Not only after MI but also after zatebradine treatment, the LF/HF-ratio remained unchanged indicating that the general variability (as reflected by SDNN and total power), and LF- and HF-bands in a proportional matter, were unaffected. This may be explained by the evidence that LF incorporates BRS components [36,37] and is largely vagally modulated in rats [21,26,29,35]. The effects of bradycardic agents on HRV have been previously studied in healthy rats with the novel I_{a} inhibitor S-16257 showing a marked increase of HRV by this drug [38].

As post-MI reduction of BRS and HRV is considered as an indirect sign of reduced vagal activity [18–20,26], it may be concluded that improving BRS and HRV by zatebradine indicates a correction of sympathovagal balance by enhancement of vagal activity. In principle the increase in BRS and HRV could be simply explained by the drug-induced HR reduction itself which might facilitate reflex bradycardia and enhance the probability of a high statistical variance of RR-intervals. However, the effects of zatebradine on BRS and HRV cannot be explained by the
Effect of zatebradine on heart rate variability (SDNN and frequency domain parameters); means±SEM. * P<0.05 before vs. after treatment.

Reduced HR alone: reflex tachycardia was also enhanced, and after normalization of the values of the HRV parameter SDNN to the simultaneously measured NN-interval, i.e. by calculation of CV, the influence of the drug on HRV was only slightly less pronounced. Moreover, despite little effect on HR, the lowest dose of zatebradine increased reflex bradycardia which is largely dependent on vagal activity [18–20,26], whereas reflex tachycardia being less controlled by the vagus remained unchanged. Theoretically

the effects of zatebradine on BRS could also be influenced by an enhanced left ventricular stroke volume which was not measured. However, neither LVEDP nor contractility were different between the three dose-groups in both SH- and MI-rats.

Since the main action of zatebradine yet known is \( I_h \) inhibition in the sinusatrial pacemaker cells, a direct increase of peripheral vagal nerve neurotransmission by the drug appears improbable. The vagal neurones in the dorsal vagal nucleus and in the nucleus ambiguus of the medulla oblongata are integrating components of HR control [28]. Even though other central nervous structures such as the dorsomedial region of the solitary tract are also involved in baroreceptor reflexes [28] it appears likely that zatebradine exerts influence on the central vagus itself because the tonic vagal control of HR as assessed by HRV is also enhanced by the drug. Zatebradine which is known to pass the blood–brain barrier [personal communication; B. Guth, Dr. Karl Thomae Ltd., Biberach/Riss, Germany] has been shown to selectively block \( I_h \) in thalamocortical neurons [11]. Even though close similarities between the channel functions mediating \( I_h \) in the heart and in neurons have been demonstrated [10], the role of \( I_h \) in the function of the central vagus is still unclear. At least in MI-rats the absolute values of BRS and HRV parameters tended to be higher in the middle- than in the high-dose-group which might suggest that: (I) the drug actually has not only electrophysiological but also intrinsic autonomic actions; and (II) these intrinsic autonomic effects are less evident when HR is very low. In summary, the present data provide evidence of an at least unspecific effect of a single i.v. dose zatebradine on the central vagus resulting in a correction of sympathovagal imbalance after MI.

4.5. Limitations of the study

The focus of the present study was the period early after MI which is characterized by the most distinct changes of HR control [4–7,26,33]. Therefore, it remains to be determined whether zatebradine has the same effects under

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**Table 4**

Effect of zatebradine on heart rate variability (SDNN and frequency domain parameters); means±SEM

<table>
<thead>
<tr>
<th>Parameters of heart rate variability</th>
<th>Sham-operation (n=5 each)</th>
<th>Myocardial infarction (n=6 each)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN (ms)</td>
<td>Total power (ms²/Hz)</td>
<td>HF-power (ms²/Hz)</td>
</tr>
<tr>
<td>Control</td>
<td>4.2±0.8</td>
<td>28.2±7.8</td>
</tr>
<tr>
<td>0.05 mg/kg Zatebradine</td>
<td>3.1±0.4</td>
<td>13.7±2.9</td>
</tr>
<tr>
<td>Control</td>
<td>4.5±0.5</td>
<td>22.1±4.3</td>
</tr>
<tr>
<td>0.5 mg/kg Zatebradine</td>
<td>8.2±0.7*</td>
<td>83.0±23.0*</td>
</tr>
<tr>
<td>Control</td>
<td>3.1±0.6</td>
<td>16.7±7.1</td>
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<tr>
<td>5 mg/kg Zatebradine</td>
<td>6.1±2.1</td>
<td>64.7±40.6</td>
</tr>
</tbody>
</table>

* Abbreviations: SDNN=standard deviation of mean NN-interval; HF=high frequency; LF=low frequency.

* P<0.05 before vs. after treatment.
chronic post-MI conditions. Moreover, the study protocol did not include internal controls before and after thoracotomy to demonstrate that BRS and HRV were not affected by the surgical procedure. Thus, it cannot be entirely excluded that the measurements as soon as 3 days after open chest surgery were performed under conditions of incomplete autonomic stabilization of the animals, which might have interfered with drug effects on BRS and HRV. The protocol with successive measurements before and after treatment in the same rat was designed to allow for analysis of intraindividual drug-induced changes of BRS and HRV. Therefore, data under treatment were obtained in a later period of the experiment which might have been associated with increasing levels of stress applied to the animals. However, the differential effects of the three doses of zatebradine, with the lowest dose appearing like a threshold dose, suggest that the modulation of BRS and HRV data is primarily drug-induced. Even though the drug had no influence on peripheral plasma catecholamines in any of the MI- or SH-groups, it cannot be completely ruled out that the overall sympathoexcitatory state increased during the experiment [39]. However, if stress would have been present this should have decreased [40,41] rather than increased HRV as observed in the present study.

The interbeat intervals for calculation of baroreflex slopes were derived from the intervals of systolic pressure peaks. Even though the BRS analysis software allowed for accurate triggering of the pulse it would have been desirable to obtain instead the RR-intervals from the R-peaks in the ECG, which was not possible due to technical reasons. The high intragroup homogeneity of BRS data is in accordance with the previously reported accuracy of the method used in the present study [18,23,26,34,42,43]. Since the duration of the experiments could not be extended too far in order to assure steady-state conditions, only short-term ECG-recordings were used to analyze HRV. Thus HRV was analyzed both in the time and frequency domain, revealing a relatively high interindividual variation of spectral data, as previously described [26,35]. This may be due to the analysis by FFT, as performed in the present study. Even though an autoregressive approach [4] would allow the most appropriate analysis of nonequidistant time series of HR, previous studies have shown that major changes of HRV in the frequency domain may also be detected by FFT [2,8,21,26,30,35,46]. However, the analysis of short-term ECG-recordings in the time domain may be sufficient to detect changes in vagal tone in rats [26].

In the only study of autonomic effects of zatebradine in humans yet available, the drug was shown to reduce HRV in a group of 24 patients without structural heart disease [44]. This contrasts with the observations in SH-rats of the present study and underscores that experimental data from small animals cannot be directly extrapolated to conditions in humans. The very high HR and the small BRS and HRV in rats as compared to humans should be taken into consideration when interpreting experimental data of drug effects on HR control. Thus, the influence of zatebradine on both BRS and HRV in patients with cardiac disease remains to be determined.

4.6. Clinical implications

Pharmacological reduction of HR is a central concept in the treatment of patients with coronary heart disease. Due to lack of negative inotropic [13–15] or proarrhythmic [14,16,17] properties, the bradycardic agents may be of interest for the therapy of those patients with coronary artery disease who have impaired left ventricular function [13]. The present experimental data suggest that the well-known reduction of BRS and HRV in post-MI-patients might be influenced by such a drug. An enhancement of the vagal markers BRS and HRV by other drugs, such as metoprolol and scopolamine, has been described [2,45,46]. In post-MI-dogs, electrical vagal stimulation has been shown to prevent exercise- and ischemia-induced ventricular fibrillation [47], and treatment with zatebradine reduced automaticity-related ventricular tachyarrhythmias [17]. In another study, however, the increase in HRV by scopolamine did not result in a decreased risk for exercise- and ischemia-induced ventricular fibrillation in post-MI-dogs [46]. In the present study, the relation between markers of autonomic tone and incidence of tachyarhythmias or mortality was not analyzed. Controlled clinical trials are required in order to clarify whether drug-induced increase of the prognosis markers BRS and HRV actually improves clinical outcome in MI-patients. Nevertheless, since there is evidence of a causal relationship between BRS depression and impaired hemodynamic tolerability of both general cardiovascular stress and sustained ventricular tachycardia [48,49], treatment with a BRS-enhancing drug like zatebradine may be beneficial not only with regard to mortality.

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