Regional left ventricular deafferentation increases baroreflex sensitivity following myocardial infarction

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Abstract

Objective: Depressed baroreflex sensitivity (BRS) has been observed following MI and has adverse prognostic implications. The mechanism for this finding is unknown. We tested the hypothesis that depressed BRS following myocardial infarction (MI) is related to augmented input from afferent receptors in the left ventricle. Methods: Conscious, chronically-instrumented dogs were trained to undergo BRS testing. This testing was performed before and 4 weeks after creation of experimental anterior MI. Animals were then randomized to undergo regional deafferentation or sham thoracotomy. One week later, BRS testing was repeated. Results: Animals with reduced BRS post-MI showed slight increases in sensitivity values after regional deafferentation. Following sham thoracotomy, animals with reduced BRS post-MI exhibited further decreases in sensitivity values. The differences in mean BRS values measured after regional or sham deafferentation were significant (17.4±2.0 ms/mmHg vs. 11.7±1.4 ms/mmHg; P<0.05). Conclusions: In animals with reduced BRS post-MI, deafferentation of the infarcted region prevented the progressive decline in sensitivity values that was noted in the control group. These data suggest that depressed BRS following MI is related to augmented afferent input from left ventricular receptors.

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

1. Introduction

Abnormalities in the reflex control of heart rate by the sinoaortic baroreflex have been demonstrated following myocardial infarction (MI) in humans and experimental animals [1–13]. Most of the investigative effort has concentrated on the effects of MI on sinoaortic baroreflex sensitivity (BRS) [1,2,6–8,11–13]. Depression of sinoaortic BRS following MI is associated with poor outcomes both in humans [7,13,15,16] and experimental animals [1,6]. The mechanisms whereby infarction of the left ventricle leads to alterations in the control of heart rate by the sinoaortic baroreceptors are unknown. Depressed BRS after MI is not related to infarct location, infarct size, extent of left ventricular dysfunction, or level of cardiac filling pressures [7,15]. These observations indicate that depressed BRS is an independent finding and is not merely due to congestive heart failure after MI.

One possible mechanism is that depressed BRS following MI is caused by interactions between the sinoaortic baroreflex and left ventricular sensory receptors. The left ventricle is richly innervated by sensory receptors with afferent fibers traveling in the vagus and sympathetic nerves. Under most physiologic conditions, these receptors appear to have little direct influence on heart rate in comparison to the sinoaortic baroreflex [17]. However, stimulation of left ventricular receptors with either vagal or sympathetic afferent fibers elicits changes in sinoaortic BRS that are similar to those observed after MI [18–26]. Based on these observations, Schwartz and colleagues

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suggested that depressed BRS post-MI could be related to augmented afferent input from the left ventricle [6]. The purpose of our experiments was to evaluate this possibility by testing the hypothesis that depressed BRS following experimental MI can be reversed by regional deafferentation of the left ventricle.

2. Methods

These experiments were performed in conscious, chronically-instrumented dogs that were trained to lie unrestrained in a quiet room. Following placement of an indwelling arterial catheter, baseline BRS testing was performed. All animals then underwent creation of an experimental anterior wall MI. Approximately 4 weeks following MI, BRS testing was repeated. Animals were then randomized to either regional deafferentation of the infarcted area of the left ventricle or to a sham operation with thoracotomy but no regional deafferentation. After a recovery period of 7–10 days from this second thoracotomy, BRS testing was repeated for a third time. Upon completion of this protocol, animals were anesthetized and a right heart catheterization was performed for hemodynamic assessment. Animals were then euthanized and the hearts were harvested for morphometric analysis. All experimental protocols and anesthetic techniques were reviewed and approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Mongrel dogs of mainly female sex weighing 15 to 25 kilograms and two to four years of age were used for this study.

2.1. Sinoaortic BRS testing

Following adequate training, the dogs were instrumented for recording of surface electrocardiograms. Using aseptic technique, a needle was placed through the skin into the port of a chronic, indwelling arterial catheter for recording arterial pressure with a fluid filled catheter–manometer system. Once stabilized, the responses of heart period and arterial pressure to a bolus intravenous injection of phenylephrine (PE) were recorded. The dose of PE (initially 60 micrograms) was adjusted so that an increase in systolic pressure of approximately 30 mmHg was obtained. During each BRS testing session, PE was injected 3–6 times. Heart period and arterial pressure returned to baseline levels before additional PE was administered. In the majority of experiments, BRS testing was done on two separate days at each point of the protocol. In the remainder, testing was done on a single day.

2.2. Surgical procedures

2.2.1. Arterial catheter placement

The dogs were anesthetized with sodium thiopental (15 mg/kg) and isoflurane (1–2%). Arterial catheters (Access Technologies, Skokie, IL) were placed in the femoral arteries and ports were tunneled subcutaneously to the back. After this and other surgical procedures, animals received post-operative antibiotics for 48 h and analgesics as needed.

2.2.2. Myocardial infarction

An anterior MI was created using the Harris two-stage occlusion of the anterior descending coronary artery [27]. Dogs were anesthetized with sodium thiopental (30–35 mg/kg) and isoflurane (2–4%), intubated, and placed on a respirator. Following left thoracotomy, a small segment of the anterior descending coronary artery was isolated and a ligature was placed to partially occlude the vessel. After 20–30 min, the vessel was completely and permanently occluded. Air was evacuated from the chest and the wound was closed in standard fashion. Oral tocainide (600 mg tid) was given for 72 h postoperatively [28].

2.2.3. Regional left ventricular deafferentation

Animals were again anesthetized as described above, left thoracotomy was performed, and the ligated segment of the anterior descending coronary artery was isolated. For the group randomized to regional deafferentation, a rapidly hardening latex solution was injected into the vessel distal to the occlusion until all visible branches were filled with latex. This technique results in transmural myocardial necrosis [29] and interrupts afferent input emanating from cardiac receptors and/or neural fibers in the infarcted region [30,31]. For the sham deafferentation group, the coronary artery was isolated but no injection was performed.

2.2.4. Hemodynamic evaluation

Once final BRS testing had been completed, the dogs were anesthetized with sodium thiopental (35 mg/kg) and alpha-chloralose (80 mg/kg loading dose and 10 mg/kg hourly). The external jugular vein was exposed and a Swan–Ganz catheter was placed to measure right heart pressures. Phasic and mean pressure measurements were made in the right atrium, right ventricle, pulmonary artery, and pulmonary capillary wedge positions.

2.3. Cardiac morphometric analysis

Following euthanasia, the hearts were harvested for analysis. The coronary arteries were perfused with 500 ml of 1% 2,3,5-triphenyltetrazoleum chloride (Sigma) warmed to 35 °C. The hearts were incubated in the tetrazoleum red solution overnight and then fixed in 10% formalin solution. Transverse sections approximately 0.5 to 1 cm thick were
made and both viable and infarcted regions were traced on clear acetate sheets. Viable areas of the left ventricle were separated from necrotic areas and each was weighed to determine percent infarction by weight. To obtain the fraction of the left ventricle infarcted by area, the tracings were imaged with a camera, digitized, and analyzed using image analysis software (Bioquant).

2.4. Data analysis

BRS was defined as the slope of the linear portion of the stimulus response relationship between heart period (ms) and systolic pressure (mmHg) during bolus PE administration [14]. Linear regression was used to calculate this slope. For each linear regression, an R value ≥0.70 was required for analysis.

Animals were stratified according to whether BRS values were reduced or preserved following MI and according to randomization to either regional or sham deafferentation. Reduced BRS post-MI was defined as a decrease in BRS of at least 5.0 ms/mmHg. This was based on data by Schwartz et al. which indicate that BRS was decreased 6.4±8.2 ms/mmHg post-MI in animals susceptible to sudden cardiac death [6]. For each group, BRS values measured at each stage of the experiment were combined and mean (±S.E.M.) values were calculated. Repeated measures analysis of variance and Bonferroni t-tests were used for within group BRS comparisons between experimental stages. Unpaired t-tests were used for BRS comparisons between regional and sham deafferentation groups. For these and all other comparisons, a P value <0.05 was considered statistically significant.

Similar analysis was used to assess hemodynamic measurements, baseline heart period and systolic pressure measurements, changes in heart period and systolic pressure elicited by PE, intra-animal variability, and cardiac morphometric parameters.

3. Results

Experiments were initiated in 66 animals. This was a technically difficult protocol with a significant failure rate. Data could not be obtained in 28 dogs due to operative mortality (n=16), high arterial pressures (n=8), arterial catheter complications (n=2), congestive heart failure after latex injection (n=1), and poor correlation coefficients (n=1). Thirty-eight dogs completed the protocol and were randomized to either regional (n=20) or sham (n=18) deafferentation. Reduced BRS was observed in 55% (n=11) of the dogs randomized to regional deafferentation and in 44% (n=8) randomized to sham deafferentation. These animals will be the focus of this manuscript. The remaining animals exhibited preserved BRS throughout the protocol and will not be discussed further.

Fig. 1. Mean (±S.E.M.) and individual BRS values for the groups with reduced BRS. Left panel shows the results for the group randomized to regional deafferentation (DEAFF) and right panel shows results for those randomized to sham deafferentation (SHAM). For each group, measurements made before (Pre) and after (Post) MI as well as after either regional or sham deafferentation (Pst Tx) are shown.

3.1. BRS values

Fig. 1 (left panel) shows the mean and individual BRS values in the dogs who were randomized to regional deafferentation. Measurements at each stage of the experiment are shown. Mean BRS following MI and after regional deafferentation were significantly lower than the value measured before MI. However, in 7 of these 11 animals, BRS improved following regional deafferentation. As a result, the mean BRS value increased slightly, but not significantly, following deafferentation.

Fig. 1 (right panel) shows the mean and individual BRS values for the group who were randomized to sham deafferentation. Mean baroreflex sensitivities measured after MI and after sham deafferentation were significantly lower than before MI. However, as opposed to the regional deafferentation group, only 2 of these 8 animals showed improvement in BRS following sham deafferentation. Of the remaining 6 animals, no change was noted in one, but further decreases in BRS were observed in the other 5 dogs. As a result, mean BRS progressively decreased at each stage of the experiment in the sham group.

Direct comparisons between mean baroreflex sensitivities in the regional deafferentation and sham groups are shown in Fig. 2. There were no significant differences in the mean values measured before or after MI. However, mean BRS increased following regional deafferentation but decreased further in the sham group leading to a difference that was statistically significant.

3.2. Heart period and systolic pressure measurements

Table 1 shows baseline heart period and systolic pressure values that were observed during conscious testing. In
and sham deafferentation compared to before MI. In the group randomized to regional deafferentation, these changes were not statistically significant ($P=0.14$).

Intra-animal variability as reflected by standard deviations for the changes in heart period and systolic pressure in response to PE were similar in both groups at each stage of the experiment. Standard deviations for heart period changes ranged from 0 to 414 ms. Standard deviations for systolic pressure changes ranged from 0 to 10.2 mmHg.

3.3. Cardiac morphometric analysis

As expected, the animals randomized to regional deafferentation who received intracoronary latex had significantly larger infarcts than the animals who received sham deafferentation. Infarcts were larger when analyzed as percent infarct by weight (15.2±1.6% vs. 6.0±1.0%) or by area (13.2±1.8% vs. 7.3±0.7%). In addition, the percentage of animals with transmural necrosis was greater in those randomized to regional deafferentation (65%) compared to shams (18%). Gross inspection of the pathologic specimens revealed no characteristics of infarct morphology which were consistently predictive of depressed BRS post-MI.

3.4. Hemodynamic measurements

None of the animals had hemodynamic evidence of congestive heart failure as indicated by elevated right heart pressures. Despite the larger infarct size in dogs who received intracoronary latex, there were no significant differences in these hemodynamic parameters. Dogs randomized to regional or sham deafferentation had similar mean right atrial (0.0±0.5 vs. 0.1±0.2 mmHg), mean pulmonary artery (11±2.6 vs. 12±2.6 mmHg), and mean pulmonary capillary wedge (3.4±0.8 vs. 3.7±1.1 mmHg) pressures.

4. Discussion

Depressed BRS following MI has been documented extensively in both humans and experimental animals [1,2,6–8,11–13]. The presence of impaired reflex control of heart rate post-MI is not merely a pathophysiologic curiosity. Rather, this finding has important prognostic implications. A canine model of sudden cardiac death [32] documented the adverse prognostic significance of reduced BRS post-MI [1,6]. These studies indicate that there are profound consequences of arterial baroreflex dysfunction. The incidence of ventricular fibrillation elicited by this experimental model is significantly greater in dogs with reduced BRS following chronic anterior MI than in those with preserved BRS [1,6]. The incidence of sudden death was as high as 91% for the group of dogs with the lowest BRS values following MI [6]. In humans, prospective
studies of post-infarct patients identified reduced BRS as an important predictor of subsequent mortality [7,13]. Thus, the occurrence of reduced BRS post-MI and its association with a variety of adverse clinical events, including sudden death and cardiac mortality, is well established.

Considering the clinical relevance of reduced BRS post-MI, therapeutic interventions aimed at correcting this autonomic disturbance could be highly beneficial. The rational development of such therapeutic strategies requires understanding of the mechanisms that are responsible for this finding. To date, no definite mechanism has been elucidated. Schwartz and colleagues hypothesized that impaired reflex control of heart rate following MI is related to the central effects of altered afferent input from the heart on the sinoaortic baroreflex [6]. This idea is supported by suggestive but indirect evidence. The left ventricle is richly innervated by sensory receptors associated with vagal and sympathetic afferent fibers. Under most physiologic conditions, these receptors contribute little to the control of heart rate in comparison to the sinoaortic baroreceptors [17]. However, afferent input from ventricular receptors has been shown to have an important effect on BRS, presumably through an interaction in the central nervous system. Activation of left ventricular receptors with vagal afferent fibers by either myocardial ischemia [18,19], volume loading [20], or intracoronary infusion of veratrum alkaloids [21] and prostaglandins [22,23] elicits striking decreases in BRS. Similar effects have been observed in response to activation of cardiac sympathetic afferents [25,26]. These acute changes in BRS in response to activation of left ventricular receptors are similar to the chronic changes observed following MI. These similarities suggest that reduced BRS post-MI may be the result of chronic stimulation of left ventricular receptors in or around the necrotic zone and/or in regions distant from the infarct.

Our experiments were designed to test this hypothesis first proposed by Schwartz and colleagues over 10 years ago [6]. Using identical methodology as in previous studies, we assessed reflex control of heart rate before and after non-transmural anterior MI and then assessed the effects of regional left ventricular deafferentation on BRS. Regional deafferentation resulted from injection of a rapidly hardening latex compound into the previously ligated anterior descending artery. Previous studies have shown that this technique creates homogeneous, transmural infarcts and that afferent input from cardiac receptors and/or neural fibers in the area of necrosis is interrupted [29–31].

The results of our experiments indicate that deafferentation of the infarcted region of the left ventricle has a beneficial effect on reduced BRS post-MI. Regional deafferentation not only restored BRS toward pre-MI levels, but also prevented a progressive impairment in BRS that was noted in the sham group over time. These data suggest that augmented afferent input from the left ventricle may be responsible for the phenomenon of reduced BRS following MI. To our knowledge, these are the first experiments to provide direct evidence in support of this hypothetical mechanism.

In our experimental cohort, reduced BRS following MI was observed in 50% of dogs. In the largest experimental study of the effects of MI on reflex heart rate control published to date, Schwartz and colleagues reported that the incidence of reduced BRS was 73% [6]. In their study, a difference of 3 ms/mmHg was considered a change in BRS. In our study, a decrease of 5 ms/mmHg was required for an animal to be classified as having reduced BRS post-MI. This more conservative definition may account for the different incidence of reduced BRS. If the 3 ms/mmHg criteria are applied to our experimental cohort, the incidence of reduced BRS post-MI increases to 63%. Because animals who were susceptible to experimental sudden cardiac death had a decrease in BRS of 6.4±2.8 ms/mmHg following MI [6], our conservative definition of baroreflex dysfunction may correlate better with this important endpoint.

Augmented input from left ventricular receptors with either vagal or sympathetic afferent fibers can reduce BRS values [18–23,25,26]. Our technique for regional deafervation interrupts both types of afferent fibers. Thus, the present results cannot differentiate whether reduced BRS following MI is related to augmented input from vagal or sympathetic afferents or both. However, observations from our experiments infer that augmented afferent input from left ventricular sympathetic afferents may be the primary factor. In animals with reduced BRS post-MI, a correlation between the changes in sensitivity and changes in baseline heart period was observed (Table 1). Baseline heart period decreased as BRS fell and improvement in BRS was associated with an increase in baseline heart period. These changes in baseline heart period during the protocol were not associated with any significant changes in baseline systolic arterial pressure. Thus, these observations suggest that reduced BRS post-MI may be associated with an alteration in tonic autonomic outflow to the sinoatrial node. For such an alteration to decrease resting heart period, withdrawal of parasympathetic outflow to the sinoatrial node and/or increase in sympathetic outflow would be required. Of note, only augmented input from left ventricular receptors with sympathetic afferent fibers elicits this pattern of altered autonomic outflow [26,33]. Augmented input from left ventricular receptors with vagal afferents produces the opposite effects on sympathetic and parasympathetic outflow to the heart [34].

In summary, regional left ventricular deafferentation by injection of latex into the infarct-related artery has two effects on dogs with reduced BRS post-MI. First, regional deafferentation returns BRS toward baseline levels measured prior to MI. Second, regional deafferentation attenuates a progressive decrease in BRS which occurs in the
control group with sham deafferentation. These results support the concept [6] that impaired reflex control of heart rate following MI is related to the effects of augmented input from left ventricular receptors with either vagal or sympathetic afferent fibers.

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