Effects of Barium on Mature and Embryonic Heart Cells

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The effect of barium ion on the electrical and mechanical activity of the heart has been reported by many investigators. The production of various degrees of heart block, idioventricular rhythm, extrasystoles, tachycardia and fibrillation have been observed. Boehm noted in 1875 that the barium ion in small doses, 10 to 30 mg/kg, increased the work of the frog's heart without changing its frequency and that relatively large doses, 50 to 100 mg/kg, given subcutaneously, produced irregularities and cardiac arrest. Kruta observed that barium can initiate automatic activity in quiescent strips of mammalian left atrium. The same effect has also been observed in quiescent strips of frog ventricle. By means of intracellular glass capillary electrodes Kleinfeld et al. observed that the transmembrane action potentials recorded from the apical strip of frog ventricle showed depolarization during diastole and slowing of repolarization. The amplitude of the transmembrane action potential was unchanged. Since this region of the ventricle is devoid of ganglia and nervous connections, it was concluded that barium can initiate automaticity and that it has a myogenic action. More recently it has been shown by Lehmkuhl and Sperelakis that barium administered to embryonic chick heart cells initiates pacemaker activity in heart cells previously quiescent.

The present study was undertaken to observe the effects of barium chloride on 1) the transmembrane action potentials of sinoatrial, atrial, atrioventricular, Purkinje and ventricular fibers of the dog heart, 2) electrical and mechanical activity of isolated rat atrium, and 3) rhythmicity of cultured embryonic chick heart cells.

Methods

For recording transmembrane action potentials from sinoatrial node, right atrium, and atrioventricular node of dog heart, the preparation included portions of superior and inferior vena cava, right and left atrium, interventricular septum and ring of tricuspid valve. The specimen was placed in a temperature-controlled perfusion chamber and bathed in a slowly flowing Tyrode solution through which a mixture of 95% oxygen and 5% carbon dioxide was bubbled continuously. The temperature of the bath was maintained between 35 and 36°C. The transmembrane action potential was recorded through an intracellular glass capillary electrode. When the action potential was recorded from sinoatrial node, the preparation was beating spontaneously. However, when action potentials were recorded from atrium or atrioventricular node, the tissue was stimulated by rectangular pulses, usually at a rate of 90 beats per minute, by a Grass stimulator. The stimulus had a duration of 5 milliseconds and was isolated from ground. In 5 experiments action potentials were recorded from the atrioventricular node in a preparation beating spontaneously. To obtain action potentials from Purkinje and ventricular muscle fibers, a false tendon-papillary muscle preparation was used and the specimen was placed in the same temperature controlled perfusion chamber described above. The preparation was stimulated at the false tendon, most distal to its attachment to the papillary muscle, by rectangular pulses in a manner similar to that described for the atrial recording, but the rate was usually maintained at 60 beats per minute. A total of 34 experiments was done on the dog heart. Of the 34 experiments 5 were on the sinoatrial node, 7 on right atrium, 10 on atrioventricular node, and 12 on the false tendon-papillary muscle preparation.

For recording the isometric tension output of rat right atrium, the atrial preparation was sus-
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Pended in a perfusion bath of Tyrode solution into which were bubbled the same percentages of oxygen and carbon dioxide mentioned previously. The temperature was maintained between 22 and 23°C. The isometric contractions were recorded by a mecano-electronic transducer, RCA 5734, simultaneously with the transmembrane action potentials. The preparation was kept in the perfusion bath for a period of about 1 hour to reach equilibrium before control action potentials were recorded. Barium chloride was administered subsequently as a constant perfusion and recordings were taken frequently during and after its administration. After a period of time varying from 30 minutes to 1 hour, control solution was returned to the perfusion chamber to ascertain whether the changes produced by barium chloride could be reversed. This series consisted of 6 experiments in all. In a second series of 6 experiments, atrial preparations were perfused initially with normal Tyrode solution and after one hour of perfusion the solution was replaced by Tyrode containing no calcium. Transmembrane action potentials were recorded simultaneously with the isometric tension output as had been done in the previous series. When the isometric tension output of the rat atria decreased to about 20% of control, barium chloride was added to the perfusate. In three instances the concentration of barium chloride added was 2.8 \times 10^{-4} M and in the remaining three, the concentration was 0.4 \times 10^{-5} M. Approximately 30 minutes after barium was added, normal Tyrode solution was reintroduced into the perfusion chamber to determine if electrical and mechanical activity would return to normal. Where recordings indicated a return to normal, barium chloride was then readministered to the perfusion bath in order to ascertain whether the modifying effects i.e., prolongation of duration of action potential and increase in isometric tension output, would again be seen as in the first series.

To observe the action of barium chloride on the rhythmicity of cultured chick heart cells the procedure was as follows. The hearts of chick embryos usually nine to eleven days old were disaggregated into single cells by treatment with trypsin after which they were passed through a narrow bore pipette. After removal of the trypsin, the individual cells were suspended in a semisynthetic liquid medium containing fetal calf’s serum and embryonic chick extract. The cells were then incubated at 37°C and cultured for a period of two to fourteen days. The cells became attached to the culture dish and assembled into monolayer communities, in which the cells of each community contracted synchronously. The cells in the culture dish to which barium was added were compared with cells in a control dish prepared from the same chick heart. Various concentrations of barium chloride, 0.4 \times 10^{-7} to 4 \times 10^{-8} M, were used and both treated and untreated cultured cells were observed at 15-minute intervals for changes in rate and/or rhythm.

Effects of barium chloride on the transmembrane action potential of a single fiber of sinoatrial node of dog heart. A: control; sinoatrial rate, 52/min. B: 5 minutes after administration of barium chloride as a constant perfusion showing prolongation of action potential duration; sinoatrial rate, 52/min. C: 10 minutes after; shows more pronounced prolongation of the action potential duration; sinoatrial rate, 54/min. D: 20 minutes after; shows an slowing in depolarization and repolarization of action potential; sinoatrial rate, 55/min. Ordinates: millivolts. Abscissa: time. Time lines, 0.04 second. Dotted lines indicate zero level of potential for the membrane. Gain: 10 mm = 50 mv.

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FIGURE 2

Effects of 4.0 \times 10^{-4} \text{ M barium chloride} on atrial and atrioventricular nodal fibers of isolated perfused dog heart. Shown are simultaneous records of the transmembrane action potential of atrial fiber (above) and atrioventricular nodal fiber (below). A: control. B: 10 minutes after barium chloride administration as a constant perfusion; there is a prolongation of the action potential duration of both atrial and atrioventricular nodal fibers. The latter also shows a slower rate of rise of the action potential, a negative after-potential and a decrease in resting potential. C: 30 minutes after; both fibers show a more marked prolongation of the action potential duration of both atrial and atrioventricular nodal fibers. The latter also shows a slowing in the rate of rise of the action potential. Ordinates, abscissas, time lines, and gain are the same as those shown in figure 1.

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Results

Barium chloride produced changes in the electrical activity of the dog heart, in the mechanical activity of rat atrium, and in the rhythmicity of the cultured chick heart cells.

DOG HEART

At concentrations of 1.4 \times 10^{-4} \text{ M} barium chloride, the duration of the action potentials from sinoatrial node was prolonged about 30%. The action potential duration was measured at 40 mV below the zero level of potential. With increased concentrations (greater than 2.1 \times 10^{-4} \text{ M}) there was, in addition, a slowing in rise time of action potential of approximately 20%, followed by a slight loss in diastolic resting membrane potential, but this occurred usually 20 or more minutes after barium was administered (fig. 1).

Action potentials obtained from the right atrium showed a progressive slowing of repolarization (fig. 2). The diastolic resting membrane potential decreased at concentrations greater than 2.7 \times 10^{-4} \text{ M}, but this usually occurred as a late event. On occasions single or multiple premature action potentials were observed.

Concentrations of 1.4 \times 10^{-4} \text{ M} barium chloride produced approximately a 20% prolongation of the duration of action potential from the atrioventricular node. The action potential duration from the atrioventricular node was measured at 40 mV below zero level of potential. At concentrations above 3.5 \times 10^{-4} \text{ M}, there was approximately a 40% decrease in rise time of the action potential which was followed by loss of about 20% in diastolic resting membrane potential (figs. 2 and 3). In three out of ten experiments, a prominent negative after-potential appeared (fig. 2).

Purkinje fiber action potentials initially showed a loss in overshoot at concentrations...
FIGURE 3
Effect of barium chloride $4.0 \times 10^{-4} \text{M}$ on the transmembrane action potential of single fiber of atrioventricular node of dog heart. A: control; A-V nodal rate, 44/min. B: 10 minutes after administration of barium chloride; there is a slowing in rate of rise of action potential and a prolongation of action potential duration; A-V nodal rate, 44/min. C: 25 minutes after; the action potential duration is appreciably prolonged; A-V nodal rate, 49/min.

Effects of $2.72 \times 10^{-4} \text{M}$ barium chloride on Purkinje and papillary muscle fibers of isolated perfused dog heart. Simultaneous records are of transmembrane action potential of Purkinje fiber (above) and papillary muscle fiber (below). A: control. B: 5 minutes after barium chloride administration; the only change noted is an increase in frequency of action potentials in both fibers. C: 15 minutes after; the transmembrane action potential of the Purkinje fiber shows a diastolic depolarization, a slowing of a rise time, loss of overshoot, and a decrease in resting potential. The papillary muscle fiber action potential shows a slight loss in overshoot and resting potential. Ordinates, abscissa, time lines, and gain are the same as those shown in figure 1.
control solution was reintroduced into the perfusion chamber.

**RAT ATRIUM**

Simultaneous recordings of the action potential and contractility in the right atrium showed several changes. With concentrations of barium chloride between 0.7 and $3.5 \times 10^{-4}$M, repolarization of the action potential became slower and was associated with an increase of about 60% in isometric tension. Subsequently a prominent negative after-potential appeared and persisted (fig. 5). Concentrations of barium chloride above $3.5 \times 10^{-4}$M produced an initial increase in the duration of action potential of the atrial fiber which was followed by a prominent negative after-potential. Simultaneously, isometric tension increased 80%. Later, both the tension induced and magnitude of the action potential decreased approximately 30 and 20% respectively. The modifying effects produced by concentrations of barium chloride less than $3.5 \times 10^{-4}$M were completely reversed by returning the atrial tissue to normal Tyrode solution. There was a 60% recovery at the higher concentrations.

When the atrial preparations were perfused with Tyrode containing no calcium, the transmembrane action potentials showed a loss in overshoot while the resting potential remained essentially unchanged. The isometric tension output decreased markedly within approximately 15 minutes. The addition of $2.8 \times 10^{-4}$M or $0.4 \times 10^{-3}$M barium chloride to the perfusate produced a prolongation of the duration of the action potential, a decrease in resting potential and a negative inotropic effect. When normal Tyrode solution was reintroduced into the perfusion chamber both the electrical and mechanical activity returned subsequently to normal. The administration of barium chloride at this stage produced the same changes in transmembrane action potential and isometric tension output described in the first series of experiments.

**CULTURED CHICK HEART CELLS**

At concentrations of $0.5 \times 10^{-4}$M barium chloride, the rate of beat increased slightly. At concentrations of $0.4 \times 10^{-4}$M, the average increase in rate of beat was 35/min from the mean control of 62/min. At higher concentrations of 60%.

**FIGURE 5**

Effects of $2.0 \times 10^{-4}$ M barium chloride on action potential of single fiber and isometric tension output of rat right atrium. A: control. B: 20 minutes after administration of barium chloride; action potential of atrial fiber shows a negative after-potential. The isometric tension developed by the right atrium is increased. C: 40 minutes after; the negative after-potential and the isometric tension output are more pronounced. The action potential shows a notch at the beginning of the negative after-potential. Ordinates, abscissa, time lines and gain are the same as those shown in figure 1. Tension output: 10 mm = 300 mg.
trations, $0.4 \times 10^{-4}M$ to $4.0 \times 10^{-3}M$, the average increase was 50. At levels of $0.8 \times 10^{-3}M$ to $4.0 \times 10^{-3}M$, the contractions became more forceful and spontaneous activity was induced in cell communities that had been quiescent previously. Concentrations above $2.0 \times 10^{-3}M$ barium chloride produced various arrhythmias including tachycardia and fibrillation. The fibrillation was observed as a rapid disorganized movement of the individual cells within the cell communities. Other toxic effects such as rounding of cells and cessation of beating were also noted at this concentration. In 80% of the experiments cells ceased to beat within 15 minutes at levels above $4.0 \times 10^{-3}M$. If the barium were removed after the cells stopped beating, and the control medium introduced, the beating resumed in 50%.

**Discussion**

The slowing of the rising and falling phases of the action potential and the production of a pronounced negative after-potential observed in cardiac cells following the administration of barium chloride have also been reported in experiments on intestinal smooth muscle, skeletal muscle cells, and nerve fibers. The mechanism responsible for the slowing of the rise time of the action potential following barium is not known. A depression of the sodium-carrying system or a decrease in permeability of the cell membrane to sodium may explain this slowing effect. Similarly the mechanism responsible for the slowing of repolarization is not clearly established. One can ascribe the slowing of repolarization to a decrease in potassium conductance. Yamasaki and Narahashi found that barium decreased the potassium conductance, both in the resting phase and depolarized state, and they speculated that the small potassium conductance is responsible for the delayed repolarization. Although their experiments were performed on the cockroach giant axon, a similar action could be expected in other cells. No satisfactory explanation is offered to elucidate the negative after-potential produced by barium in cardiac cells. Two views prevail as to its origin in voluntary muscle. One is that the negative after-potential is simply the passive return of the potential to its resting value after the rapid permeability changes of the spike, and the other is that the negative after-potential is determined by a metabolic process. Agents which depress metabolism reduce the negative after-potential. The available evidence supports the first view. Hence, the onset of the negative after-potential in voluntary muscle signals the end of potassium permeability changes associated with the repolarization phase of the action potential. One can conjecture that the negative after-potential in the cardiac cell produced by barium is due to a sustained impermeability of the membrane to potassium. In some experiments, the action potentials of the barium-treated rat atrium showed a notch or hump at the beginning of the negative after-potential (fig. 5). This may be due to a rise in sodium conductance in the wake of the after-depolarization as with cathodal polarization. The hump would then represent a subthreshold response which, under certain conditions, can become regenerative.

The increase in contractility of the rat atrium following the administration of barium has also been observed in isolated strips of smooth muscle obtained from rat ileum, guinea pig ileum, and rat uterus. Yukisada and Ebashi, in their experiments on smooth muscle, have reported that barium does not require the presence of calcium for its stimulatory action and have speculated that calcium and barium act directly on the contractile protein of smooth muscle. It is noteworthy that in the present study barium chloride in concentrations of $2.8 \times 10^{-4}M$ or $0.4 \times 10^{-3}M$ produced a negative inotropic effect in the rat atrium when the Tyrode solution perfusing the atrial tissue did not contain calcium. The different effects observed in the smooth muscle as compared to the rat atrium are not readily explainable. They cannot be ascribed to differences in concentration because the concentrations used by Yukisada and Ebashi were not appreciably different from those administered in the present study. Species and tissue differences may account for the difference in effects.
observed. The positive inotropic effect in the rat atrium observed when barium was added to the perfusate containing normal Tyrode cannot be attributed primarily to a prolongation of the duration of the action potential since other divalent cations namely, cadmium, copper, and manganese also produce a prolongation of the duration of the action potential but decrease the isometric tension of the rat atrium (unpublished data of authors).

The cultured embryonic heart cell was more sensitive to the action of barium than the adult heart cell. In the cultured embryonic heart cells, the effective concentration for producing an increase in rate of contraction was $10^{-6}$M barium chloride whereas in the dog heart, the increase in heart rate was first observed at concentrations of $10^{-4}$M barium chloride. The comparatively sharp rise in rate of beat at concentrations of $0.4 \times 10^{-4}$M barium chloride in the embryonic chick heart cells may correspond to the ventricular tachycardia observed in the barium-treated adult heart of the dog. The induction of beating in cell communities previously quiescent cannot be due to a propagated activity from an adjacent cell community but must be due rather to local automatic activity. This conclusion is supported by the distance separating the cells originally contracting from those that develop automatic activity and, moreover, by the rate of beat which, in the induced cells, frequently differed from the rate of those cells that were beating originally.

Summary

Barium chloride was administered to isolated preparations of dog heart and rat atrium and to trypsinized embryonic chick heart cells cultured in vitro. Transmembrane action potentials recorded from pacemaker, specialized conductive and contractile fibers of the dog heart, and atrial fibers of rat heart showed a slowing in the rising and falling phases of the action potential and a prominent negative after-potential. The action potential of the Purkinje fiber of the dog heart showed in addition a depolarization during diastole. A decrease in resting potential was observed later.

The isometric tension developed by the rat atrium showed a significant increase which was greater at the higher concentrations (greater than $3.5 \times 10^{-4}$M) of barium chloride. The cultured embryonic heart cells were appreciably more sensitive to barium than the mature heart cells. An increase in rate of beat of the embryonic heart cells was observed at concentrations of $10^{-4}$M whereas a change in heart rate was first observed in the adult heart at concentrations above $10^{-4}$M. The induction of beating in cell communities previously quiescent is due to a local automatic activity rather than to a propagated activity from an adjacent cell community.

The slowing in rise time of the action potential can be attributed to a depression of the sodium-carrying system. The slowing of repolarization can be ascribed to a decrease in potassium conductance. The pronounced negative after-potential can be explained on the basis of sustained impermeability of the membrane to potassium. The mechanism by which barium produces a positive inotropic effect in the rat atrium is open to conjecture. It cannot be attributed solely to a prolongation of the duration of the action potential nor can a direct effect on the contractile proteins be excluded. The presence of extracellular calcium is essential in order to obtain a positive inotropic effect in the rat atrium when barium is added.

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