Effects of Barium Chloride on Resting and Action Potentials of Ventricular Fibers of the Frog

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By means of an intracellular capillary electrode the effects of barium chloride on the resting and action potentials of ventricular fibers of the frog were recorded in hearts with circulation intact and hearts isolated and perfused. The changes were essentially similar in both preparations. The major change observed was a statistically significant (p < 0.01) increase in duration of the action potential. Changes were also observed in the magnitude of the resting and action potential, in the rate of depolarization, and in rhythm, but these did not occur invariably. By a series of exclusion experiments it was demonstrated that barium has a direct effect on the ventricular fibers of the frog's heart.

The application of the intracellular capillary electrode developed by Graham and Gerard to single myocardial fibers has provided a new tool for studying effects of various influences such as temperature, ionic concentrations, electrolytes, drugs and induced arrhythmias on the electric potential of the cell.

Numerous other investigators have reported on the intracellular potentials obtained on hearts with intact circulation and in isolated heart muscle strips. The purpose of the present study was twofold: (1) to confirm their observations and to extend the investigation to perfused hearts, (2) to study the effects of barium on the intracellular potentials of the ventricular fibers of the frog.

The effect of barium ion on the electrical and mechanical activity of the heart has been studied by many investigators. The production of various degrees of block, idioventricular rhythm, and extrasystoles, tachycardia, and fibrillation of ventricular origin have been reported. Boehm in 1875 noted that barium ion in small doses (0.01 to 0.03 Gm.) increased the work of the frog's heart without changing its frequency and that relatively large doses (0.05 to 0.10 Gm.) subcutaneously, produced irregularities and cardiac arrest. Kruta's observation that barium ion can initiate automatic activity in quiescent strips of mammalian left atrium has been confirmed by others. Minute amounts of barium ion have been shown to have the same action on small muscle bundles from the frog's ventricle and on the isolated papillary muscle of the cat's heart.

Gmelin in 1824 postulated that the cardiac effects were mediated through its nervous connections. Marceau similarly concluded that the effect of barium chloride on the frog heart is on the ganglia and nerves and not on the muscle cell. The rapidity of the effect is faster than can be accounted for by the rate of penetration of barium. however, have postulated both a vagotropic and myotropic effect of barium chloride.

Methods

Frogs (Rana pipiens) in winter stage were used. The animals were pithed and the heart exposed by removing the chest wall and pericardial sac. The frog with intact circulation was placed in a lucite container which was fixed to a suitable platform that could be raised or lowered. Frog Ringer's solution was circulated through the container and the temperature of the solution was recorded with each experiment. In the experiments with the perfused heart the method of Bülbring was employed, since this procedure permits the heart to be maintained within the chest cavity. Frog Ringer's solution was the perfusing medium. The venous pres-
The frequency-response curve was flat to approximately 100 cycles per second with a resonant peak above that value. The over-all time constant of the recording system was of the order of 500 microseconds. The resistance of the capillary electrodes varied between 10 and 30 megohms.

Microelectrodes were pulled from special glass capillary tubing to a diameter tip of approximately 1 micron, and filled with 3 molar potassium chloride by boiling under pressure. The electrode was placed in a special holder and the latter mounted in a micromanipulator. A fine sodium chloride-agar wick acted as the indifferent electrode and was inserted into a similar holder and mounted in an adjacent twin micromanipulator. Chlorided silver spirals were used to lead off from both the intracellular and indifferent electrode to the recording system. A block diagram of the latter is illustrated in figure 1. The adequacy of the recording system was determined by measuring the frequency response with the circuit as used in the experiment. The frequency-response curve was flat to approximately 100 cycles per second with a resonant peak above that value. The over-all time constant of the recording system was of the order of 500 microseconds. The resistance of the capillary electrodes was between 10 and 30 megohms.

Before attempting to record with the microelectrode a small glass loop 3 mm. in diameter was placed about 5 mm. above the frog's heart. A dissecting microscope of 35X magnification was employed to guide the intracellular electrode into the glass loop and then into the myocardial fiber. The tip of the agar wick electrode was placed on the epicardium within the loop, 1 to 2 mm. from the exploring electrode. The lucite container was elevated until the heart made light contact with the glass loop. The intracellular electrode was then lowered until it penetrated the heart tissue. The success of implantation was determined by observing the size and shape of the membrane action potential on the cathode ray oscilloscope. When satisfactory, the electrical potential was recorded on a Hilger string galvanometer (Cambridge Model No. 3) and after 5 to 10 beats were obtained, the electrode was withdrawn to the surface to obtain the zero level of potential and a calibration was made. The container was then lowered to remove the pressure on the loop on the heart. A fresh site was selected and the procedure repeated.

For the simultaneous recording of the surface electrocardiograms with the electrical events of single fibers a Sanborn Twin Beam electrocardiograph was employed. For the recording of the action potential the output of the electrometer tube, used as a cathode follower, was directly coupled to the direct current amplifier stage of the electrocardiograph. A series of control records were obtained from each preparation. Every effort was then made to obtain records during, immediately after, and at frequent intervals following the administration of barium chloride.

**RESULTS**

**Observations on Normal Controls.** The configuration and magnitude of the action potentials recorded from both the intact and perfused hearts were essentially similar. The typical normal control curve revealed a rapid rise time, with overshoot followed by a slow repolarization stage. Not infrequently, various degrees of hyperpolarization were also seen (figs. 2A and 3A).

In 150 measurements of 23 preparations with intact circulation taken at 22 C. to 29 C., the range of the resting membrane potential (MRP) was between 40 and 80 mv. with a mean of 58.4 ± 8 mv. The membrane action potential (MAP) varied between 50 and 110 mv. with a mean of 74.2 ± 9 mv. The overshoot averaged 27.1 per cent of the membrane resting potential.

The duration of the membrane action potential varied between 0.42 to 1.0 second and increased approximately linearly with the cycle length over this range (fig. 4).
**Fig. 2.** Effect of barium chloride (5 mg. per kilogram) injected into the aorta on the membrane action potentials of ventricular fibers of frog heart. See text for discussion. Ordinates, millivolts; abscissas, time. Time lines, 0.04 second. All measurements made from top of string shadow. X indicates mark where standardization voltage was introduced.

**Fig. 3.** Effect of 1:5000 barium chloride on membrane action potentials of the ventricular fibers of the frog heart, isolated and perfused. Lead I (above). Membrane action potential (below). Discussion in text. Ordinates, millivolts; abscissas, time. Time lines, 0.04 second. Gain, 0.9 cm. = 50 mv.

**Fig. 4.** Graph showing duration of action potential in seconds (ordinate) as a function of heart rate per minute (abscissa).

**Modifications Produced by Barium on the Transmembrane Potential.** The effects of barium chloride in doses of 5 mg. per kilogram were variable and not easily summarized. The most consistent change was an increase in the duration of the action potential (APd) and this was mainly due to a lowering and lengthening of the terminal portion of the recovery phase. A lengthening of the plateau portion of repolarization was also a contributing factor but less frequently. The action potential of the experimental as compared with the control preparations with the same heart rates was increased. This increase was found to be statistically significant (p <0.01).

In 30 per cent of the observations a moderate increase in the duration of depolarization was observed. The magnitude of the membrane resting potential was not significantly changed in the majority of the experiments. When the membrane resting potential was decreased...
A

FIG. 5. Intracellular potentials from an isolated ventricular tip of frog heart as modified by barium and potassium (see text). A, 20 minutes after injection of 5 mg. of barium chloride into dorsal lymph sac; B, six minutes after immersion in 1 per cent potassium chloride solution.

during the course of an experiment the membrane action potential was decreased proportionately, and this was usually observed when the heart contractions were feeble or when ventricular arrhythmias occurred.

When 15 mg. of barium chloride per kilogram were injected (six experiments) ventricular asystole was observed in all, with recovery in four. The membrane resting potential was greatly diminished or unobtainable during mechanical asystole. With recovery the ventricles contracted with greater force than normal and both the membrane resting potential and the membrane action potential returned to normal or above control values.

Figure 2 shows the effects of the 5 mg. of barium chloride per kilogram on an intact heart and reveals the following significant points: (1) The voltages of the membrane resting potential and the membrane action potential are maintained relatively unchanged throughout the experiment (2A-G); (2) the increase in duration of the action potential appears quite early—even during the period of injection (2B)—while the lengthening occurs predominantly in the third phase of recovery as seen in 2B and C; (3) when a transient ventricular arrhythmia develops, the action potentials following a longer cycle have a shorter time of depolarization (2D); (4) the effect of the barium passes off after 20 minutes (2G).

The modifications of the transmembrane potential in the perfused frog heart were essentially similar to those in the heart with an intact circulation. Figure 3 shows the characteristic changes. Figure 3B shows the prolonged action potential obtained with 1:5000 barium chloride. With return to normal frog Ringer's solution, the action potential assumed a normal configuration.

DISCUSSION

The ability of barium chloride to produce various degrees of block, idioventricular rhythm, ventricular extrasystoles and tachycardia has been observed in the course of this study and confirms the observations of others.7-9 Many investigators have commented on the hypertensive effects of barium chloride.7-18 Dawes17 suggests that the rise in blood pressure may be due to an enhanced sympathetic outflow and the liberation of adrenaline and noradrenaline in the heart from the adrenals and elsewhere. Hazard and Quinquand18 have shown that this rise is abolished by yohimbine, a sympatholytic agent. The perfusion technic was adopted essentially to exclude this or any other hormonal effect on the heart. The observation that barium chloride produced similar modifications of the transmembrane potential both in the perfused and intact heart appears to justify the exclusion. It did not, however, rule out a direct effect of barium chloride on ganglia and nerves within the frog heart. To demonstrate that barium chloride has a direct effect on the ventricular fiber of the frog, the following procedure was employed: Five to 10 mg. of barium chloride were injected into the dorsal lymph sac of a frog and after 10 minutes the heart was exposed and the ventricular tip excised. The action potentials of these rhythmically beating tips were recorded. Figure 5A shows the characteristically prolonged action potential. When additional barium chloride (1 per cent solution) was applied topically an exaggeration of the barium effect was observed. On the other hand, addition of a 1 per cent potassium chloride solution to the rhythmically beating tip antagonized the barium effect by shortening the action potential and almost abolishing its rhythmicity (fig. 5B).
It is noteworthy that in the intact frog heart, the injection of potassium chloride in proper concentration also antagonized the effect of barium.\textsuperscript{12}

The results obtained in ventricular tip experiments point to a direct effect of barium chloride on the myocardial fiber. They, therefore, confirm the opinion of Agnoli and Bussa\textsuperscript{16} who have postulated both a neurogenic and myotropic effect of barium chloride on the heart.

The exact mechanism responsible for the modifications of the transmembrane potential by barium ion is not known. If we accept the postulate of Hodgkin and Huxley\textsuperscript{19} that sodium permeability is principally associated with the phase of depolarization and potassium permeability with the phase of repolarization of the action potential, then the prolonged action potential occurring with barium ion can be explained on the basis of its inhibition of potassium ion migration. Davson,\textsuperscript{20} studying the comparative effects of environmental changes on the permeability of the cat erythrocyte membrane, demonstrated that barium ion retarded permeability of the red cell membrane of the cat to potassium ion (at all concentrations above 0.505 M) while calcium ion (in concentrations above 0.01 M) accelerated the penetration of potassium ion. An excess of calcium ion should therefore produce a shortened action potential; this has been observed when calcium was increased in the perfusate. Of further interest is the observation of Hoffman and Suckling,\textsuperscript{21} confirmed by us, that ethylene diamine tetra-acetate (EDTA), a chelating agent which preferentially binds divalent cations, also produces a prolonged action potential but primarily of the plateau rather than the terminal portion of recovery. The finding suggests that barium ion and ethylene diamine tetra-acetate act at different metabolic levels, but both are associated with a retardation of membrane permeability to potassium.

**SUMMARY AND CONCLUSIONS**

The configuration and magnitude of the membrane action potential obtained in the intact and perfused hearts were similar. Resting potentials averaged 58.4 ± 8 mv. and action potentials averaged 74.2 ± 9 mv. giving an average overshoot of 27.1 per cent. The duration of the membrane action potential varied between 0.42 and 1.0 second, being a function of heart rate. A linear relationship exists between the logarithm of the action potential duration and the logarithm of the heart rate.

The modifying effects of barium chloride on the transmembrane potential of the frog's ventricle were essentially similar in the perfused heart and in the heart with intact circulation. The major change observed was a statistically significant (p < 0.01) increase in duration of the action potential. Changes were also produced in the magnitude of the resting and action potential, in the rate of depolarization, and in rhythm, but these did not occur invariably.

By means of a series of exclusion experiments it was demonstrated that barium chloride has a direct effect on the ventricular fiber of the frog's heart.

It is postulated that the prolonged duration of the action potential caused by barium is associated with a retardation of migration of potassium out of the cell during its electrical activity.

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