Effects of anodal vs. cathodal pacing on the mechanical performance of the isolated rabbit heart

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Thakral, Anshul, Louis H. Stein, Mahesh Shenai, Boris I. Gramatikov, and Nitish V. Thakor. Effects of anodal vs. cathodal pacing on the mechanical performance of the isolated rabbit heart. J Appl Physiol 89: 1159–1164, 2000.—Previous studies have suggested that anodal pacing enhances electrical conduction in the heart near the pacing site. It was hypothesized that enhanced conduction by anodal pacing would also enhance ventricular pressure in the heart. Left ventricular pressure measurements were made in isolated, Langendorff-perfused rabbit hearts by means of a Millar pressure transducer with the use of a balloon catheter fixed in the left ventricle. The pressure wave was analyzed for maximum pressure (Pmax) generated in the left ventricle and the work done by the left ventricle (Parea). Eight hearts were paced with monophasic square-wave pulses of varying amplitudes (2, 4, 6, and 8 V) with 100 pulses of each waveform delivered to the epicardium. Anodal stimulation pulses showed statistically significant improvement in mechanical response at 2, 4, and 8 V. Relative to unipolar cathodal pacing, unipolar anodal pacing improved Pmax by 4.4 ± 2.3 (SD), 5.3 ± 3.1, 3.5 ± 4.9, and 4.8 ± 1.9% at 2, 4, 6, and 8 V, respectively. Unipolar anodal stimulation also improved Parea by 9.0 ± 3.0, 12.0 ± 6.0, 10.1 ± 7.7, and 11.9 ± 6.0% at 2, 4, 6, and 8 V, respectively. Improvements in Pmax and Parea indicate that an anodally paced heart has a stronger mechanical response than does a cathodally paced heart. Anodal pacing might be useful as a novel therapeutic technology to treat mechanically impaired or failed hearts.

anodal pacing; cardiac stimulation; pacing waveforms; myocardial contraction

PERMANENT PACEMAKERS ARE THE standard treatment for a variety of symptomatic bradycardias, such as sinus node dysfunction and atrioventricular block. Cardiac stimulation and pacing have focused on such parameters as stimulation site, waveform polarity, stimulus strength, and pulse duration. Clinically, artificial pacemakers have classically utilized standard unipolar cathodal (depolarizing) waveforms (≤1 ms in duration) because it is believed to be safer in regards to inducing arrhythmia. Typically, unipolar anodal stimulation has a shorter absolute refractory period than does unipolar cathodal stimulation. It is presumed that this fact explains the smaller risk of inducing ventricular fibrillation with cathodal pacing. Dekker (4) showed that anodal currents can be used to excite the myocardium; however, their threshold level for excitation is generally higher than that of cathodal stimulation. The same author showed that, although late diastolic stimulation thresholds are lower with cathodal stimulation, with closely coupled stimuli, stimulation thresholds for anodal pacing may become lower than cathodal thresholds. The threshold levels may drop below those of unipolar cathodal pacing during the relative refractory period. Cranefield et al. (3) hypothesized that, for relative refractory tissue, anodal excitation may be more efficient. Indeed, with shorter coupling intervals, during the relative refractory period, anodal stimuli increase the amount of sodium available for depolarization, accelerate repolarization, and increase depolarization amplitudes and upstroke. Previous work in our laboratory showed that the increased thresholds of anodal pacing can be brought down to the level of cathodal pacing by pacing with equipolar biphasic pulses (18). Another recent study (5) suggested that pulses of opposite polarity with some time delay in between might produce a similar effect.

Recent work done in humans with biventricular pacing, as a possible treatment of congestive heart failure, has raised speculation that the polarity of the stimulating pulse may have positive effects on the contraction of the myocardium (1, 2, 17). Experimental work in our group demonstrated that anodal pacing shows marked improvement in electrical conduction velocities over cathodal pacing (14, 18). The possible mechanism of enhanced electrical conduction may be associated with an increased amount of sodium available and improvement in action potential (AP) upstroke (16). In the present study, we hypothesize that the improvements of electrical conduction and AP upstroke due to anodal pacing will lead to a faster activation process and hence to a possible improvement in the mechanical response of the myocardium. A pilot study done in mongrel dogs first suggested that anodal unipolar pacing might improve cardiac output (7). A

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preliminary study done in our laboratory in isolated hearts appeared to support this claim. We observed that changing the pacing waveform slightly improved the ventricular pressure. We seek to examine the statistical significance of this pacing therapy on the mechanical response of the heart under different pacing conditions.

The central goal of this project was to test the hypothesis that anodal stimulation would have a statistically significant, beneficial effect on the contraction of myocardium and would show an improvement in mechanical performance compared with standard cathodal stimulation. We investigated the effects of the pacing waveforms on intraventricular pressure by measuring the maximum pressure ($P_{\text{max}}$) generated by the left ventricle (LV) and the work performed by it ($\text{Parea}$) in response to various waveforms.

METHODS

Eight New Zealand White rabbits, weighing between 2.5 and 3.0 kg, were used for this study. The animals were anesthetized intravenously with a 50 mg/kg dose of pentobarbital sodium with 2,000 USP units of heparin added as an anticoagulant. The heart was then rapidly excised through median sternotomy, and the aorta was cannulated. A modified HEPES buffer (in mM: 108 NaCl, 5 KCl, 5 HEPES, 20 sodium acetate, and 1 MgCl$_2$) mixed with 32 ml of 0.5 M CaCl$_2$, 14.2 g dextrose, 4.4 g sodium pyruvate, 4 ml insulin, and up to 8 liters of deionized water was perfused in the heart in a classic retrograde apparatus. Physiological pH of 7.4 and perfusate temperature of 37°C were maintained throughout the experiment. Perfusion was delivered through a vertical column to ensure that the mean aortic pressure was maintained at 80 mmHg. Once the perfusion was stabilized, Ag-AgCl needle electrodes were inserted epicardially into the myocardium for stimulation, as well as for electrogram recording. The stimulation was delivered to the apex of the LV with the reference kept at the base of the LV; thus the stimulating electrodes were separated by the distance of the LV, which was ~4 cm. The heart was hanging freely from the apparatus to avoid mechanical strain and was submerged into a perfusion bath connected to the pacing reference and amplifier ground through a large-surface conductive plate (10 cm$^2$). Anodal and cathodal thresholds were determined qualitatively to ensure that the myocardium was being stimulated ("capturing"). These thresholds were determined quantitatively in previous studies done in our laboratory (18). A bipolar electrogram was recorded by means of epicardial needle electrodes inserted longitudinal to fibers in the middle of the right ventricle. The electrogram was recorded for the purposes of monitoring electrical activity and ensuring capture due to stimulation. A balloon catheter was then inserted into the LV via the left atrium. Intraventricular pressure measurements were recorded from the balloon catheter by means of a Millar pressure transducer (model SPC-470, Millar Instruments, Houston, TX).

An NB-MIO-16L data-acquisition board, along with a customized program written in LabVIEW (National Instruments, Austin, TX) running on a Macintosh Quadra 650, was used for stimulation as well as data acquisition. The program generated waveforms with varying amplitudes, shapes, durations, and interstimulus coupling intervals. These stimulation waveforms were then delivered through an end-stage amplifier and Ag-AgCl needle electrodes to the heart. The program was also set up to record the bipolar electrogram, as well as LV pressure, simultaneously. The stimulus was also recorded so that pressure and electrogram readings could be correlated with the characteristics of the individual stimulus pulses.

Once excised and set up on the apparatus, the heart was stabilized for a period of controlled pacing, which was adjusted in amplitude, duration, and coupling interval, 5–10% shorter than spontaneous R-R interval of the heart (generally between 400 and 600 ms), to ensure capturing. The stimulus

<table>
<thead>
<tr>
<th>Voltage Level, V</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>8</th>
<th>Across All Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>P$_{\text{value}}$</td>
<td>0.050</td>
<td>0.015</td>
<td>0.050</td>
<td>0.036</td>
<td>0.014</td>
<td>N/A</td>
<td>N/A</td>
<td>0.516 &lt;0.05</td>
</tr>
<tr>
<td>P$_{\text{value}}$</td>
<td>0.066</td>
<td>0.83</td>
<td>0.22</td>
<td>0.36</td>
<td>0.496</td>
<td>0.130</td>
<td>0.117</td>
<td>2.87 &lt;0.05</td>
</tr>
<tr>
<td>P$_{\text{value}}$</td>
<td>0.077</td>
<td>0.10</td>
<td>0.28</td>
<td>0.34</td>
<td>1.91</td>
<td>1.41</td>
<td>3.16</td>
<td>2.34 &lt;0.05</td>
</tr>
<tr>
<td>P$_{\text{value}}$</td>
<td>0.30</td>
<td>0.87</td>
<td>0.70</td>
<td>0.85</td>
<td>9.41</td>
<td>4.38</td>
<td>5.85</td>
<td>5.3  &lt;0.05</td>
</tr>
<tr>
<td>P$_{\text{value}}$</td>
<td>0.66</td>
<td>0.015</td>
<td>0.06</td>
<td>0.37</td>
<td>0.003</td>
<td>0.252</td>
<td>0.516</td>
<td>0.29 &lt;0.05</td>
</tr>
<tr>
<td>P$_{\text{value}}$</td>
<td>1.85</td>
<td>0.026</td>
<td>0.87</td>
<td>0.73</td>
<td>5.52</td>
<td>3.93</td>
<td>3.33</td>
<td>4.8  &lt;0.005</td>
</tr>
<tr>
<td>P$_{\text{value}}$</td>
<td>0.010</td>
<td>0.013</td>
<td>0.047</td>
<td>0.035</td>
<td>0.501</td>
<td>0.210</td>
<td>N/A</td>
<td>3.33  &lt;0.005</td>
</tr>
</tbody>
</table>

Values are means ± SD of percent improvement due to anodal pacing: [(A - C)/C] × 100%, where A is anodal and C is cathodal. SDs and $P$ values result from pairwise $t$-test. Data marked as N/A (not applicable) represent failure to capture for either waveform.
was delivered at 1-ms duration. The pacing protocol was started 30 min after the heart was excised. It consisted of controlled pacing with two different waveforms at four different voltage levels. The stimulation was given in sets of 100 pulses of each waveform. The order of the waveform was alternated between sets, i.e., anodal followed by cathodal and cathodal followed by anodal. The acquisitions were stepped alternated between sets, i.e., anodal followed by cathodal and pulses of each waveform. The order of the waveform was different voltage levels. The stimulation was given in sets of 100 controlled pacing with two different waveforms at four different voltage levels. The protocol was repeated three times for each experiment. In experiments 1 and 6, the heart was only able to capture for two or three acquisitions per stimulation level.

The data-acquisition program recorded from three channels (stimulation, bipolar electrogram, and pressure) at a sampling frequency of 1,000 Hz. These data were then analyzed via a custom program written in MATLAB (Mathworks, Natick, MA). The acquired pressure waves were analyzed for two parameters: maximum systolic pressure generated (Pmax) and work done (Parea). To ensure a "steady-state" condition, only the last 20 beats of each acquisition were analyzed; only captured stimulation and corresponding pressure responses were considered. The Pmax of each pressure wave was defined as the Pmax value after the onset of each captured depolarization. Parea was calculated by estimating the integral of the pressure wave from pressure wave onset to the time when the pressure had fallen to 5% of Pmax.

Both parameters were calculated for each beat for both cathodal and anodal pacing. The results were then represented as means ± SD for every acquisition. This was repeated for each voltage level. Data were then compared in terms of percent improvement due to anodal pacing. The governing equation for each parameter was \((A - C)/C \times 100\)%, where A is anodal and C is cathodal.

For each experiment, the averages across all acquisitions for a particular voltage and particular parameter were represented as means ± SD and were then run through a paired t-test to establish statistical significance. (A P value < 0.05 represented a statistically significant difference due to anodal pacing.) An ANOVA test (repeated measures) was then run across all experiments for each voltage level and each parameter to analyze the variance due to two factors: waveform (anodal vs. cathodal) and subject (experiment). The goal of the test was twofold: 1) to analyze the impact of changing the waveform, and 2) to check whether differences among subjects were significant. A P value < 0.05 indicates that the variable was a statistically significant factor in the variance of data.

**RESULTS**

Figure 1 shows a typical recording of average pressure waves resulting from a train of anodal (solid line) and cathodal (dashed line) stimulation. It is important to note that the averages for the pressure waves are taken for the last 20 beats of the recording, thus avoiding variances due to the transient adaptation resulting from switching pacing waveforms. The overall averages for Pmax and Parea across all experiments for every voltage level are shown in Tables 1 and 2, respectively. The data shown are in three parts: mean percent improvement, SD, and P value (for pairwise t-test). In general, we found that a higher Pmax was generated due to anodal stimulation, as well as a larger integral under the pressure wave. From Tables 1 and 2, respectively, we see that Pmax is, on average, ~4–6%
greater with anodal pacing, and Parea is \( \sim 10-12\% \) greater with anodal pacing. Figure 2 gives insight into the statistically significant range of improvement due to anodal pacing for Pmax at 4 V. The data displayed are the average improvements for all the acquisitions in each experiment with the corresponding SD (represented in percentages), as well as the \( P \) value results of the pairwise \( t \)-test. The pairwise \( t \)-test failed to show significance in experiments 1 and 6 of Fig. 2. This can be attributed to the fact that too few data points were evaluated by the \( t \)-test in these experiments, which is due to the fact that the heart was only able to capture for two or three acquisitions per stimulation level. During the experiments, capture and noncapture were determined qualitatively for obvious cases, and, during data analysis, our software detected noncapture.

Variances in the data were analyzed by means of the ANOVA test (repeated measures) for two factors: waveforms (within experiments) and subject (between experiments). Waveform was found to be a statistically significant factor, and the results of this test are shown in the last columns of Tables 1 and 2 for Pmax and Parea, respectively. The data shown in these columns are represented as the average percent improvement across all experiments for each voltage level, the SD, and the \( P \) values resulting from the ANOVA test discussed above. One can see that waveform is in fact a statistically significant factor for all parameters at all voltages (\( P < 0.05 \)). These data are shown graphically in Figs. 3 (Pmax) and 4 (Parea). The results of the ANOVA test showed that subject was not a significant factor. We conclude from these tests that the improvements in Pmax and Parea reported in Tables 1 and 2, respectively, are not due to the variances in subjects or experimental conditions but, rather, are due to the changing of the pacing waveform.

**DISCUSSION**

Most present day pacemakers use either unipolar cathodal (negative polarity) or bipolar pacing, where the cathode is closer to the myocardium and thus is
responsible for most of the stimulation. It is also known that anodal pacing has higher excitation thresholds and a potential for proarrhythmia. A period of vulnerability for arrhythmia begins at the end of the refractory period and terminates later in the cardiac cycle, and Mehra et al. (12) showed that anodal stimulation has a shorter refractory period than cathodal stimulation. Thus the period of vulnerability is longer for anodal stimulation. It has been indicated that differences between arrhythmia vulnerability to various stimulation waveforms are dependent on their excitability characteristics, such as strength-interval curves (11, 13).

Another area of active interest in applying pacing and pressure waveforms is the multisite-pacing experiment in which the anode is used as the stimulus side. In congestive heart failure models, some signs of increase in effectiveness of myocardium contraction have been shown (1, 2, 7, 18). However, not much has been done to correlate pacing to the heart’s electromechanical coupling, contractility, and other mechanical work. This study presents new data on the effect of pacing waveform on the heart’s mechanical response. The results from our study show that the Pmax generated and the Parea vary with changing waveforms. On average, a 4–6% improvement is seen in the Pmax generated by the LV due to anodal pacing, and a 10–12% improvement is seen in the average Parea due to anodal pacing.

The mechanism of anodal pacing-based stimulation and mechanical performance enhancement in hearts is not fully understood. The question arises as to how anodal stimulation can lead to stronger myocardial contraction. At rest, cardiac myocytes exhibit closed sodium and calcium channels that are slightly inactivated. However, during hyperpolarization, these channels become less inactivated, thereby permitting a larger current flow after stimulation (6). Presumably, faster upstroke allows for stronger contraction as more calcium channels open sooner and for a longer period of time, promoting the contraction cascade (8). Also, because intracellular calcium is at very low concentrations, small improvements are largely amplified by the calcium-triggered calcium-release mechanism. Thus, despite larger activation thresholds, it is conceivable that anodal stimulation provides a measurable advantage in myocardial excitation-contraction coupling. Furthermore, because calcium is a sensitive intracellular signal, accumulation over longer periods of time may modulate contractility (8).

Extensive prior studies have shown that, during anodal stimulation by bipolar electrodes, a "dog bone" shaped region of the cardiac tissue under the stimulating electrodes becomes hyperpolarized. At the same time, the tissue lying in the convexity of the dog bone becomes depolarized and is commonly referred to as "virtual cathodes" (10, 15, 19). Generally, it is believed that the excitation wavefront of anodal stimulation propagates from these virtual cathodes. Therefore, differences in anodal and cathodal responses can be attributed in part to such virtual electrode stimulation in the case of bipolar pacing. This might be applicable in unipolar pacing as well. Employing optical imaging, Knisley (9) showed in rabbit hearts that unipolar anodal stimulation consistently produced early excitation at spots away from the electrode on the fast (fiber longitudinal) propagation axis. At those spots, polarization was found to be positive for anodal stimulation, which is the opposite of polarization immediately adjacent to or under the electrode. It is possible that such a virtual electrode might alter conduction not only locally, but globally as well. Also, our group previously showed that anodal stimulation enhances the conduction velocities of the myocardium (18). An increase in conduction velocity invariably derives from a faster upstroke due to stronger inward ionic current. This observation was verified in our earlier studies, in which APs recorded with the use of floating microelectrodes showed a faster upstroke (dV/dt) for anodal vs. cathodal stimulation (18). A faster upstroke may result from increased intrinsic sodium channel activity, decreased outward K+ current unitary conductance, or a recruitment or decruitment of novel ionic channels (15, 16). A recent study presented the existence of a novel hyperpolarization-induced potassium channel. Computer simulations incorporating this channel kinetics do indeed show altered conduction spread as a result of anodal pacing (14). We hypothesize that these faster conduction velocities resulting from anodal stimulation would alter the mechanical contraction of the heart. We suggest here that increased conduction velocity may lead to higher contractility and thus an increase in mechanical response produced by the anodal stimulation. However, we do not have any data supporting the hypothesized link between conduction and the observed mechanical response. Considerable fundamental research remains to be done to connect these possible relationships.

Limitations. The studies reported here, as well as our previous preliminary studies, were done in isolated, Langendorff-perfused hearts. Several experimental conditions may have contributed to the variabilities in the data. Some factors causing variences in our data included the inherent condition of some hearts compared with others, because some animals were in better physical condition than others. Over the course of the experiment, we observed that the absolute Pmax generated by the LV varied between 10 and 25 mmHg. This suggests the possibility that the heart was weakening over time or previous damage could have occurred in the heart. In some experiments, toward the end of the study, the heart would be expected to be ischemic. We also noticed that anodal pacing still showed a significant improvement in performance. This is an intriguing subject for future studies to show the effects of anodal pacing under ischemic conditions.

We have so far reported electrophysiological and pressure recordings only from isolated hearts. Studies in situ anesthetized, as well as nonanesthetized, animals need to be done next. More comprehensive measurements, including cardiac output, under different loading conditions are warranted. Implications of
our work will be most relevant if benefits can be shown in diseased, ischemic, or failed hearts.

This study would be much more comprehensive if it examined the precise depolarization patterns with the two stimulation waveforms studied. Isochrone maps obtained through extracellular electrical recordings or optical methods would shed light on the shape of the electric field during stimulation and, later, the activation front during propagation.

The promising findings presented here create a need to review the benefits of anodal pacing to enhance mechanical contractility. Benefits of this therapy would be even more appreciated if electrical pacing is shown to enhance heart performance in ischemia or heart failure. In conclusion, use of stimulation pulses involving an anodal component may offer a way for implanted pacemakers to enhance the electromechanical response of the heart. This line of research seeks ultimately to expand the potential applications of pacemaker therapy.

REFERENCES


