Beat-to-beat modulation of heart rate is coupled to coronary perfusion pressure in the isolated heart

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Beat-to-beat modulation of heart rate is coupled to coronary perfusion pressure in the isolated heart. J. Appl. Physiol. 86(2): 694–700, 1999.—A goal of clinicians caring for heart transplant recipients has been to use heart rate variability as a noninvasive means of diagnosing graft rejection. The determinants of beat-to-beat variability in the surgically denervated heart have yet to be elucidated. We used an isolated, blood buffer-perfused porcine heart preparation to quantitatively assess the relationship between coronary perfusion and sinus node automaticity. Hearts (n = 9) were suspended in a Langendorff preparation, and heart rate (HR) fluctuations were quantified while perfusion pressure was modulated between 70/50, 80/60, 90/70, and 100/80 mmHg at 0.067 Hz. In 32 of 32 recordings, the cross spectrum of perfusion pressure vs. HR showed the largest peak centered at 0.067 Hz. In eight of nine experiments during nonpulsatile perfusion, HR accelerated as perfusion pressure was increased from 40 to 110 mmHg (mean increase 24.2 ± 3.0 beats/min). HR increased 0.34 beats/min per mmHg increase in perfusion pressure (least squares linear regression y = −25.8 mmHg + 0.34x; r = 0.88, P < 0.0001). Administration of low- and high-dose nitroglycerin (Ntg) resulted in a modest increase in flow but produced a significant decrease in HR and blunted the response of HR to changes in perfusion pressure (HR increase 0.26 beats·min−1·mmHg−1, r = 0.87, P < 0.0001 after low-dose Ntg: 0.25 beats·min−1·mmHg−1, r = 0.78, P < 0.0001 after high-dose Ntg). These experiments suggest that sinus node discharge in the isolated perfused heart is mechanically coupled to perfusion pressure on a beat-to-beat basis.

Heart rate variability; heart transplantation

REduced heart rate variability (HRV) serves as an important marker in several states in which autonomic regulation of the heart is impaired, including aging, diabetes, and end-stage heart failure, and for patients at increased risk for sudden cardiac death (3, 7, 10, 26). A goal of clinicians caring for heart allograft transplant recipients has been to use HRV as a noninvasive means of diagnosing graft rejection (25, 32). After heart transplantation, the cardiac vagal and sympathetic efferent nerves that normally regulate heart rate are absent, and only a low-amplitude HRV remains (2, 23, 25). Beat-to-beat oscillations of heart rate after heart transplantation are thought to result from mechanical coupling between the right atrium and sinus node (5, 21, 23, 27). However, clinical studies on heart transplant recipients reveal that simple entrainment of heart rate to respiration cannot account for the observed dynamics (2, 28).

It is likely that other factors play a role in chronotropic regulation of the denervated heart, including changes in perfusion pressure (19), alterations in flow rate (12, 17), and stretch of the vascular endothelium, which may release locally active mediators that alter sinus node discharge. For example, nitric oxide (NO) is released in response to pulsatile flow and shear stress and may influence sinus node automaticity (18, 20). Previous studies have not investigated the relationship between perfusion pressure and heart rate on a beat-to-beat basis (12, 16, 19). Such an analysis is a prerequisite to elucidating the determinants of HRV in the surgically denervated heart. Moreover, there has been a long-standing interest in detecting cardiac allograft rejection by using the surface electrocardiogram instead of the endomyocardial biopsy (9, 15, 25). It is known that cardiac sympathetic reinnervation can occur within 6–12 mo after heart transplantation (31). The incidence of acute graft rejection is highest within the first 6 mo and rare beyond 1 yr after transplant (30). Thus the vast majority of rejection episodes occur before cardiac reinnervation, an observation that underscores the importance of elucidating the determinants of heart rate control in the early postoperative period.

The present study was designed to determine whether changes in coronary perfusion pressure play a role in regulating heart rate after heart transplantation. We used an ex vivo model of cardiac perfusion to quantitatively assess the relationship between coronary perfusion and sinus node automaticity. An isolated heart preparation enables us to examine the determinants of heart rate without the confounding effects of autonomic input and respiration. Our findings suggest that sinus node discharge responds to changes in perfusion pressure on a beat-to-beat basis.

METHODS

Experimental Preparation

All procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with institutional and National Institutes of Health guidelines. Outbred swine of either sex weighing 30–40 kg were sedated with Telazol (300 mg im) and anesthetized with intravenous pentobarbital sodium via the marginal ear vein. The animals were intubated and ventilated while anesthesia was maintained with a mixture of 4 l/min oxygen and 2% isoflurane. The heart was exposed through a median sternotomy. The

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venae cavae and carotid and brachiocephalic arteries were isolated, and the pericardium was incised. Heparin (25,000 U) was administered intra-arterially, and the heart-lung block was procured after arrest with topical cold (4°C) saline. After the heart was dissected free from the remainder of the heart-lung block, the pulmonary artery was ligated. Silastic catheters were sutured to the apex of the right and left ventricles to permit drainage. A 16-Fr arterial cannula (Bard, Tewksbury, MA) was placed in the aorta with its tip above the coronary ostia to allow retrograde perfusion of the coronary arteries. The cannula was secured with umbilical tape, filled with cold saline, and clamped. We eliminated the potential for right atrial distention by creating a large opening in the right auricular appendage that allowed blood to drain freely from the right atrium at all perfusion pressures. During each experiment, we assessed the right atrium and verified that it remained collapsed.

Hearts were suspended in a Langendorff perfusion circuit, warmed, and defibrillated with 10–25 J to reestablish sinus rhythm. Blood was drained from the venae cavae and ventricles into a 3,000-ml cardiotomy reservoir equipped with a 20-µm filter, circulated with a centrifugal pump (Biomedicus 550 Bio-console, Medtronic, Eden Prairie, MN), passed through a Minimax Plus Hollow Fiber Oxygenator (model 3381, Medtronic), and returned to the heart via the aortic cannula. We digitized the electrogram, pressure, and flow at 500 Hz/channel (CODAS, Dataq Instruments, Akron, OH) on an IBM-compatible computer for later analysis.

Experimental Protocol

We assessed the effects of changes in perfusion pressure on sinus node automaticity in a total of nine preparations during the following manipulations.
Basal state, pulsatile perfusion. The electrocardiogram was recorded while perfusion pressure was modulated between 70/50, 80/60, 90/70, and 100/80 mmHg at 0.067 Hz. Recordings were for 4 min. In our recent clinical study of heart rate control in heart transplant recipients, we observed heart rate fluctuations synchronous with low-frequency oscillations in pulse pressure (28). In each case, the rise in pulse pressure preceded the increase in heart rate. These fluctuations in peripheral vasomotor tone, referred to as Mayer waves, have their spectral peak between 0.04 and 0.1 Hz. In the present study, we wanted to simulate the effect of these fluctuations in arterial pressure on heart rate. Thus we chose 0.067 Hz because it lies within the limits of the Mayer wave frequency.

Basal state, nonpulsatile perfusion. Perfusion pressure was increased from 40 to 110 mmHg and then decreased from 110 to 40 mmHg in 10-mmHg increments. We recorded the electrocardiogram after waiting a minimum of 30 s for heart rate to equilibrate at each new pressure. This portion of the experiment examines further the effect of small changes in perfusion pressure on sinus node automaticity.

Nitroglycerin (Ntg), nonpulsatile perfusion. Ntg was administered to determine whether the changes in heart rate observed with changes in pressure were related to coronary blood flow. Low-dose (5 mg/ml reservoir volume) and high-dose (20 mg/ml reservoir volume) Ntg were given, and the electrocardiogram was recorded as perfusion pressure was increased incrementally from 40 to 110 mmHg.

Epinephrine (1 mg/ml reservoir volume) was administered to determine whether the sinus node remained responsive to catecholamine stimulation after up to 6 h of ex vivo perfusion. The electrocardiogram was recorded as pressure was increased from 40 to 110 mmHg.

All experiments were terminated within 6 h of suspension of the heart in the perfusion circuit. In preliminary experiments, we determined that the preparation remains stable for up to 6 h.

Data Acquisition and Analysis

P waves were marked by using a peak-capture algorithm and edited interactively (CODAS). Pulse interval was computed by taking the difference between successive peaks. P-P interval, rather than R-R interval, was chosen to represent cardiac cycle length because the P waves correspond with sinus node discharge.

In experiments with pulsatile perfusion, a smoothed instantaneous time series of heart rate was constructed at 4 Hz from the series of P-P intervals (1). Significant trends were removed from the heart rate time series by subtracting from them a straight line through the first and last points (24). The perfusion pressure corresponding with each P wave was identified and resampled at 4 Hz so that the values of the two time series were synchronized (1). In cases where the resampled time series was <1,024 points, periodic extension was employed to bring the data sequences to 1,024 points.

We computed the Fourier transform of heart rate and defined the frequency components as follows: 0.03–0.15 Hz, low frequency; 0.16–0.5 Hz, high frequency. We did not consider the length of data recording sufficient to resolve spectral peaks in the ultra-low-frequency band. Power spectra were quantified by using the one-sided power spectral density and compared in terms of the amplitude of low-frequency power, high-frequency power, and total power (24). Additionally, we calculated the cross-amplitude spectrum between perfusion pressure and heart rate by taking the cross product of the heart rate transform times the complex conjugate of perfusion pressure. Finally, we calculated two widely accepted estimates of HRV: P-P standard deviation and P-P range (longest minus shortest P-P interval of the sequence).

In experiments with nonpulsatile perfusion, mean heart rate was determined from a minimum of 10 P-P intervals at each perfusion pressure. Instantaneous heart rate was calculated by dividing each pulse interval into 60 s. Before linear regression analysis was computed (see below), heart rate data were normalized by subtracting the mean heart rate at each perfusion pressure from the mean heart rate for each series. We refer to the normalized data as the “heart rate index.” In these experiments, flow rate was normalized to flow at 40 mmHg under basal conditions.

Where indicated in the text, values are for means ± SE. Significant differences were determined by Student’s t-test for paired observations or ANOVA. The relationship between perfusion pressure and heart rate was obtained by linear least squares regression analysis. Data sets were analyzed by using Matlab (MathWorks, Natick, MA) and Superanova (Abacus Concepts, Berkeley, CA). Statistical significance was set at P < 0.05 (2 tailed).

RESULTS

Basal State, Pulsatile Perfusion

Figure 2 shows a time series from a single experimental recording (pH22) in which perfusion pressure oscillated between 50 and 70 mmHg at 0.067 Hz. Heart rate appears entrained by the fluctuations in perfusion pressure. As perfusion pressure increased, heart rate increased; as pressure decreased, heart rate decreased. The acceleration in heart rate occurred ~2.9 s after peak perfusion pressure was reached. In contrast, the decay in heart rate as pressure decreased was more rapid (1.5–2.0 s; Fig. 2).

Although several studies showed unequivocal evidence of entrainment, in most cases it was difficult to discern by inspection of the relationship between perfusion pressure and heart rate. Thus we relied on spectral analysis to determine whether the sinus node re-
responded to fluctuations in perfusion pressure. The power spectrum of the heart rate time series (Fig. 3A) depicted in Fig. 2 shows a single peak similar in both shape and center frequency to that of the perfusion pressure signal (Fig. 3B). In 27 of 32 studies, the dominant peak in the power spectrum was located at the fundamental forcing frequency. In the remaining studies, the heart rate spectrum contained large amplitude peaks at integer multiples of the forcing frequency (i.e., harmonics). As a control, we performed power spectral analysis on heart rate sequences recorded during nonpulsatile perfusion at 70 mmHg. No consistent spectral peaks were identified.

The concordance between the pressure and heart rate spectra is demonstrated by the cross-amplitude spectrum, which shows a large amplitude peak at the fundamental frequency (Fig. 3C). In 32 of 32 recordings, the largest peak of the cross spectrum of perfusion pressure vs. heart rate was centered at 0.067 Hz. Increases in mean perfusion pressure produced a significant increase in the amplitude of high-frequency components (70/50 mmHg 0.14 ± 0.03 Hz vs. 80/60 mmHg 0.18 ± 0.03 Hz, P < 0.05; vs. 100/80 mmHg 0.21 ± 0.04 Hz, P < 0.01; repeated-measures ANOVA) but not low-frequency components or total power (data not shown). The increase in high-frequency components in the cross spectrum reflects the appearance of harmonics in the heart rate power spectrum.

Fluctuations in perfusion pressure produced low-amplitude HRV in each of the recordings. Overall, as was the case during nonpulsatile perfusion, the mean heart rate increased as perfusion pressure increased (see above). The increase in mean heart rate approached, but did not reach, statistical significance (P < 0.06, repeated-measures ANOVA). No significant change in HRV measures (PP standard deviation and PP range) was observed over the four different ranges of perfusion pressure (Table 1).

Basal State, Nonpulsatile Perfusion

In eight of nine experiments, heart rate accelerated as perfusion pressure was increased from 40 to 110 mmHg (mean heart rate increase 24.2 ± 3.0 beats/min; Fig. 4). Basal heart rate varied between experimental preparations (range 90–149 beats/min at a perfusion pressure of 70 mmHg). Consequently, we used normalized heart rate to quantify the relationship between perfusion pressure and heart rate. Bivariate linear regression revealed a strong positive relationship between pressure and heart rate (r = 0.82, P < 0.0001). Heart rate decreased as perfusion pressure was decreased from 110 to 40 mmHg (r = 0.89, P < 0.0001). Because there was no evidence of hysteresis, we took the average of the two baseline measurements when we examined the effect of Ntg on heart rate (Fig. 5). The slope of the regression equation indicates that heart rate increased by an average of 0.34 beats/min for each mmHg increase in perfusion pressure (y = −25.8 mmHg + 0.34x; P < 0.001). The increase in heart rate did not appear to saturate at higher perfusion pressures.

Ntg, Nonpulsatile Perfusion

Ntg produced a modest increase in flow over the entire pressure range studied (10.5% after low-dose Ntg; 12.7% after high-dose Ntg). Both concentrations of Ntg produced a significant decrease in heart rate at perfusion pressures between 60 and 110 mmHg. Compared with basal state, the response of heart rate to increases in perfusion pressure was blunted after administration of Ntg. Heart rate increased 0.26 beats·min⁻¹·mmHg⁻¹ after low-dose Ntg (r = 0.87, P < 0.0001) and 0.25 beats·min⁻¹·mmHg⁻¹ after high-dose Ntg (r = 0.78, P < 0.0001; Fig. 5). Compared with basal state, the difference in heart rate response did not reach statistical significance (P < 0.09).

Catecholamines, Nonpulsatile Perfusion

Epinephrine was administered before the conclusion of each experiment. Hearts retained chronotropic competence despite ex vivo perfusion times of up to 6 h. Compared with basal state, heart rate increased an
average of 66.6 ± 14.1 beats/min after administration of epinephrine (P < 0.005). Additionally, in three of eight preparations, heart rate remained dependent on perfusion pressure (1 preparation was excluded from this analysis because it developed a junctional rhythm). The mean heart rate increase was 25.8 ± 6.6 beats/min as perfusion pressure was increased from 40 to 110 mmHg. Overall, linear regression revealed a significant positive correlation between perfusion pressure and heart rate during catecholamine stimulation (r = 0.45, P < 0.001).

**Rhythm**

Normal sinus rhythm was present in three preparations, while atrioventricular (AV) dissociation (AVD) was present in the remaining preparations. AVD was diagnosed when the atrial and ventricular rhythms were separate and independent; i.e., there was a changing relationship between P waves and QRS complexes and the ventricular rhythm was regular despite the changing P-R relationship. Results of linear regression analysis for hearts in normal sinus rhythm and first-degree AV block (basal state r = 0.86, P < 0.0001) were nearly identical to results for hearts in AVD (basal state r = 0.89, P < 0.0001), which suggests that P waves in both groups originated from the sinus node.

**DISCUSSION**

The objective of the present study was to employ beat-to-beat analysis to identify the determinants of HRV in the surgically denervated heart. We found that heart rate was modulated by pulsatile and nonpulsatile perfusion. In 27 of 32 studies during pulsatile perfusion, the dominant peak in the heart rate power spectrum was located at the fundamental forcing frequency of the perfusion pump. In the remaining studies, large-amplitude peaks were present at harmonics of the forcing frequency. Inspection of the time series in a smaller number of studies showed unequivocal evidence of entrainment between perfusion pressure and heart rate. Loeb et al. (17) observed similar oscillations in cardiac cycle length related to pulsatile perfusion of the sinus node region by using a denervated canine preparation. In that study, however, the authors did not quantify the effect of pressure on beat-to-beat changes in heart rate. We also found that, during nonpulsatile perfusion, heart rate accelerated in 89% of preparations as perfusion pressure was increased from 40 to 110 mmHg. Our findings are consistent with those of Musgrave (19), who employed an isolated sinus node preparation and found that heart rate decreased below pressures of 40 mmHg and increased with perfusion pressures from 45 to 95 mmHg. In both our study and that of Musgrave, the rich sympathetic innervation to the sinus node has been severed. Thus we believe it unlikely that increase in sinus node discharge was triggered by perfusion-related increases in local catecholamine release, as Loeb et al. (17) found using an intact canine preparation.

Our findings contrast with those of Hashimoto et al. (8), who found an inverse relationship between perfusion pressure and sinus node discharge. Hashimoto et al. employed large (50-mmHg), sudden changes in perfusion pressure to the sinus node in an open-chest, intact canine model. Rapid bolus injections into the sinus node artery result in injection bradycardia followed by postinjection tachycardia [see Fig. 4 of Hashimoto et al. (8)]. In the present study, we employed significantly lower pressure increments and allowed
time for the heart rate to equilibrate at each perfusion pressure.

Other studies have shown a direct correlation between decreased perfusion pressure, sinus node ischemia, and reduced heart rate (4, 6). There are several factors that make it unlikely that the sinus node was ischemic in our study. First, there was no abrupt change in the relationship between perfusion pressure and heart rate as pressure decreased from normal physiological range (70–110 mmHg) to lower pressures (40–60 mmHg). Second, the sinus node is supplied by extensive anastomoses and collateral connections. In a study by White et al. (29), even after the sinus node artery was ligated and flow to the sinus node decreased by 36%, heart rate was not altered. Finally, low perfusion pressures lead to reduced P-wave amplitude and, ultimately, loss of atrial depolarization, neither of which we observed in the present study (4, 19).

Under basal conditions, there was a linear relationship between perfusion pressure and flow, raising the possibility that changes in heart rate could be linked to flow. Ntg was given to address this possibility. We found that Ntg produced a significant decrease in basal heart rate over most perfusion pressures despite an increase in coronary blood flow. Moreover, the response of heart rate to increases in pressure was blunted. Only the presence of a single extreme outlier in the high-dose Ntg group prevented this result from reaching statistical significance. Although flow may have modulated heart rate, if anything, increases in flow were associated with a decrease in heart rate.

It is conceivable that Ntg affects the sinus node directly. In the isolated rat atrium, high concentrations of the NO donor 3-morphino-sydnonimine lead to a decrease in heart rate (14). Paulus et al. (22) found a small but significant decrease in heart rate in normal volunteers after intracoronary infusion of sodium nitroprusside, an NO donor. The results of Paulus et al. are significant because they used a nitroprusside dose (<4 µg/min) that was devoid of systemic effects. We cannot exclude the possibility that products of NO metabolism, including peroxynitrites (product of NO and superoxide) or oxygen radicals, exert a negative chronotropic effect (14). Further studies are required to elucidate the role of NO in sinus node automaticity.

Diagnosing rejection noninvasively remains an important goal of clinicians caring for heart transplant recipients. Essential to the development of such a test is a clearer understanding of the sources of HRV and the mechanisms of cardiovascular control in the nonrejecting patient. We speculate that, in heart transplant recipients, the denervated sinus node responds to two sources of mechanical stretch: intrathoracic pressure and perfusion pressure. The presence of mechanoelectrical feedback in the heart has been well established (11). In many instances, stretch on the sinus node induced by changes in intrathoracic pressure exceeds that induced by perfusion pressure, and heart rate changes with respiration. In other cases, stretch caused by pulse pressure exceeds that caused by intrathoracic pressure, and the sinus node responds preferentially to pulse pressure. The results of the present study support the hypothesis that perfusion pressure plays a role in regulating heart rate after surgical denervation. During both pulsatile and nonpulsatile perfusion, the amplitude of heart rate fluctuations was similar to that observed in our recent study on heart transplant recipients (28). The interaction among perfusion pressure, respiration, and heart rate has yet to be explored fully in intact preparations.

In summary, sinus node discharge in the isolated perfused heart is mechanically coupled to perfusion pressure on a beat-to-beat basis. Despite increases in flow after administration of low- and high-dose Ntg, heart rate was reduced and the response of heart rate to changes in perfusion pressure was attenuated. These results extend our understanding of the mechanisms of beat-to-beat control of heart rate after heart transplantation.

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