SIMPLE EXPERIMENTS TO UNDERSTAND THE IONIC ORIGINS AND CHARACTERISTICS OF THE VENTRICULAR CARDIAC ACTION POTENTIAL

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Electrophysiological experiments are helpful for students to understand the role of electrical activity in heart function. Papillary muscle, which belongs to the ventricle, offers the advantage of being easily studied using glass microelectrodes. In addition, there is commercially available software that simulates ventricular electrical activity and can help overcome some difficulties, such as voltage clamp experiments, which need expensive apparatus when used for studies on living preparations. Here, we present a class practical session that is taken by undergraduate students at our University. In the first part of this class, students record action potentials from papillary muscles with the use of glass microelectrodes, and they change extracellular conditions to study the ionic basis of the action potential. In the second part of the class, students simulate action potentials using the Oxsoft Heart model (v. 4.0) and model their previous experiments on papillary muscle to quantify the effects. In particular, the model is very helpful in promoting understanding of the effect that extracellular potassium has on cardiac action potential by simulating voltage clamp experiments. This twin approach of papillary muscle experiments and computer modeling leads to a good understanding of the functioning of the action potential and can help introduce discussion of some abnormal cardiac functioning.

Key words: cardiac muscle; action potential; microelectrode; computer simulation; Ca²⁺, K⁺

It is of importance to observe, understand, and underline the ionic specificities of the action potential from excitable cells in class experiments. It is quite easy, and relatively cheap, to record action potentials (AP) from a papillary muscle by use of a glass microelectrode (2). Also, it is an important technique for undergraduate students to learn, because it is required for basic studies but is also used by private companies to detect unwanted cardiac side effects of potential new therapeutic agents (1).

In this article, we present a practical class taken by B.Sc. students that has now run for several years in our University. Technically, the impalement of a pap-

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illary cell with a microelectrode is not difficult to achieve. Extracellular conditions (e.g., ionic concentrations or pharmacological agents) can be easily changed to test their impact on the AP. In parallel, a computer stimulation is performed to quantify the experimental effects and to further understand the characteristics of the electrical activity of heart muscle (see Ref. 5).

The general approach is to change a given ion concentration to see which part of the AP is modified. Thereafter, a blocker of the channel carrying the given ion is used to determine the conductance involved. The model allows the quantification of the effects, and, in the particular case of K⁺, it helps students understand why K⁺ has an effect on the AP that might not be initially predicted.

The ions that are studied are Ca²⁺ and K⁺. Ca²⁺ plays a key role in contraction, the physiological function of ventricular muscle. K⁺ is very important in controlling the excitability of cardiac cells and in the occurrence of some arrhythmias (see Ref. 6).

Our general approach can be extended to other cardiac tissues from different species.

MATERIALS AND METHODS

Papillary muscle preparation. Experimental procedures conform to national guidelines. Guinea pigs are killed by intraperitoneal injection of pentobarbital sodium (50 mg/kg) and heparin (1,000 IU/100 g). The thorax is opened and the heart quickly removed, washed in a beaker filled with a physiological saline solution (PSS; see Solutions for composition). A 5-ml syringe filled with PSS is used to cannulate the aorta to wash the coronary arteries of blood. Discrete papillary muscles are then dissected from both ventricles and mounted in an experimental chamber warmed to 37°C (see Fig. 1). Stimulation electrodes are used to mechanically secure the muscle by pinning the papillary tendons and a small piece of ventricular wall at the base of the papillary.

Solutions. To keep the papillary muscle close to physiological conditions, it is submerged in a given volume of PSS, prepared by the students, having the following composition (in mM): 120 NaCl, 5.4 KCl, 2 CaCl₂, 1 MgCl₂, 0.6 NaH₂PO₄, 25 NaHCO₃, 5.6 glucose, and 5 Na-pyruvate, bubbled with 5% CO₂-95% O₂, pH 7.45. This solution is modified by addition of aliquots of modified PSS such that their addition gives the desired concentration within the experimental chamber. After each experiment, the experimental chamber is washed three times with PSS.

Electrophysiology. Glass microelectrodes (GC-150 TF10; Clark Instrument) are pulled by students using a "robust" Narishige puller (PE-2). After the microelectrode is backfilled with 3 M KCl, it is connected to an impedance adapter (HS 170, Biologic) linked to an amplifier (VF-180, Biologic). The resistances of the microelectrodes range between 15 and 40 MΩ.

Hard copies of the electrical recordings are made with a digital oscilloscope (TDS-210; Tektronik) connected to a deskjet printer (Hewlett Packard 690C). An example of such a recording of an AP is given in Fig. 2A.

The AP are triggered by injecting a suprathreshold current, through the stimulation electrodes, via a homemade stimulator. Commercially available stimulators such as the Grass SD9 can also be used. Whatever the stimulator, the initial stimulation setting is used: square pulses of 100 mV and 1 ms at 1 Hz are sent to the preparation. Then, the voltage amplitude is increased until an AP is triggered.

Computer simulations. to simulate guinea pig ventricular AP, we use Oxsoft Heart v. 4.0 (written by and available from Professor D. Noble, Oxford University). An input file as follows is used to run the computation:

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$***PREP:GPCELL***

TEND = 0.25% duration of the computation
ON = 0.05% current injection starts after 0.05 s
OFF = 0.052% and stops after 0.052 s
CA12 = 0.05% free calcium concentration in the uptake compartment (mM)
CA13 = 0.05% calcium concentration in the release compartment (mM)
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GTO = 0.003% conductance for the transient outward current in nS

STEEPK1 = 1.5% factor to set the negative slope of the $I_{K1}$-voltage relationship (5)

GRAFCA = 0% no graph of intracellular calcium

GRAFINC = 0% no trace of $I_{NaCa}$

GRAFA = 0% no trace of $I_{CaL}$

GRAFIK = 0% no trace of $I_{K1}$

GRAFIF = 0% no trace of $I_f$ (peculiar $I$)

GRAFCONT = 0% no trace of contraction

YRANGE1 = 160% voltage scale in mV

YBASE1 = 0.6% position of the 0 mV on the screen

With such parameters, an AP is obtained as shown in Fig. 2B. Parameters such as ionic concentration or conductance (to mimic channel blocker) can easily been changed using the interactive menu of the software.

All figures (except Fig. 1) were obtained by students during a class. They are presented in the article as seen by the students.

RESULTS

Role of Ca$^{2+}$ on the AP. In a first experiment, EGTA (final concentration 1 mM) is added to chelate and
thus reduce the extracellular Ca$^{2+}$ concentration. With such a concentration of EGTA, the concentration is not clamped but slightly reduced. As shown in Fig. 3A, this treatment induces a slight decrease of the AP duration (APD) and a more pronounced decrease of the plateau amplitude. The same effects can be obtained using Oxsoft Heart by reducing the extracellular Ca$^{2+}$ concentration from 2 to 1.6 mM.

To block the L-type calcium current ($I_{CaL}$ in the model), 10 μM nifedipine is used. This blocker induces a reduction of the APD (Fig. 4A) that can be reproduced by blocking 20% of $I_{CaL}$ with the computer model.

From these experiments, it is clear that the plateau is maintained by a calcium current. When reproducing
the nifedipine experiments, students typically begin by blocking 90% of the channels. In this case, the AP becomes very short, strengthening the conclusion about the role of the I_{CaL} on the duration and maintenance of the plateau.

Role of K⁺ on the AP of cardiac muscle. To check the role of K⁺, students increase the extracellular K⁺ concentration to 15 mM. As shown in Fig. 5A, this increase in K⁺ has two main effects, which can be reproduced using Oxsoft Heart (Fig. 5B): it depolarizes the resting membrane potential, and it reduces the APD.

To understand both effects, papillary muscles are exposed to normal K⁺-PSS, containing 1 mM BaCl₂, which is known to block I_{K1}, a background K⁺ current. As shown in Fig. 6A, an increase of the APD, due
to a slowing of the rapid phase of repolarization, is observed. These effects can be reproduced with the computer model by blocking 75% of the $G_{K_1}$ (conductance of the channel carrying K⁺ and responsible for $I_{K_1}$).

From these experiments, we can conclude that the resting membrane potential is due to the opening of the channels responsible for $I_{K_1}$. With respect to APD, Ba²⁺ experiments show that $I_{K_1}$ is involved in the repolarization of the AP, but the decreased APD observed when increasing extracellular K⁺ is difficult to explain. Indeed, increasing the extracellular K⁺ concentration must lead to a reduced outward repolarizing K⁺ current and thus an increased APD. This is the case in nerves and skeletal muscles but not in ventricular muscles (8). To understand this particularity, voltage clamp experiments are needed. It is difficult (technically and financially) to perform such experiments on living preparations with undergraduate students. Thus the use of the model is helpful to build an instantaneous $I_{K_1}$ voltage curve. Such a curve shows

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**FIG. 4.** Effects of blocking L-type calcium current ($I_{CaL}$) on the AP. **A:** adding 10 μM nifedipine, an $I_{CaL}$ blocker, to the bath induces a reduction of the APD. E is the AP in the presence of nifedipine; the time base is 25 ms/division. Other parameters are the same as in Fig. 2A. **B:** modeling a blockade of 20% of the calcium channels can reproduce the experimental results.
how current amplitude, here $I_{K1}$, which is characterized by an anomalous rectification (the outward current is reduced with depolarization), varies with membrane voltage and with external K$^+$ concentration. In Fig. 7, it is shown that increasing the external K$^+$ concentration induces an increased outward K$^+$ current, explaining the reduction of the APD.

**DISCUSSION**

The aim of this class is for students to learn a classic and widely used electrophysiological technique (the recording of membrane potentials with microelectrodes) to understand the role of ions on the cardiac AP.

To understand the role of key ions (Ca$^{2+}$, which is responsible for contraction, and K$^+$, which is important for the stabilization of the resting membrane potential and has a particular role in AP repolarization), we always use the following experimental scheme: changes in external ionic concentration and use of ion channel blockers. The role of sodium chan-
nels in the rapid depolarization phase can also be showed by applying 3–10 μM tetrodotoxin or another sodium channel antagonist to block them. Removing sodium ions cannot be performed, because it will induce a tonic contraction of the papillary leading to death of the tissue. When students are more specialized in electrophysiology, this approach helps students understand the two parts of the Hodgkin-Huxley (H-H) equation (4):

\[ I_x = G_x (E_m - E_x), \]

where \( I_x \) is the current carried by the \( x \) ion, \( G_x \) the conductance of the channel, \( E_m \) the membrane potential and \( E_x \) the equilibrium potential of the ion \( x \) calculated from the Nernst equation

\[ E_x = \frac{RT}{zF} \cdot \log\left(\frac{[x]_o}{[x]_i}\right), \]

where \( R \) is the constant of the perfect gas, \( T \) is the temperature in Kelvin, \( z \) is the valence of the ion \( x \), \( F \) is the Faraday constant, and \([x]_o\) and \([x]_i\) are, respectively, the extracellular and intracellular concentrations of \( x \). By changing the external concentration, the main param-
eter affected is $E_x$ and so the driving force ($E_m - E_x$) in the H-H equation. Thus the student can clearly see where a given ion acts on the AP. By using a blocker, the involved conductance can be more precisely determined ($G_x$ in the H-H equation). In this latter case, the potency of the blocker can be evaluated using the computer model.

The use of a computer model offers many advantages; e.g., rather than spending time calculating free $Ca^{2+}$ in an EGTA-containing solution or the percentage of channels blocked from apparent binding constants, a model rapidly supplies students with the relevant information, the aim being to maintain the focus of their thinking on “the physiological approach”: where does a current play a role; what is its importance; is there a link between the observations and the function of the organ studied? Additionally, pharmacological experiments do not always give a ready explanation of some phenomena, as is the case with the surprising effect of increasing extracellular potassium where, from H-H equations, an increase of the APD, as is seen in nerves, is expected. Modeling allows the simulation of voltage clamp experiments, helping teach students how to interpret $I-V$ curves and, in this example, showing that increased outward current is responsible for the decreased APD. In contrast, performing real voltage clamp experiments is both expensive and difficult.

It is easy for less specialized students to understand the role played by $Ca^{2+}$ ions in the presence of the plateau. The observed effects can be linked to the function of the ventricle: to pump blood into the...
circulation by contracting. The roles of $K^+$ ions in the repolarization phase and in the resting membrane potential are also underlined. Thus this class can be easily adapted to different kinds of students (medical students, scientists). Also, it can lead to discussion about some arrhythmias and/or pharmacological specificities, e.g., hypokalemia, having a renal origin, leading to a longer AP (due to $I_{K1}$'s particular sensitivity to $K^+$) and thus arrhythmias. Also, by using nifedipine to block $I_{CaL}$, students generally find a relatively small decrease of the APD (see Fig. 4), which is quantified as an ~20% blockade. We can also discuss the fact that nifedipine (or molecules of the same family, the dihydropyridines) is generally used clinically to treat hypertension, which has a vascular origin. The differential sensitivity of vascular and cardiac calcium current to nifedipine explains why there is no side effect on cardiac pump functioning. This can be developed further by coupling this class with one on aorta rings (see Refs. 3 and 7) in which the increased sensitivity to nifedipine is shown. The other advantage of such coupled classes is an economizing of the use of animals. Presently, with one guinea pig, we can get four papillaries and six rings from the thoracic aorta. Because the papillary muscle class is quite demanding, one papillary muscle is used per three students. For aortic rings, students work in pairs. Thus with one animal, we are able to serve a class of up to 24 students.

In conclusion, in this article, we have presented a practical class that we have developed over several years. The original feature of this class is the dual simulation/“wet” experiment, which allows students to concentrate on physiology and to bypass certain technical difficulties such as the use of voltage clamp techniques.

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