ACTION POTENTIALS OF OYSTER MYOCARDIUM

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Although many studies have been reported on the transmembrane potentials of the vertebrate heart, little is known about those of the invertebrate heart\(^5,11,26\). According to Prosser\(^{17}\), molluscan heart can be classified as a myogenic heart. Previous histological observations indicate that many of the molluscan myocardium are striated\(^{10}\), suggesting that they have a close similarity to the vertebrate myocardium. The electrogram of molluscan heart was studied by many authors with various materials, including octopus, aplysia, helix, freshwater mussel and oyster\(^{17}\). In general, they have a rapid diphasic deflection at the beginning of the contraction followed by a slow wave. Since the pattern of the surface action potential varies according to the electrode position, monophasic potential recordings are required for discussing the electrical nature of the myocardium. For this reason, the following studies were made to record the action potentials of the myocardium of an oyster (Ostrea laperousei Schrenk), using an ultramicroelectrode and a suction electrode.

METHODS

Glass ultramicroelectrodes with resistance ranging from 30 to 50 MΩ were used for intracellular recording. The flexibly mounted electrode system\(^{20}\) was employed for the recording from moving heart. However, electrodes were easily dislodged probably because of a slow and long myocardial contraction and this in turn prevented the use of the ultramicroelectrode for long continuous observation. Thus, the suction electrode was employed instead of the ultramicroelectrode in long recording. It consists of a 5 ml multifit syringe with No. 20 needle with tip ground so that little harm is applied to the surrounding tissues, and finally a lead was soldered to this needle. A piece of myocardium was sucked through this needle and served as a suction electrode. A cathode follower amplifier with a well selected 1/2 12AU7 as head tube was used, whose output was amplified and recorded by both the inkwriting oscilloscope and the cathoderay oscilloscope. Registration of the mechanical tension was also made through the bonded strain gauge and the Sanborn strain gauge amplifier. The heart was isolated from the shell and placed in artificial sea water.
RESULTS

I. Intracellular recording: Out of the 200 preparations, in only twenty the action potentials were successfully recorded continuously over ten beats. In the other instances, the electrodes were pulled out after one or two beats. In 232 instances of successful penetration two different patterns of action potentials were classified. One of them is illustrated in Fig. 1 A-1. It shows a slowly rising prepotential followed by a spike potential. It then falls down with relatively long plateau phase. Resting potential of this type is about 57 MV and action potential height ranges from 62 to 23 MV (mean 53.3±10.0 MV). In three instances, overshoot was observed, although their magnitude is only of the order of 3 to 8.5 MV (mean 5.7 MV). The duration of action potential ranges from 0.2 to 1.16 sec., and has an average value of 0.75 sec. Slowly rising phase followed by a rapidly rising phase has a magnitude of 10.8±1.9 MV (ranging between 22.4 and 1.4 MV). The duration of this slowly rising phase also varied widely, but it averaged about 0.7 sec. Frequently, a small notch is superimposed on the course of repolarization (Fig. 1 A-2).

The second type also shows a slowly rising potential prior to the rapid depolarization, the magnitude of the potential being 10.5±3.3 MV (Fig. 1 A-3). Therefore, no significant difference can be seen between the first type and the second type with respect to prepotential. Also, the height of the action potential (49.1±10.0 MV) and the resting potential (57.0±6.8 MV) are both very

![Comparison of intracellular and extracellular records. A: Intracellular potentials recorded by ultramicroelectrode. B: Extracellular records led off through a suction electrode.](image)
similar in value to those of the first type. The main difference is then the absence of the long plateau phase. Thus, the duration of the action potential ranges from 166 to 42 msec. (mean 75.4±18.8 msec.) and it is one tenth of the first type potential. In two out of the 50 instances, overshoot of three to five millivolts is recorded. In rare occasions, another spike is observed immediately after the first spike potential (Fig. 1 A-4). The spike height of the second spike is always less than the first one. In this type the magnitude of resting potential is 60.5±4.7 MV and that of action potential is 51.5±7.6 MV in 18 instances. A slowly rising phase, which resembles the other two in both shape and magnitude, is also illustrated in Fig. 1 A-4.

II. Recording from suction electrode; The pattern of action potential recorded by a suction electrode (Fig. 1 B) resembles that obtained through an intracellular electrode (Fig. 1 A), though its magnitude is about one tenth of the latter. Whenever the monophasic action potential is changed into a diphasic one, it is considered to be a development in sufficient suctioning. The suction electrode recording is also superior in that a steady potential can be recorded over five minutes, during which time a series of experiments can be conducted. Although the rapid spike and the slow potentials were both typical features in the myocardium, those potentials which lack spike component were also obtained by the extracellular lead. As can be seen later (Fig. 3), the slow potentials were observed after adding acetylcholine to the heart, and these slow fluctuations were considered to be similar to that described by Bozler\(^2,3\) and Melton\(^15\).

III. Effect of GABA and acetylcholine on the action potentials; Florey\(^6\) applied GABA (\(\gamma\)-Amino-n-butyric acid) on a crayfish heart and observed an inhibition of the heart beat. The action of this chemical on molluscan heart was not so definite as in crustacea heart. After an administration of concentrated solution of GABA an acceleration of the heart beat rather than an inhibition was obtained although the height of mechanical displacement and the magnitude of action potential were remarkably reduced. The spike component appeared to be unchanged, while the plateau phase of the control action potential changed from the dome type to the spike type when tachycardia occurred (Fig. 2). A remarkable inhibition of heart rate is observed after the administration of acetylcholine as expected\(^17\). Fig. 3 A gives a series of records obtained by a suction electrode. A drop of 10\(^{-4}\) acetylcholine was applied between 1 and 2 of the figure, where the heart rate immediately decelerated and the heart ceased to contract. Although there was no visible contraction, potential tracing showed a small undulatory fluctuation which gradually developed into a spike potential. After 30 seconds, the potential was restored to its original shape. Another instance of acetylcholine effect is given in Fig. 3 B, where a drop of 10\(^{-4}\) acetylcholine was applied at the arrow shown in Fig. 3 B-2. The heart beat stopped after one second, while the potential fluctuated continuously. During the recovery from acetylcholine, the premature slow potentials which failed to develop spike potentials were observed.
Fig. 2. Effect of GABA on the action potential (lower curve) and the mechanical tension (upper curve).
A: Control. B: 5 sec after the administration of GABA $1 \times 10^{-2}$. C: 10 sec after B curve. D: 1 minute after C curve.
Time 2 sec; Voltage calibration 2 MV. Noted the acceleration of heart beat while the magnitudes of the potential and tension are both inhibited.

Fig. 3. Effect of acetylcholine on the action potential of an oyster heart (suction electrode).
A: Acetylcholine is applied between 1 and 2. Noted an undulatory potential in 2 previous to the spike. The duration of action potential is also shortened in 2. Time in second.
B: Acetylcholine is applied at an arrow in 2. Noted two premature slow potentials at 3 shown by upward arrows.

It can also be noticed that the duration of action potential is shortened, and therefore, the shape of the action potential changes from plateau type to spike type. This change can be comparable to that of the vertebrate atrial potentials$^{8,18}$. IV. Effect of picrotoxin: On the other hand, Figs. 4 and 5 are the instances of picrotoxin administration where a tremendous increase of the duration of action potential was recorded. In Fig. 4, a drop of $10^{-2}$ picrotoxin was applied to the heart surface at the beginning of B. The tension curve immediately increased. Heart rate temporarily increased but after a few seconds it gradually slowed down and continued until the preparation was washed away with fresh sea water. The
FIG. 4. Effect of picrotoxin on the action potential (upper curve) and the mechanical tension (lower curve) of an oyster heart.

A: Control. B: Immediately after the administration of picrotoxin $1 \times 10^{-2}$. Noted increase in both heart rate and tension.

C: One minute after B. D and E: heart rate decreased remarkably, and the durations of both action potential and mechanical tension remarkably increased. Noted a close similarity of action potential to the vertebrate myocardium. Time 2 sec. Vertical line represents 2 MV.

FIG. 5. Effect of picrotoxin on the action potential (upper curve) and the mechanical tension (lower curve) of an oyster heart.

A: Control. Gradual prolongation of action potential plateau from B to E. Two or three small potentials appear to be superimposed on the plateau phase. Time 2 sec. Vertical line indicates 2 MV.

action potential shows a small initial negative deflection followed by a prolonged potential change. Similarity of this curve to that of the myocardial monophasic potential is striking. Fig. 5 shows other instances where the prolongation of the plateau phase is also remarkable. In this instance, the plateau phase of the action potential (C, D and E) is not likely to be a single smooth event. There appears another depolarization at the end of each potential. The fact that the contraction time is much longer than the relaxation time suggests that the muscle contracts tetanically. Since these curves are obtained by a suction electrode, the activity of many fibers must be taken into consideration during this plateau phase. The prolonged action potential after picrotoxin can be shortened by the administration of GABA and acetylcholine.
DISCUSSION

Two types of action potentials were described, but they all have both spike and slow component, suggesting that in this myocardium, the potential consists of the basic depolarization reinforced by a superimposed spike potential. This finding agrees with that of the vertebrate smooth muscle potential\(^1,5\), rather than myocardial action potentials.

Histological observation was made parallel with this study. Difficulty in inserting the microelectrode is attributed to the small size and the coarse orientation of the muscle fibers. With Azan staining some of the myocardial fibers showed striation, while in the others, striation was hardly recognizable. Presence of striation in myocardium of molluscan has already been known\(^10\), but in some species striation is reported to be absent\(^13\). The suggestion can thus be made that the similarity of the oyster myocardial action potential to the vertebrate smooth muscle potential may not largely be due to morphological reasons, such as the existence of striation.

The presence of an overshoot and the double spike (Fig. 1 A-4 and 1 B-3) is the characteristic feature of the vertebrate smooth muscle\(^21\). A group of potentials which have a remarkable plateau resembles the typical monophasic action potential recorded from stomach surface\(^4\) or sinus node\(^8,10\). The difference in pattern of an intracellular action potential appears not due to the different type of myocardial fibers, since the transition from spike type to the spike-dome pattern has frequently been observed with a suction electrode.

Hoffman et al.\(^7\) found in the mammalian myocardium that, if the suction electrode is properly used, the monophasic potentials recorded with it may be taken as a reliable index of the shape of the repolarization phase of an action potential. The above experiments provide the possible generalization of their concept into the oyster heart. Presence of slow depolarization prior to the spike, or the slow waves, appears to be the prepotentials as stated above. The presence of this potential without obvious mechanical contraction after the administration of acetylcholine provides another criterion of the characteristics of this potential. It was previously reported that there are no localized pacemaker regions in this heart studied by a local heating method\(^12\). This is in good agreement to the fact that the prepotential can be seen everywhere in this heart.

GABA appears to have no appreciable effect on molluscan heart, although in a very concentrated solution, it has an excitatory effect.

A remarkable prolongation of plateau was formerly observed by the administration of low potassium solution and by veratrine\(^14\) in vertebrate myocardium. The significance of this finding is that the spike type action potential is converted into a plateau type. In other words, smooth muscle type potential is changed into a cardiac type.
SUMMARY

Membrane resting and action potentials of the oyster heart were studied, using both an ultramicroelectrode and a suction electrode. Intracellular action potential and the monophasic extracellular action potential led off from the suction electrode show very similar patterns. Action potential can be classified into two types, but they all have a basic slow potential change superimposed by a rapid spike component. The close similarity of action potential to the vertebrate smooth muscle action potential or the pacemaker potential was discussed. With the application of picrotoxin, remarkable prolongation of action potential resembling the vertebrate cardiac action potential was obtained.

REFERENCES

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