Use of frog ventricle to examine mechanical and electrical activity of heart

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Knights, V. A., D. R. Richardson, and B. Makoba. Use of frog ventricle to examine mechanical and electrical activity of heart. Am. J. Physiol. 256 (Adv. Physiol. Educ. 1): S9-S13, 1989.—This report describes a Langendorff heart preparation, which utilizes frog rather than mammalian hearts to demonstrate both mechanical and electrical events of the cardiac cycle. The preparation is durable in that it can be maintained for several hours while being perfused with room-temperature solutions that are not oxygenated. Ventricular pressure can be correlated with simultaneous measurements of ventricular electrocardiogram (ECG). Ventricular ECG can in turn be correlated with ventricular demarkation, or injury, potentials that reflect changes in ventricular action potentials. The preparation can be set up and used as a wet lab exercise or as a classroom demonstration. It can be maintained for several hours while being perfused with room-temperature solutions that are not oxygenated.

METHODS

Relatively large (4-6 in. body length) African frogs, Cape Rana and giant pyxie (9) were used for this study. After stunning and pithing, the heart was quickly excised and massaged to remove all blood while being suffused with a Ringer solution similar to that described by Gore (4). The ion constituents of the solution (in mmol/l) were 116 NaCl, 4 KCl, 2.6 CaCl2·(H2O)6, 2 MgSO4. The solution used in the present study had a glucose concentration of 11 mmol/l and was phosphated buffered to a pH of 7.4. This solution was later used to perfuse the ventricle of the heart.

The truncus arteriosus or one of its aortic branches was then catheterized, and the catheter was advanced into the ventricle for measurement of intraventricular pressure. Previous studies have recorded intraventricular pressure in an isolated heart via a needle inserted into the ventricle (8). After the catheterization, the excised heart was mounted to the frame of a Langendorff system (Bioscience 8582).

An illustration of the complete preparation along with a base-line polygraph tracing of the variables that can be recorded is presented in Fig. 1. The cannula was coupled via flexible Tygon tubing to a reservoir containing the Ringer solution described above. In this manner, the heart was retrograde perfused via perfusate flowing from the ventricle through leaks in the aortic valve, due to the catheter, then into the truncus arteriosus. Note that a side port on the perfusion tubing was coupled to a pressure transducer for the recording of ventricular pressure. With this arrangement, the ventricle pumped its...
stroke volume into the reservoir system, and the height of the reservoir, which could be adjusted, determined the afterload on the heart, i.e., the aortic pressure. Furthermore, since the reservoir was in direct communication with the ventricle, via the perfusion tubing, the reservoir height also determined ventricular filling, i.e., preload on the heart.

The generation of a ventricular pressure in this system is dependent on there being a finite resistance of the perfusion tubing between the reservoir and the pressure port, here indicated by a narrowing in the tubing labeled R (Fig. 1). In the present experiments, we used a fixed resistance composed of a narrow-bone plastic luer adapter. However, an adjustable clamp on the tubing could also be used to give the demonstrator an additional means of manipulating the preparation.

Electrical activity of the heart was measured in two ways: 1) by measuring potential changes, via a needle electrode, that are reflective of ventricular action potentials; and 2) by detecting the integrated ventricular ECG on the surface of the heart. For the former, a silver needle electrode (Bioscience 814-81470-0) was inserted into the ventricular wall near the apex but without penetrating into the ventricular chamber. The needle was positioned such that no part of it rested against the outside of the ventricular wall. It was then coupled to one side of a high-impedance amplifier/coupler, the indifferent electrode of which was “grounded” into the side port of the perfusion tubing. As shown in Fig. 1 (middle tracing), this system detects a demarkation or “injury” potential that resembles a ventricular action potential. The high resistance of the measuring circuit accounts for the small but repeatable potential changes measured. To measure these injury potentials in a consistent manner, it was necessary to manipulate the electrodes and preparation, such that the penetrating electrode made minimal contact with the small amount of perfusate that oozed over the surface of the heart. Because the size of the needle electrode shaft (350 μm) is more than 10 times the diameter of a ventricular cell (5), it is very unlikely that the injury potential recorded from this electrode was directly measuring intracellular potentials. A much more probable explanation is that the injury potential was the result of extracellular ion fluxes that reflect the action potentials of intact cells within the immediate vicinity of the needle electrode. The small magnitude of the injury potential change during a cardiac cycle (Fig. 1) supports this notion. Additional evidence that this is the case is presented in RESULTS. However, from the point of view of the purpose of this study (to develop an in vitro heart demonstration) it is not necessary to know the exact nature of an injury potential so long as it can be reasonably assumed that such a potential is reflective of a ventricular action potential.

The second type of electrical recording was a simple ECG. This was obtained by placing a pair of silver electrodes coupled to an ECG amplifier against the moist surface of the ventricle.

All oscillograph recordings of pressure and electrical potentials were measured with a Bioscience DC 10 polygraph.

RESULTS

The injury potential. The correlation of this potential to an ECG is presented in the middle and bottom panels of Fig. 1. The close correspondence of the R wave to the rapid increase in voltage on the injury potential tracing and the approximate correspondence of the T wave to the rapid reduction in the injury potential supports our contention that the injury potential is an extracellular recording that is reflective of a ventricular action potential.

A different and slightly more stable injury potential, but one without a corresponding ECG, is presented in Fig. 2. The various phases of this potential have been labeled 0-4 in keeping with standard cardiac action potential nomenclature (6). The sudden but short decrease in the potential toward the middle of phase 4 probably represents atrial depolarization. This notion is supported by the fact that the approximate 300-ms time lag between
not observe a pronounced peak at phase 1, and pacemaker-like activity (phase 4) was noted only in the sinus venosus region. However, the upward drifting phase 4 of the injury potential (Fig. 2) may have been a manifestation of this being an extracellular recording as opposed to being reflective of pacemaker activity. This notwithstanding, the duration of the injury potential (~750 ms from the start of phase 0 to the end of phase 3) agrees quite well with the ventricular action potential duration of 700 ms reported for a frog heart paced at a rate of ~1 beat per 1.2 s (2). Note that the inherent rate of the ventricular rhythm presented in Fig. 2 is ~1 beat per 1.6 s. In summary, the fact that phases 0 and 3 of an injury potential correspond in time to, respectively, the R and T waves of an ECG (Fig. 1) and the fact that the duration of an injury potential is similar to that of a ventricular transmembrane potential supports the notion that the injury potential, as recorded in this study, is reflective of a ventricular action potential. These considerations coupled with the simplicity and minimal equipment necessary to record an injury potential, make it a potentially useful tool for demonstrating dromotropic and chronotropic effects of physiological and pharmacological perturbations.

Correspondence of electrical and mechanical events. This information is easiest to obtain by simultaneously recording the surface ventricular ECG and ventricular pressure, with the latter being used as a measure of contractile activity. An example of such a recording is presented in Fig. 3. Note the close correspondence between the R wave and the onset of ventricular pressure rise. The ~160-ms delay between these two events is within the range of latency periods observed by Brady (2) between the onset (phase 0) of a frog ventricular
action potential and the development of isometric twitch tension. Thus the time lag between the R wave and the subsequent rise in ventricular pressure (Fig. 3) could be due to the time required for excitation contraction coupling to occur. Alternatively, some of this delay could represent a lag between the beginning of contraction and the onset of pressure rise. We did not attempt to distinguish between these two possibilities.

On the diastolic side of the cardiac cycle, note that the T wave, which represents ventricular repolarization, occurs during ventricular relaxation. Thus this comparison of electrical and mechanical (pressure) events can be used to discuss why a cardiac muscle cannot normally contract tetanically. Other potentially useful demonstrations would include the simultaneous chronotropic and inotropic effects of pharmacological agents, e.g., epinephrine.

Pressure-volume relationships: Starling’s law of the heart. To demonstrate this relationship, ventricular pressure is recorded after placing the perfusion reservoir at different heights above the heart. One sequence of such measurements is presented in Fig. 4. Shown are the polygraph tracings of a series of five measurements, each made in the steady state. Note that, as the reservoir height is increased, ventricular diastolic pressure, systolic pressure, and the rate of pressure rise during systole (dP/dt) all increased as shown graphically in Fig. 5. The increase in systolic pressure with increasing end-diastolic pressure is reflective of Starling’s law (10), whereas the increase in dP/dt is considered to be reflective of an increase in contractility (11).

In the teaching of cardiac physiology, Starling’s law and contractility are often considered separate phenomena. Indeed, until the 1970s the consensus among cardiac physiologists was that myocardial fiber length (Starling’s law) and inotropic state (contractility) were independent mechanisms (3). In this regard, the various indexes of contractility derived over the past half century have all been aimed at providing a measure of cardiac performance that is dependent on the inotropic state of the heart but is independent of muscle length. However, in a 1977 review article aimed at reexamining the influence of muscle fiber length on myocardial performance, Jewel (7) concluded that “there can be no such index of contractility (one independent of muscle length) because the inotropic state of the muscle is strongly influenced by its length.” In other words, Starling’s law of the heart and contractility cannot be separated. A recent study by Babu et al. (1) examined possible mechanistic links between myocardial fiber length and inotropic state of the heart. They concluded that the most likely mechanism connecting muscle length to inotropic state is that stretching of the myocardium increases the sensitivity of troponin C to calcium. This results in an increase in the number of cross-bridge attachments during systole for a given level of free intracellular calcium.

The increase in dP/dt observed with increasing filling pressure in the present experiments is consistent with the notion discussed above that Starling’s law and contractility are mechanistically linked. Thus the frog heart preparation is a good teaching tool to demonstrate both Starling’s law and contractility and to point out that these two determinants of cardiac force may be different manifestations of the same basic mechanism rather than totally separate phenomena.

Because in our preparation the ventricle was in constant communication with the reservoir system, the diastolic decline observed in Fig. 4 must have been due to the ventricle relaxing faster than fluid could flow into it. With this consideration in mind it is important to note that not only did the dP/dt increase with increasing

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FIG. 6. Effects of changing the reservoir height from 30 to 60 cm above the heart on ventricular pressure and corresponding electrocardiogram.
reservoir height but the rate of pressure decline during diastole did as well. This is an important point, which demonstrates that an increase in contractility (positive inotropism) is manifested in both an increase in the rate of systolic force development and an increase in the rate of diastolic relaxation. The former affects an increase in stroke volume, whereas the latter assures that this increase will be matched by an adequate diastolic filling.

Interaction of contractility with electrical activity of the heart. An example of this is presented in Fig. 6. In this instance, increasing the reservoir height from 30 to 60 cm above the heart had the predicted effect on contractility, an increase in dP/dt. However, with the exception of some modest effects in the shape of the ECG patterns, which were probably due to a change in the geometric shape of the ventricle, there was no effect on the ECG. This indicates that Starling's law and/or contractility per se do not affect electrical properties of the heart. Further evidence of this is presented in Fig. 7, which shows virtually no change in an injury potential before, during, or after the elevation of reservoir pressure. These results indicate that cardiac action potentials, at least in the frog heart, are not modified by mechanical activity of the myocardium.

In conclusion, this study has shown the following. 1) The frog heart can be used as a Langendorff demonstration of electrical and mechanical activity of the myocardium. The advantage of this over a mammalian heart is that the preparation can be maintained with room temperature perfusates and suffusates that are not supplemented (bubbled) with oxygen. 2) Recordings can be made of at least three variables, albeit not always simultaneously: intraventricular pressure, surface ECG, and an extracellular injury potential. Evidence was presented that the injury potential is reflective of most aspects of a ventricular action potential. 3) Recording of ventricular pressure simultaneously with at least one of the two electrical recordings allows a direct comparison of electrical and mechanical events. 4) An increase in filling pressure of the ventricle results in an increase in the force of contraction, as manifested by an increase in systolic pressure, and a corresponding increase in contractility, as evidenced by an increase in the rate of pressure change during systole. The increase in absolute systolic pressure with an increase in filling pressure is reflective of Starling's law of the heart; whereas the corresponding increase in the rate of pressure change is reflective of an increase in contractility. The fact that filling pressure affects both the Starling mechanism and contractility is consistent with the current opinion of cardiologists that myocardial contractility is not independent of fiber length (7). That is, the Starling mechanism and contractility cannot be separated.

REFERENCES


