Changes in Sinus Node Function in a Rabbit Model of Heart Failure With Ventricular Arrhythmias and Sudden Death

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Background—Heart failure is associated with profound changes in the balance of the autonomic nervous system, such as vagal withdrawal and increased catecholamine levels. It is not known whether the intrinsic sinus node function changes during the progression of heart failure.

Methods and Results—We implanted transmitters for Holter recording in an established rabbit model of heart failure (n=9) and observed changes in sinus cycle length and the occurrence of arrhythmias during the progression of heart failure. The in vitro sinus cycle length and the responses to acetylcholine and norepinephrine in the isolated right atria were analyzed in 12 rabbits with heart failure and in 6 control rabbits. In vivo cycle length increased in some animals and decreased in others. Sudden death occurred in 3 of 9 rabbits. These rabbits had developed a shorter cycle length than the surviving rabbits. Ventricular tachycardias developed in all but 1 rabbit. The in vitro sinus cycle length increased in heart failure. The response to acetylcholine also increased in heart failure, whereas the response to norepinephrine was unchanged.

Conclusions—Changes in intrinsic sinus node function during the progression of heart failure cannot explain the observed decreases in heart rate variability and/or baroreflex sensitivity in this disease, because increased responsiveness to acetylcholine would be expected to cause the opposite. (Circulation. 2000;101:2975-2980.)

Key Words: heart failure • sinoatrial node • nervous system, autonomic • acetylcholine • norepinephrine • death, sudden • arrhythmia

Congestive heart failure carries a poor prognosis, with a 5-year survival well below 40%.1 Approximately 50% of deaths are classified as sudden2 and are probably caused by ventricular tachycardias (VTs), bradycardias, or electromechanical dissociation.3 Ambulatory monitoring in patients who then died suddenly demonstrated two patterns of cardiac rhythm immediately before the fatal arrhythmia: (1) a decrease in cycle length and (2) a sinus pause.4,5 Increased heart rate has been shown to be a risk factor in postinfarction patients with depressed left ventricular function.6 These observations suggest an important role for the autonomic nervous system in triggering arrhythmias. Indeed, in patients with heart failure, the occurrence of ventricular premature beats (VPBs) is related to increased sympathetic activity.7

Combined activation of the sympathetic nervous and renin-angiotensin systems and parasympathetic withdrawal have been described in patients with heart failure.7,8 Autonomic tone is assessed by heart rate variability or baroreflex sensitivity.8,9 Therefore, a change in heart rate variability or in baroreflex sensitivity is considered the consequence of a change in the autonomic balance. However, the intrinsic responsiveness of the sinus node to autonomic transmitters or a change in the intrinsic sinus nodal cycle length itself may be altered by the process of heart failure. Thus far, information on these latter issues is lacking.

The purpose of this study was to assess long- and short-term cycle length changes in relation to spontaneous arrhythmias and sudden cardiac death in a rabbit model of heart failure.10 We measured the intrinsic sinus node cycle length and the responses to acetylcholine and norepinephrine in right atrial preparations obtained from rabbits with heart failure. In rabbits in which the in vivo cycle length and the occurrence of arrhythmias during the progression of heart failure had been assessed by telemetry, the in vitro observations could be matched with the in vivo data.

Our results indicate that heart failure causes an increase in the intrinsic cycle length of the sinus node. Moreover, the sinus node develops a larger responsiveness to acetylcholine. Still, sudden death occurs at short in vivo cycle lengths. This implies that these two negative chronotropic factors, increased intrinsic sinus cycle length and larger responsiveness to acetylcholine, are eventually outweighed by other neurohumoral factors.
Methods

Surgical Procedures and Postoperative Care
The experimental protocol was approved by the institutional animal experiments committee and complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 85-23, revised 1985). Two experimental groups were formed: a control group (n=16) and a heart failure (HF) group (n=17) of male New Zealand rabbits (body weight 3 to 4 kg). In the HF group (n=17), the animals were subjected to surgically induced volume and pressure overload as previously described (including anesthesia).10 A biopotential transmitter (TA10CTA-F40, Data Sciences) was implanted in the abdomen of 9 of the 17 animals. In the control group, 12 of 16 animals received a transmitter. The rabbits, housed individually in stainless steel cages (50×40×53 cm) with light on between 6 AM and 6 PM, were inspected daily to detect signs of heart failure (see below).

Telemetry
The radio transmitter transformed the ECG into a frequency-modulated signal and sent it to the telemetry receiver KLA2000 (Data Science, Inc) positioned at the ceiling of the cage. The frequency-modulated signal was transformed back to an ECG signal and recorded on an Oxford Medilog MR35 Holter tape recorder. The recordings were analyzed with Medilog Excel hardware and software (Oxford Instruments). After implantation of the transmitters, 24-hour recordings were made every 2 weeks. The tapes were digitized, and the ECG was displayed on a monitor and visually scanned for arrhythmias. In the absence of differences between cycle length for day and night, 6 hours of the ECG recording (10 AM to 4 PM) were printed on 24 pages displaying 15 minutes each. On every page, the mean value of the RR interval was calculated by counting the number of R waves over the first line of each page (22.5 seconds). By averaging of the 24 values, the mean cycle length over these 6 hours was calculated.

Definitions
VPBs were defined as premature complexes with a QRS morphology different from that during sinus rhythm and without resetting of the sinus rhythm; VT as >3 consecutive VPBs; severe dyspnea as forced respiration at increased rate; and sudden death as death without preceding dyspnea.

Echocardiography
Echocardiography was performed in 12 of the 16 control rabbits and in 6 of the 9 instrumented HF rabbits (ie, not in the animals with sudden death) just before the animals were euthanized (5-MHz ultrasonic transducer; Ultramark 9, Advanced Technology Laboratories). Measurements of left ventricular diameters were made from M-mode recordings of the parasternal long-axis view from which left ventricular dimensions and fractional shortening were calculated (Table 1).

Assessment of Heart Failure: Failure Index
A failure index (0.0 to 1.0) based on relative heart weight, relative lung weight, left ventricular end-diastolic pressure (LVEDP), third heart sound, and ascites was calculated as described previously.10 All 5 parameters were assessed except in animals that suffered sudden death, in which the third heart sound and LVEDP could not be determined, for obvious reasons.

Sinus Node Preparation and Protocol
Right atrial preparations were made as described previously.11 The preparation was pinned on silicon with the endocardial side up in a 5-mL tissue bath and superfused with Tyrode’s solution. An extracellular electrode on the crista terminalis provided an atrial electrogram. Conventional microelectrodes were used to record transmembrane potentials. Temperature was monitored continuously (37.8°C and 38.2°C). Acetylcholine and norepinephrine were obtained from Sigma Chemical Co and from Centrafarm. Oxidation of norepinephrine was prevented by 50 μmol/L EDTA (Merck). Flasks with norepinephrine were protected from light. In pilot experiments, 50 μmol/L EDTA did not exert a chronotropic effect.

In 9 of 17 HF rabbits, a transmitter was implanted. Of these 9 rabbits, 3 died suddenly, 1 was excluded because its failure index was 0, and 1 preparation was lost for technical reasons. We measured the in vitro sinus node function in 12 HF rabbits (4 with previous Holter recordings). These data were compared with a control group of 6 sinus node preparations (2 with previous Holter recordings). After 1 hour of equilibration in the tissue bath, transmembrane recordings were made from the sinus node. We impaled cells that discharged before the atrial reference signal under all experimental conditions to warrant sinus node control over pacemaking.12,13 The administration of 5 μmol/L acetylcholine was the first intervention. The chronotropic effect was measured after 10 minutes. Thereafter, we switched back to normal Tyrode’s solution. The responses to norepinephrine (0.5, 1, and 5 μmol/L) were measured in random order.

Statistics
All data of control and HF rabbits were first compared concerning variance by the F test. Only when permitted, ie, when variance in the 2 groups was not statistically different, was ANOVA applied. Otherwise, the nonparametric Wilcoxon test was used in its exact version. Thus, exact probabilities are given when the latter test was applied. For digital parameters (absence or presence of third heart sound or ascites), we used Fisher’s exact test. Comparison of changes in cycle length relative to the failure index was performed by linear regression analysis. Numerical data are given as mean±SEM unless otherwise stated.

Results

Heart Failure Parameters and Echocardiographic Data
Table 1 (top) shows the results of the induction of heart failure. Significant differences are scored for all tested parameters except body weight. Ascites and a third heart sound were found exclusively in HF rabbits. The failure index was 0.67±0.07 in the HF group. The variance of the parameters relative heart weight, relative lung weight, and LVEDP was significantly larger in the HF group than in the control group.

| TABLE 1. Parameters of Heart Failure in Control and HF Groups |
|----------------|----------------|
|                | Control (n=12) | HF (n=17) |
| Body weight, kg | 3.53±0.14      | 4.17±0.19 |
| Relative heart weight, g/kg | 3.3±0.17 | 5.5±0.38* |
| Relative lung weight, g/kg | 3.2±0.09 | 4.5±0.46* |
| LVEDP, mm | 3.4±0.52 | 12.8±2.59* (n=14) |
| Third heart sound, present/total, n | 0/12 | 10/14* |
| Ascites, present/total, n | 0/12 | 7/17* |
| Failure index, 0.0–1.0 | 0 | 0.67±0.07* |
| Fractional shortening, % | 39.1±1.2 | 24.0±5.7* (n=6) |
| Left atrial diameter, cm | 0.7±0.1 | 1.4±0.04* (n=6) |
| Left ventricular posterior wall thickness, cm | 0.3±0.03 | 0.5±0.04 (n=6) |
| Left ventricular end-diastolic diameter, cm | 1.3±0.1 | 2.4±0.2* (n=6) |
| Left ventricular end-systolic diameter, cm | 0.8±0.1 | 1.9±0.2* (n=6) |
| *P<0.05.
Thus, the results of induction of heart failure are heterogeneous, despite identical procedures.

Atrial and ventricular dilatation was significant in the HF group, whereas left ventricular posterior wall thickness was not significantly increased. Fractional shortening was significantly decreased in the HF group (Table 1, bottom).

In Vivo Cycle Length and Sudden Death

Figure 1A shows the long-term response of the basic cycle length after the induction of heart failure (second operation performed at time zero). Two important features of the group with heart failure are apparent: (1) sudden death in 33% of the animals and (2) a variable response of cycle length during the progression of heart failure between individual animals. Interestingly, animals demonstrate either an increase (n=5) or a decrease (n=3) in cycle length during the progression of heart failure. In 1 animal, there was no change. Figure 1A suggests that cycle length was shorter in animals with sudden death (rabbits 2, 3, and 4; dagger) or dyspnea (rabbits 5 and 6; arrow) than in animals that either survived the observation period or were killed at the first/early occurrence of arrhythmias (rabbits 1, 7, 8, and 9). Figure 1B shows the ultimate in vivo cycle length in 12 control rabbits (265 ms) and in the 9 rabbits of the HF group. The latter group was separated into survivors (304 ms; n=4), rabbits with sudden death (237 ms; n=3), and in rabbits killed because of dyspnea (255 ms; n=2). The differences between the groups survivors, dyspnea, and sudden death were significant (ANOVA, P<0.025). The specific difference between control rabbits and survivors was also significant.

Isolated Sinus Node

Figure 2A shows that the basic cycle length of the sinus node is significantly longer in HF rabbits than in control rabbits (406±13 versus 353±9 ms; ANOVA; P<0.025). Figure 3A shows the significant correlation between failure index and basic cycle length.

Cycle length increased by 74±14 ms in response to 5 μmol/L acetylcholine in control rabbits and by 133±31 ms in HF rabbits (Figure 2B). Therefore, the difference in cycle length between HF rabbits and control rabbits was even larger in the presence of acetylcholine than in normal Tyrode’s solution (Figure 2A). Figure 2B shows that the response to acetylcholine in the HF group is much larger at high heart failure index.

Figure 2A shows that the significant difference in cycle length between HF and control sinus nodes persisted at 1 μmol/L norepinephrine and at 0.5 and 5 μmol/L norepi-
nephrine (not shown). The responses to the 3 different concentrations of norepinephrine were similar in the control and HF groups (Figure 2B).

Cycle Length of Isolated SA Node and In Vivo Cycle Length

Figure 4 shows the relation between the failure index and the in vivo and in vitro cycle length in 2 rabbits from the control group and 4 rabbits from the HF group. It demonstrates that (1) the sympathetic predominance in normal rabbits is preserved in heart failure and that (2) even in this small subgroup, there is a significantly positive correlation between the failure index and the in vitro sinus node cycle length (compare with Figure 3A).

The in vivo cycle length of the rabbit with HF index 1.0 is only 238 ms, although the in vitro cycle length was 420 ms. This particular rabbit showed a large in vitro prolongation of cycle length (+170 ms, compare with Figure 2B) in response to acetylcholine and a subnormal response to 5 μmol/L norepinephrine (−125 ms, compare with Figure 2B). The combined presence of 5 μmol/L acetylcholine plus 5 μmol/L norepinephrine led to a cycle length of 495 ms, although this cycle length was, on average, only 362 ms in the 6 control sinus nodes. This suggests that in this animal with severe heart failure, the sympathetic predominance had increased dramatically, probably in combination with massive vagal withdrawal, because the in vitro sinus node was very responsive to acetylcholine.

Arrhythmias During the Progression of Heart Failure

Table 2 summarizes the degree of heart failure, the moment of death, and the arrhythmic events after the second surgical procedure in the 9 instrumented animals. All 9 rabbits suffered from arrhythmias (8 from ventricular arrhythmias, 1 from a supraventricular tachycardia), and 3 of them died suddenly after 40, 122, and 300 days after the second operation (see also Figure 1A). The first occurrence of VPBs and VTs was after 58±20 and 123±28 days, respectively. We euthanized 3 rabbits (rabbits 7, 8, and 9 in Figure 1A and Table 2) early after the first appearance of arrhythmias. Interestingly, these rabbits had an HF index of only 0.27±0.18, whereas the HF index for the whole HF group was 0.67±0.07 (Table 1). These rabbits had a 44% increase of the left ventricular end-diastolic diameter compared with their own preoperative control values. This underscores the significance of left ventricular dilatation as an arrhythmogenic parameter, because rabbits 8 and 9 (Figure 1A and Table 2) had VTs but failure indices of only 0 and 0.20. The 3 rabbits with sudden death (rabbits 2, 3, and 4 in Figure 1A and Table 2) had a failure index of 0.78±0.11.

Discussion

The main findings of our study were that in this heart failure model, because of combined volume and pressure overload, the in vitro sinus node cycle length increased as a function of the severity of heart failure. To our surprise, the in vitro response to acetylcholine was increased, whereas the re-
response to norepinephrine was unchanged. Moreover, VTs occurred in all but one rabbit. Three animals died suddenly, and they had a short sinus cycle, in contrast to the survivors, in which the sinus cycle was prolonged.

**In Vivo Cycle Length**
In our study, we demonstrated an increase in cycle length in some animals and a decrease in others. A decrease in cycle length seemed to be associated with the occurrence of sudden death. In contrast, prolongation of sinus cycle length was associated with survival. Interestingly, patients with heart failure treated with a β-adrenergic receptor blocker have a better prognosis. The increase of the in vivo cycle length in our study may be due to changes in autonomic tone, but the increase of the in vitro cycle length of the sinus node, as demonstrated in our study, may also play a role. The in vivo cycle length depends on (1) the in vitro (intrinsic) cycle length, (2) the autonomic balance, and (3) the severity of heart failure, which affects both other parameters.

**In Vitro Cycle Length**
Our study shows for the first time that the basic cycle length of the isolated sinus node increases in the setting of dilatation, hypertrophy, or heart failure. We observed a longer cycle length as a function of the failure index. However, we also found an increased basic cycle length when the failure index was zero and only dilatation was present, as demonstrated by echocardiography (not shown). It is of interest to compare our data with in vivo data on cycle length in humans and dogs with heart failure. These data, in humans and dogs, are in good agreement with our observations on increased sinus node cycle length in rabbits with heart failure.

**Increased Response to Acetylcholine**
We have demonstrated an increased response of the isolated sinus node to acetylcholine during the progression of heart failure. The reduction in vagal input to the sinus node during the progression of heart failure therefore has to be substantial enough to compensate for this increased responsiveness to be compatible with high heart rate and decrease in heart rate variability in heart failure. Our findings are in contrast to previously reported decreased sinus node responsiveness to infused acetylcholine and to vagal stimulation in conscious dogs with right-heart failure.

In the dog, the density of muscarinic receptors is 5 times higher in the sinus node than in the atrium. We know of no data on changes in muscarinic receptor density in the sinus node in any species during the progression of heart failure.

**Unchanged Response to Norepinephrine**
In none of the rabbits with heart failure do we have indications for downregulation and/or desensitization of β-adrenergic receptors in the sinus node, because the response to norepinephrine was unchanged. We infer that short in vivo cycle lengths are due to very high catecholamine levels. Our experiments were performed on animals that so far had survived the induction of heart failure. Therefore, we cannot exclude the possibility that the victims of sudden death had altered responses to catecholamines.

Downregulation of β-adrenergic receptors is a well-established phenomenon in failing or hypertrophic ventricular myocardium. In the normal canine sinus node, the β-adrenergic receptor density is ~3 times as high as in the atrium. Data on changes in adrenergic receptor density in the sinus node during heart failure are not available.

**Implications and Limitations**
Our study suggests that the increase in intrinsic sinus cycle length and the increased responsiveness to acetylcholine are adaptive mechanisms during the development of heart failure. Obviously, this is a speculative teleological interpretation, which will be difficult to prove. A lower heart rate may
protect the failing heart from arrhythmias and contraction abnormalities. Only in the face of excessive catecholamine levels and massive vagal withdrawal might these protective mechanisms fall short. Pharmacological agents specific for the control of heart rate may have different effects in failing and in normal hearts, as has been demonstrated with the specific I\(_{\text{f}}\) blocker zatebradine.\(^{22}\)

It should be taken into account that rabbits have a prevailing sympathetic tonus, whereas humans have a prevailing vagal tone. This limits extrapolation of our findings to patients.

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