Effects of Post-Extrasystolic Potentiation on the *in situ* Heart of *Rana catesbeiana*

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Writer's Comment: *This paper was originally written as a laboratory report for a Physiology class I took in my Junior year. I've always found it somewhat disheartening that after papers I've poured my heart into have been graded -- they're relegated into a monastic existence on a desktop folder, never to be seen or heard from again. That's why I was thrilled to learn that I'd be breathing new life into this old lab report for my UWP 104E class by turning it into a scientific journal article. Part of the assignment was to pretend my work represented original research and to convince my audience and peers that I'd made a significant contribution to my field. The work also needed to target a specific journal (in this case,* PNAS*) in format, tone, and style. I'd like to thank Shannamar Dewey, my physiology TA, and Dr. Victor Squitieri, my UWP professor, for their patience, wise counsel, and unwavering encouragement.*

Instructor's Comment: *For UWP 104E Scientific and Technical Writing, Heather McConnell revisited one of her old physiology lab write-ups and reconfigured that document in accordance with the rigorous editorial and content specifications of a major peer-reviewed scientific journal:* Proceedings of the National Academy of Sciences. *This task required that Heather carefully study not only* PNAS's *formidable "Information for Authors" rubric but also a selection of recently published research papers that had successfully passed this journal's stringent review process.*

To her credit, Heather has crafted an admirably polished and insightful experimental report on the inotropic effects of an induced post-extrasystolic potentiation on the in situ heart of Rana catesbeiana: the familiar American bullfrog. After concisely reviewing the prior research on the topic and articulating an explicit research question, Heather chronicles her experimental procedures, delivers her results, and cautiously discusses their significance. That Heather has managed to record the process of experimental inquiry with such elegance, grace and poise renders her accomplishment all the more remarkable.

—Victor Squitieri, University Writing Program

Premature ventricular contractions (PVC) are a type of arrhythmia that, in conjunction with existing cardiomyopathies, can have deleterious effects on cardiac health. PVCs are largely idiopathic and have no known cure to date. We elicited PVCs in the in situ heart of R. catesbeiana by electrically inducing a premature impulse (extrasystole) in the ventricle and then determined the inotropic effects. Using in vivo techniques, we were able to show that post-extrasystolic potentiation had negative inotropic effects on the extrasystolic contractions and positive inotropic effects on post-extrasystolic contractions.

post-extrasystolic contraction | potentiation | premature ventricular contraction

The muscular heart organ is responsible for pumping blood throughout the body and contains specialized cells for both autorhythmicity and contraction. The autorhythmic cells of the heart, also known as pacemaker cells, are unique in that they can generate their own action potentials, independent of neural stimulation. The electrical activity generated from pacemaker cells is ultimately translated into mechanical activity, namely into ventricular contraction and relaxation (1).

An alteration in the heart's electrical or mechanical activity can have a range of inotropic effects. One common alteration is premature ventricular contraction (PVC), a serious affliction that, in conjunction with existing cardiomyopathies, is associated with an increased risk of mortality (2). It has been previously demonstrated that post-extrasystolic potentiation in the heart will induce a premature ventricular contraction, followed by a compensatory pause and a post-extrasystolic contraction (3).

In this study, we sought to determine the inotropic effects of an induced post-extrasystolic potentiation on the in situ heart of Rana catesbeiana. To determine the inotropic effects of post-extrasystolic potentiation on myocardial contractility, we performed direct ventricular stimulation during various periods of ventricular diastole and at various stimulus intensities. We then evaluated the resulting force of contraction as a function of stimulus timing and intensity. Our results provide direct in vivo evidence that post-extrasystolic potentiation has negative inotropic effects on the

extrasystolic contraction and positive inotropic effects on the post-extrasystolic contraction.

Results

Our results indicated that post-extrasystolic potentiation had negative inotropic effects on the extrasystolic contraction and positive inotropic effects on the post-extrasystolic contraction. To examine the relative changes in the heart's force of contraction, we obtained baseline traces of the heart's mechanical and electrical activity (Fig 1). The average heart rate, atrial contraction force and ventricular contraction force were 41 bpm, 0.14g and 0.77g, respectively.

To examine the inotropic effects of post-extrasystolic potentiation on myocardial contractility, we performed direct ventricular stimulation during late and early diastole using the minimum voltage to elicit a PVC. This voltage was determined to be 2.0 V. The effect of the stimulus voltage used to elicit PVC was determined by using twice the previous minimum (threshold) voltage during late diastole.

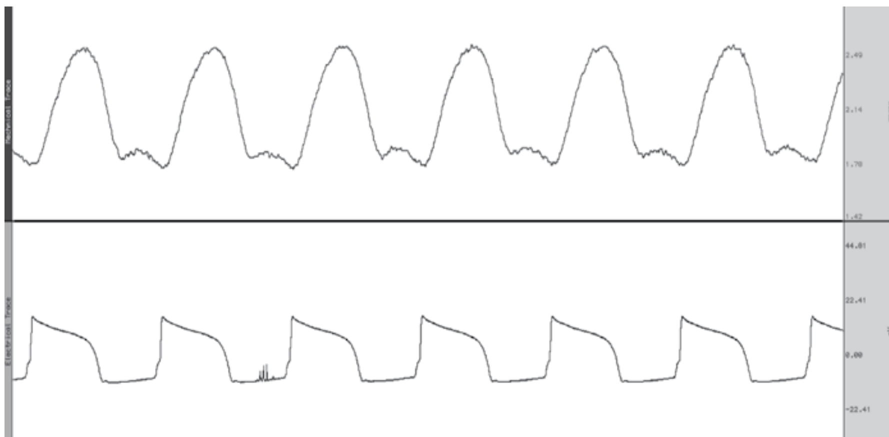

Figure 1. Baseline traces of the mechanical and electrical activity of the in situ heart of R. catesbeiana. The mechanical trace represents the contractile response, the amplitude of which was used to measure contractile force for atrial and ventricular contractions. The smaller peaks represent atrial contraction and the larger peaks represent ventricular contraction. The electrical trace represents the action potential(s) of the cardiac contractile cells, the amplitude of which gives the electric potential.

Contractile force of the heart before the extrasystolic contraction, for the extrasystolic contraction, and for the ventricular contraction after the extrasystole was 1.53g, 1.28g and 1.58g, respectively, for potentiation induced during late diastole. When twice the threshold voltage was used, these respective values were 1.41g, 1.07g and 1.45g.

Although the values were not identical as predicted, the 1X and 2X threshold-induced potentiations resulted in similar, expected, data trends. Relative to the baseline contractile force, the contractile force for the extrasystole itself decreased, and the contractile force for the ventricular contraction following the extrasystole increased for both the 1X and 2X threshold induced potentiations. The contractile force for the 1X and 2X threshold induced extrasystolic contraction was 19% and 24% less, respectively, than the contractile force generated immediately before the induced PVC.

The contractile force for the ventricular contractions that followed the 1X and 2X threshold induced extrasystolic contraction both showed a 3% increase relative to the contractile force before extrasystolic contraction. A PVC could not be elicited during early diastole.

The length of the compensatory pause was about two seconds for both the 1X and 2X threshold induced extrasystolic contractions.

Discussion

In this study, we investigated the effects of post-extrasystolic potentiation on the *in situ* heart of *R. catesbeiana*. We showed that post-extrasystolic potentiation had negative inotropic effects on the extrasystolic contraction and positive inotropic effects on the post-extrasystolic contraction.

Although the heart of *R. catesbeiana* shares several similarities with the human heart, there are differences between the two that are worth noting and were experimentally relevant. Morphologically, the amphibian heart differs from the human heart in that it contains a single ventricular chamber, instead of two, from which all blood exits. Therefore, direct ventricular stimulation in this experiment was performed on the frog's single ventricle. The identity and location of the pacemaker cells responsible for controlling the intrinsic heart rate also differ. In the frog heart, these cells make up the sinus venosus and are located

between the vena cava and right atrium (4). In the human heart, these cells make up the sinoatrial node and are located within the right atrium (1).

Despite their variant identities and anatomical locations, the pacemaker cells of both species control the autorhythmic generation of electrical activity in an analogous fashion (5). This electrical activity is ultimately translated into mechanical activity, which is responsible for the pumping action of the heart. Initiation of activation begins in the pacemaker cells and cell-to-cell spread of activation occurs via intercalated discs, from the atria to the ventricle. Electrical stimulation of the muscle fibers in the atria and ventricle causes contraction and effectively ejects the blood out of the chamber. Electrical activity must occur for the muscle to be activated and contract (1).

The time delay between electrical and mechanical activity is called latency. In our frog, the average latency was 0.15 seconds. Mechanical activity in the heart is broken down into periods of diastole, during which the chambers are relaxed and filling with blood, and systole, when the chambers are contracting and ejecting blood (1).

In our experiment, the ventricle was stimulated directly during late ventricular diastole, causing a PVC. Because the contraction was elicited prematurely (before the ventricle was finished filling with blood), the stroke volume ejected from the ventricle was relatively smaller. The force of contraction used to eject this relatively smaller volume of blood was also smaller. The extrasystolic contractions induced in our frog heart showed (on average) a 21.5% decrease in their force of contraction relative to the contractile force being generated before the induced PVC.

The weak extrasystolic contraction was followed by a compensatory pause, averaging 2.05 seconds. During this compensatory pause, the wave front of activation from the autorhythmic pacemaker cells was still arriving at the ventricular muscle cells, but since the ventricular muscle fibers were still in the absolute refractory period of the extrasystole, no spread of activation occurred; the intrinsic activation process was incapable of activating those cells (6). As a consequence of the compensatory pause, "extra" filling of the ventricle occurred and the subsequent force of contraction was larger (relative to baseline).

Both the weak premature contraction and the large extrasystolic contraction are in accordance with the Frank-Starling law of the heart, which states that the strength of cardiac muscle contraction is a function of the volume residing in the heart at the end of diastole (7). The physiological mechanisms underlying this law involve the increased cardiac muscle fiber stretch that accompanies an increase in end diastolic volume. The contractile energy available at any muscle fiber length is a function of the end diastolic volume and the stretch on the muscle. Increased cardiac muscle fiber stretch affects stretch-induced calcium (Ca^{2+}) release from the lateral sacs. The Ca^{2+} then mobilizes more Actin and Myosin cross-bridges, resulting in more contractile energy. This increase in contractile energy is used to eject the relatively larger end diastolic volume (8).

Therefore, during our compensatory pause, extra filling of the ventricle occurred, which resulted in a larger volume of blood residing in the ventricle at the end of diastole. This larger volume of blood stretched the ventricular muscle fibers, resulting in an increase in stretch-induced Ca^{2+} release, a larger number of Actin and Myosin cross-bridges and therefore a stronger contractile force. Our frog's post-extrasystolic contraction showed a 3% increase in contractile force relative to baseline. These same factors explain the weak extrasystolic contraction we elicited when at a smaller than normal end diastolic volume.

Eliciting a PVC during late diastole using twice the threshold was expected to produce nearly identical results due to the fact that cardiac myocytes are stimulated in an all or none fashion. This attribute means that any arriving stimulus, as long as it is at or above threshold, will elicit an action potential.

Like skeletal muscle, initiation of cardiac muscle excitation-contraction coupling process involves the spread of the action potential along the sarcolemma into the T-tubules, ultimately activating the Dihydropyridine receptor, leading to Ca^{2+} release. This Ca^{2+} activates Rianadine receptors, which opens up additional Ca^{2+} channels, resulting in additional Ca^{2+} release. This phenomenon is known as calcium-induced calcium release. The resultant Ca^{2+} will determine the number of Actin and Myosin cross-bridges formed and therefore the contractile energy available for that contraction (9, 10, 1). However,

because the initial Ca^{2+} release was due to the cardiac action potential, which was generated independent of magnitude above threshold (all or none), nearly identical trends for the forces of contraction were seen for the 1X and 2X threshold stimulations (namely, that relative to baseline, the contractile force for the extrasystole itself decreased, and the contractile force for the ventricular contraction following the extrasystole increased). The small discrepancies in the values between them may have been because the PVCs were elicited at slightly different periods of late diastole. Attempts to elicit a PVC during early diastole were, as expected, unsuccessful as the cardiac myocytes are absolute refractory during this period (11).

Our results suggest two physiologically important consequences of premature ventricular contraction: that it causes a decrease in tension in the extrasystolic contraction and an increase in tension in the post-extrasystolic contraction. The results of this study may change our understanding of the role of PVCs in healthy, diseased, or otherwise compromised hearts.

Materials and Methods

This experiment followed procedures outlined in the NPB 101L Physiology Lab Manual, 2nd ed. (12). A double-pithed frog was obtained and placed in a ventrodorsal position before a ventral midline incision was made through the sternum to expose the heart and surrounding vessels. The heart was released from the pericardial sac, and the vagus nerve was isolated and placed onto a hook electrode in circuit with a stimulator. The apex of the heart was perforated and secured with an uninsulated section of copper wire. The remaining length of wire was then tied at a 30° angle to a force transducer to measure the relative force and frequency of contraction of the heart.

Throughout the experiment, Frog Ringer's solution and DI H_2O were intermittently applied to the frog's heart and body, respectively. Baseline traces of mechanical and electrical heart activity were obtained over a 2-minute period.

The lowest voltage to elicit a PVC was determined and then used to stimulate the heart during late and early diastole. Additionally, twice this voltage was used to stimulate the heart during late diastole. The relative force of contraction was measured as the amplitude given during the periodic cycle.

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