Influence of right atrial pressure on the cardiac pacemaker response to vagal stimulation

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Bolter, Chris P., and Suzanne J. Wilson. Influence of right atrial pressure on the cardiac pacemaker response to vagal stimulation. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1112–R1117, 1999.—We have recently shown that the intrinsic rate response to an increase in right atrial pressure is augmented when cardiac muscarinic receptors are activated. This present study examines the cardiac pacemaker response to vagal stimulation at different values of right atrial pressure in isolated rat right atrium and in the rabbit heart in situ. In the rat atrium, when pressure was raised in steps from 2 to 10 mmHg, there was a progressive reduction in the response to vagal stimulation [40.5 ± 7.2% reduction (mean ± SE) at 8 mmHg, P < 0.01], which was independent of the level of vagal bradycardia, that persisted in the presence of the β-adrenergic agonist isoproterenol. In barbiturate-anesthetized rabbits with cervical vagal cut and β-adrenergic blockade, raising right atrial pressure ~2.5 mmHg by blood volume expansion reduced the bradycardia elicited by electrical stimulation of the peripheral end of the right vagus nerve (9.1 ± 1.1% reduction, P < 0.0001). These results demonstrate that vagal bradycardia is modulated by the level of right atrial pressure and suggest that normally right atrial pressure may interact with cardiac vagal activity in the control of heart rate.

ALTERATIONS IN RIGHT ATRIAL pressure modify heart rate through intrinsic mechanisms. An increase in pressure in preparations of the isolated right atrium and in the right atrium of the heart in situ after cardiac autonomic blockade results in an increase in pacemaker frequency (2–5, 15–17). Application of autonomic agonists to the right atrial preparation modifies this intrinsic response. In particular, application of the cholinergic agonist carbamylcholine, which slows the pacemaker, greatly amplifies the rate response to an increase in atrial pressure (3, 4). A similar effect is obtained when the isolated atrium (4) or heart in situ (3) is slowed by electrical stimulation of the peripheral end of the cut right cervical vagus nerve. These observations suggest that the cardiac pacemaker’s response to electrical stimulation of the vagus nerve might depend, in part, on the background right atrial pressure (RAP). Here we report experiments in which we examined this possibility. They were conducted on isolated, vagally innervated preparations of the rat right atrium and on the rabbit heart in situ.

METHODS

Ethics

The experiments described here were conducted with the approval of the Committee on Ethics in the Care and Use of Laboratory Animals, University of Otago, protocol numbers 2–96 and 101–96.

Preparation of the Isolated Rat Right Atrium

Wistar rats of either sex weighing 250–300 g were anesthetized with pentobarbital sodium (45 mg/kg ip). The trachea was cannulated, and the animal was placed on positive pressure ventilation. The chest was opened by a sternotomy, and the heart and lungs were exposed and the pericardium removed. In the neck, the right vagus was carefully separated from adjacent tissue and the cervical sympathetic nerve and cut just below the nodose ganglion. The inferior vena cava and the right and left superior vena cavae were ligated. Respiration was terminated, and the heart, with the lungs, thoracic vessels, and vagus nerve attached, was removed from the animal to a dish of saline at 5°C gassed with 95% O2-5% CO2. Flared polyethylene cannulas were inserted into the inferior and right superior venae cavae and secured in place. A thread was tied around the atrioventricular ring, and the ventricles and lungs were removed.

The preparation was transferred to an organ bath containing Krebs-Henseleit solution maintained at 37.0 ± 0.1°C. The cannula in the right superior vena cava was connected to a pressure transducer mounted level with the surface of the bathing solution. The other cannula was connected to a port at the base of the bath. A glass coil, an integral component of the bath, led directly to this port. The connecting tubing, coil, and right atrium were flushed with Krebs-Henseleit. The cut end of the vagus nerve was taken up into a glass suction electrode. The composition of the Krebs-Henseleit solution was (in mmol/l) 143.2 Na+, 5.3 K+, 1.2 Mg2+, 2.5 Ca2+, 127.6 Cl−, 25.1 HCO3−, 1.2 SO42−, 0.9 H2PO4−, 10.0 d-glucose, 5.0 Na pyruvate, 1.0 L-arginine, and 0.01 choline chloride.

Atrial rate was determined on a beat-to-beat basis using an instantaneous ratemeter (Narco biotachometer coupler type 7302) triggered by the atrial pressure signal. After analog-to-digital conversion (MacLab, ADI, Castle Hill, NSW, Australia) atrial rate and pressure signals were stored on disk for later analysis. Simultaneously, a hard copy of the data was obtained using a chart recorder.

Protocols. In all experiments, the reported pressure is the end-diastolic transmural pressure of the right atrium. Baseline pressure was set at 2.0 mmHg, and preparations were exposed to step changes in pressure for 3 min by altering the height of a reservoir attached to the coil. Vagal stimulation usually comprised a 15-s train of impulses of 8 V and 1-ms duration delivered at 20 Hz. This produced a 30- to 50-beats/min decrease in atrial rate at baseline RAP. The vagus was stimulated at baseline pressure just before a pressure step, near the end of the 3-min step in pressure, and 6 min after return to baseline pressure. The baseline response was
obtained as the average of the responses to vagal stimulation immediately before and 6 min after the pressure step.

Three experiments were performed. 1) We examined whether the level of RAP influences the response of atrial rate to vagal stimulation. The response to a standard 15-s period of vagal stimulation was obtained at baseline pressure and at the end of 3-min steps in atrial pressure to values ranging from 4 to 10 mmHg. The response to vagal stimulation at each pressure was expressed as a fraction of the response at 2 mmHg. 2) Next, we looked at the interaction of vagal stimulation and RAP. The responses to three different levels of vagal stimulation (10–30 Hz, 2–10 V) were obtained with RAP set at 2 and 8 mmHg. 3) In each preparation, the atrial rate response to vagal stimulation at different RAPs was examined in the presence of sustained β-adrenergic receptor activation. The response to a standard 15-s period of vagal stimulation was obtained at 2 and 8 mmHg, before and during three cumulative applications of isoproterenol, with each application sufficient to raise atrial rate by a further 50–60 beats/min.

Preparation of the Rabbit Heart In Situ

New Zealand White rabbits of either sex weighing 3.0–4.5 kg were anesthetized with pentobarbital sodium (35 mg/kg iv). Colonic temperature was maintained between 37 and 38°C. The trachea was isolated and cannulated. Catheters were placed in the right femoral artery to monitor systemic arterial pressure and in the right femoral vein for infusion of drugs and solutions. The cervical portions of both vagus nerves were isolated and cut just below the nodose ganglion. The peripheral end of the right vagus nerve was placed over a pair of platinum stimulating electrodes. To record RAP, a catheter was passed through a branch of the right external jugular vein and its tip was positioned in the right atrium. Pleural pressure (PP) was recorded using a catheter terminating in a metal tube that had a polished round tip and communicated with the pleural space through two side holes. This catheter was advanced through the muscle layers between the 4th and 5th ribs into the pleural cavity and secured with a purse-string suture. The transmural RAP was derived as RAP – PP. An electrocardiogram was recorded from surface electrodes positioned to give a prominent R wave, which then triggered an instantaneous ratemeter (Narco biotachometer coupler type 7302). We considered the output of this meter to be the heart rate because both before and during vagal stimulation every R wave was preceded by a P wave. To prevent the cardiac sympathetic nerves and circulating catecholamines influencing heart rate, the β-adrenergic antagonist propranolol was administered at 0.35 mg/kg every 30 min. After the second and subsequent doses of propranolol there was no change in heart rate; this indicated that adequate blockade of β-adrenergic receptors was achieved during the period of data collection. During the experiments, data were monitored continuously on a pen recorder and were saved to disk after analog-to-Digital conversion (MacLab, ADI, Castle Hill, NSW, Australia) for subsequent analysis. Before the collection of data, 50 ml of a solution of dextran in normal saline (50 g/l) were slowly infused intravenously. Five minutes later, 50 ml of blood were withdrawn into a
heparinized syringe and held in a water bath at the animal’s temperature. During the experiment, we infused a volume of this blood (30–40 ml) sufficient to raise transmural RAP by 2 mmHg.

**Protocol**. The heart rate response to electrical stimulation of the vagus was recorded before, during, and after blood volume expansion. This protocol was performed at least twice in each preparation; the data from these trials were averaged to obtain a preparation mean. During stimulation, a train of supramaximal pulses, 1 ms in duration, was delivered to the vagus nerve for 60 s [10 s at each of 6 frequencies (3, 6, ..., 18 Hz)].

**Statistical Analysis**

All data are presented as means ± SE. Statistical analysis was performed using ANOVA and, where appropriate, a corrected paired t-test for post hoc comparisons.

**RESULTS**

**Isolated Rat Right Atrium**

As previously reported, an increase in RAP in the isolated rat right atrial preparation resulted in an increase in atrial rate. For example, raising pressure from 2 to 6 mmHg resulted in a 9 ± 2% increase in rate. Electrical stimulation of the vagus nerve resulted in a brisk reduction in atrial rate and tension development, which reached new steady values by the end of the 15-s period (Fig. 1). The decrease in atrial rate in response to vagal stimulation was reduced as atrial pressure was increased, with a maximum effect seen at 8 mmHg (Figs. 1 and 2). At RAPs of 4 and 8 mmHg, the decrease in atrial rate in response to vagal stimulation was 79 ±
In six preparations we obtained the response to three different levels of vagal stimulation at RAPs of 2 and 8 mmHg. Over the range of vagal stimulation examined, there were no significant differences between the ratios of the response at 8 and 2 mmHg (Fig. 3; P > 0.1). The ratio of the response at 8 and 2 mmHg for the pooled data was 53 ± 3% (P < 0.0001).

The influence of isoproterenol on the response to vagal stimulation at RAPs of 2 and 8 mmHg is shown in Fig. 4. The response to vagal stimulation was increased in the presence of isoproterenol. For example, when atrial rate was increased by ~100 beats/min, the responses to vagal stimulation were approximately twice as large as those obtained under control conditions. As previously demonstrated, when RAP was raised from 2 to 8 mmHg the rate response to vagal stimulation was reduced. In the control condition, the response to vagal stimulation at 8 mmHg was 61 ± 9% the value obtained at 2 mmHg (n = 6, P < 0.01). The effect of an elevated RAP on the response to vagal stimulation was also evident at all three concentrations of isoproterenol, with no significant difference in the percent reduction observed at each concentration. Consequently, these data were pooled; in the presence of isoproterenol the response to vagal stimulation at 8 mmHg was 67 ± 5% the value obtained at 2 mmHg (n = 6, P < 0.01) and was not significantly different from the ratio obtained before the application of isoproterenol.

Rabbit Heart In Situ

Blood volume expansion increased the transmural RAP (right atrial end-diastolic pressure minus end-expiratory PP). During the 60-s period of vagal stimulation, the mean difference in transmural pressure with and without blood volume expansion was 2.5 ± 0.4 mmHg (P < 0.01) (Fig. 5). There was a significant reduction in the heart rate response to vagal stimulation during blood volume expansion (Fig. 5). For the pooled data (data pooled for all 6 frequencies of vagal stimulation), the response during expansion was 91 ± 1% of the control response (P < 0.0001; n = 6).

**DISCUSSION**

The results from this study are consistent with the idea that there is an interaction between RAP and the vagus nerve in the control of the cardiac pacemaker. We have previously shown that when heart rate is slowed by vagal stimulation or by application of carbamylcholine, the increase in heart rate evoked by an increase in RAP is amplified (2–4). Here we have demonstrated that when RAP is raised, the bradycardia elicited by vagal stimulation is reduced. In the isolated rat right atrial preparation, the response to vagal stimulation at 4 mmHg was 78% of the response at 2 mmHg, and at 8–10 mmHg it fell to 50–60% of the baseline response. The results obtained from the experiments on the rabbit heart in situ were consistent with those obtained from the isolated atria. When RAP was raised by 2.5 mmHg, the heart rate response to vagal stimulation fell by 9%. The size of these effects suggests that, normally, RAP may play a significant role in modulating vagal control of heart rate.

In a previous study on the isolated rat right atrium, the rate response to a 6-mmHg rise in RAP was increased fivefold when the preparation had been slowed by the administration of carbamylcholine (4). One
might expect that the magnitude of the influence of RAP on the atrial rate response to vagal stimulation would be similar; however, this was not the case. With RAP elevated, the response of atrial rate to vagal stimulation was reduced at most to 50% of the response observed at the lower baseline atrial pressure. As illustrated in Fig. 6, the difference between the size of these two related, interactive effects probably reflects differences in the magnitudes of the independent rate responses to an increase in RAP and to vagal stimulation.

The design of the experiments on the rabbit right atrium in situ prevented us from examining the responses to increments in RAP greater than ~2.5 mmHg. On the assumption that the response to vagal stimulation would have diminished linearly with further increments in RAP, it is unlikely that the maximum effect would have exceeded that recorded from the isolated rat right atrium. A rise in RAP from 2 to 10 mmHg could be expected to reduce the response to vagal stimulation by almost 30% and thus play a quantitatively significant part in heart rate control. As discussed in a previous paper (3), the pressure-strain relationship of the right atrium in situ will differ from the relationship in the isolated preparation. For the in situ preparation, a larger transmural pressure will be required to generate the same passive wall strain and the maximum strain that can be achieved may be lower.

There are distinct differences in the ionic mechanisms by which vagal nerve stimulation and directly applied muscarinic agonists affect the sinoatrial node membrane potential and slow the cardiac pacemaker (6–8). In this study, however, there was no evidence suggesting that RAP might interact differently with preparations slowed by vagal stimulation and by carbachol application. Nevertheless, as we have previously demonstrated, the enhancement of this intrinsic response to an elevation in RAP during a bradycardia generated by application of carbachol cannot be attributed simply to a reduction in pacemaker frequency (4).

In the isolated preparation, in the presence of the β-adrenergic agonist there was a larger reduction in atrial rate in response to vagal stimulation, a phenomenon referred to as accentuated antagonism. The influence of RAP on the response to vagal stimulation was evident and similar in proportion to that seen in the absence of the β-adrenergic agonist. This suggests that in the most usual situation, where there is tonic activity in both cardiac parasympathetic and sympathetic effenter nerves, the inhibitory influence of RAP on the vagal control of heart rate will vary in proportion to the bradycardia generated by the vagal efferent activity.

Not much is known about the mechanism behind intrinsic rate regulation, but there are some obvious candidates. One of these is the operation of stretch-sensitive channels (1, 11, 17). As a consequence of the syncytial nature of the myocardium, the operation of stretch-activated channels in the atrial myocytes would influence the sinoatrial node through electrical coupling (18). Alternatively, as suggested by the few published studies on this topic, the stretch-activated channels may be in the sinoatrial node itself (1) or in electrically coupled fibroblasts (13). A second mechanism may involve sensory feedback from the atrial myocardium. There is an extensive distribution of intrinsic cardiac neurons, of which some are sensory and have projections to the many ganglia and to the nodal regions (10, 12, 19). Projections to the sinoatrial node could directly influence pacemaker activity, whereas projections to the node and cardiac ganglia may be able to modify autonomic nervous control of the pacemaker. The function of these neurons has not been established, although some have been shown to respond to pressure changes in the heart (9). Depending on the mechanism(s) that are involved, the site of interaction of the intrinsic response to alterations in RAP and vagal nerve activity could be within extrinsic or intrinsic cardiac ganglia or the sinoatrial node or atrial myocardium.

Perspectives

What role can the interaction of this intrinsic mechanism and vagal efferent nervous control play in cardiovascular control? We have previously suggested that the intrinsically generated increase in heart rate that occurs when RAP increases may serve to unload the right ventricle (2). This will become more effective with increasing levels of background vagal tone. The results from this study also suggest that the interaction of this intrinsic atrial mechanism and vagal efferent control could operate synergistically in the control of heart rate. In addition to having a direct effect on heart rate, a rise or fall in RAP will either reduce or increase, respectively, the expression of vagal inhibition on the heart rate. The sum of these influences would help to adjust heart rate, in a beat-by-beat fashion, to suit the level of right heart filling.

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