Barium-Induced Automaticity in Right Ventricular Muscle in the Dog

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ABSTRACT

Pacemaker activity was induced in trabecular muscle fibers of canine hearts using oxygenated Tyrode solution with added barium chloride. Following exposure to barium (0.2 to 5 mM) the resting potential decreased toward zero from 10 to 60%, depending on the concentration. Simultaneously, the duration of the action potential increased and early diastolic hyperpolarization appeared with diastolic depolarization characteristic of latent pacemaker activity. In concentrations above 0.75 mM, spontaneous activity appeared with presystolic upsweep at the threshold potential. The changes were reversible when normal Tyrode solution was readministered. Suppression of the induced pacemaker activity occurred on cooling or following imposed rapid stimulation. The changes produced by barium are consistent with a decrease in the conductance of the membrane for potassium.

ADDITIONAL KEY WORDS cardiac pacemaker potential
pacemaker activity diastolic depolarization potassium conductance
membrane depolarization membrane resistance hypothermia
ventricular action potential postdrive depression

The adult mammalian heart might be considered to consist of two populations of cells which are separable by their biochemical, physiological, and anatomical differences. One population is represented by cells of the conduction system which exhibit automaticity; the other consists of the cells of the atrial and ventricular myocardium which remain quiescent unless driven by other cells or exogenous stimuli (1). Moreover, pharmacologic agents (e.g. ouabain and epinephrine) (2, 3) which enhance pacemaker activity in Purkinje fibers do not initiate automaticity in fragments of myocardial tissue which do not contain Purkinje cells.

Microelectrode studies of cultured cells from the hearts of 6- to 8-day-old chick embryos suggest that pacemaker activity may not be restricted to the cells of the conduction system in early cardiogenesis; a majority (up to 90%) of the isolated cultured cells exhibited spontaneous activity with the characteristic slow diastolic depolarization of pacemaker cells (4). Crill et al. (5) found slow diastolic depolarization in all the cells cultured from the ventricle of the 4-day-old chick embryo. If the embryonic ventricular cell has the ability to develop pacemaker activity, as these studies imply, it is not unreasonable to suppose that under certain circumstances the adult ventricular cell could be induced to generate spontaneous activity.

Recently Müller produced pacemaker activity in calf and sheep ventricular fibers by omitting both potassium and calcium ions from the perfusate (6). The capability of the barium ion to induce spontaneous activity has been demonstrated in a variety of excitable tissues including mammalian nerves (7) and smooth muscle (8), crayfish skeletal muscle (9), and frog cardiac and skeletal muscle (10). Several reports have appeared (11-13), beginning with Kruta's in 1934, indicat-
ing that barium also produced automaticity in fragments of mammalian atrial and ventricular myocardium. The possibility of the presence of Purkinje fibers in these preparations as a source of the pacemaker activity was not eliminated. In fact, the probable presence of Purkinje fibers in the preparation of Kruta, and of Greiner and Garb is suggested by their observation that automaticity occurred under the influence of epinephrine and other pharmacologic agents. These are known to be incapable of initiating spontaneous activity in ventricular muscle preparations which are free of Purkinje fibers.

Microelectrode studies by Sperelakis and Lehmkühl indicated that barium chloride (5 to 10 mM) produced pacemaker activity in the quiescent cells cultured from the 7- to 8-day-old chick embryo heart. Antoni and Oberdisse have produced pacemaker activity in the papillary muscle of guinea pigs and rabbits with barium (2 to 4 mM). Furthermore, they showed the actions of acetylcholine, potassium, calcium and cooling on the barium-induced pacemaker activity of the papillary muscle to be similar to their actions on natural pacemaker cells.

Here we have studied the effects of barium on canine ventricular fibers, and the effect of cooling and rapid electrical stimulation of preparations exhibiting barium-induced automaticity in an attempt to determine the mode of action of this ion on cardiac ventricular muscle fibers and to suggest further studies on the effect of bivalent cations on ionic conductance of excitable tissues.

Methods

Mature dogs weighing 10 to 15 kg were anesthetized with sodium pentobarbital (30 mg/kg) iv. A left lateral thoracotomy was rapidly performed and the heart was removed. Fragments of trabecular muscle were dissected from the right ventricle near the atrioventricular ring. The tissue was placed in a 5-ml bath maintained at 36° ± 1°C and perfused with Tyrode solution, equilibrated with 95% oxygen–5% carbon dioxide, at a rate of 4 ml/min. The composition of the control Tyrode solution, in millimoles per liter, was: NaCl, 137; NaHCO₃, 12; KCl, 2.7; CaCl₂, 1.8; Na₂HPO₄, 0.4; MgCl₂, 1.1; and glucose, 5.6. Transmembrane potentials were recorded through glass microelectrodes, filled with 3 M KCl, with a resistance of 15 to 25 MΩ. Membrane potentials were displayed on a Tektronix 565 dual-beam oscilloscope via an electrometer tube cathode follower and photographed with a 35 mm Grass kymograph camera. A 100-mv rectangular pulse was introduced between the preparation and the ground for calibration. The stimuli, rectangular wave pulses, 5 msec in duration, were produced by an American Electronic Laboratories stimulator and passed through an isolation unit to a bipolar silver electrode placed against one end of the muscle.

Each preparation remained in the control solution for at least one-half hour before recording. Most of the specimens were quiescent in the Tyrode solution and remained inactive following the introduction of 0.1 ml 1:1000 epinephrine into the bath. Preparations that showed activity were discarded. Following the control observation, 0.2 to 5 mM BaCl₂ was added to the Tyrode perfusate. Observations were made on the effect of BaCl₂ on the resting and action potentials of the ventricular fibers. In addition, observations were made on the effect of rapid cooling, and the superimposition of a rapid electrical drive on the pacemaker activity induced by barium. For observation of the action potential before spontaneous activity, the fiber was stimulated at a rate of 0.5/sec. Overdrive in the presence of spontaneous activity was accomplished by stimuli at a rate of 2/sec.

Ten out of the 20 specimens were serially sectioned and stained with either hematoxylin and eosin or Masson trichrome stain and examined for the presence of Purkinje fibers. No obvious Pur-
kinje fibers could be recognized in any of the quiescent preparations.

**Results**

Pacemaker activity was induced in canine trabecular cardiac muscle fibers in every instance in a series of 16 experiments in which barium chloride was added to the Tyrode perfusate in concentrations of .75 to 5 mM (Fig. 1). In several instances the automatic activity arose when barium was added and there was no electrical stimulus (Fig. 2). In these cases, the onset of automaticity was heralded by a short series of incremental oscillations of the membrane potential. Automaticity was then initiated when an oscillation reached the threshold potential for excitation. Usually, however, an electrical stimulus was required in addition to the barium to initiate automatic activity (Figs. 1 and 3). The automatic activity exhibited the characteristics of a pacemaker cell, i.e. an early diastolic hyperpolar-

**FIGURE 2**

Appearance of spontaneous activity in ventricular fiber exposed to barium (1 mM). The upper tracing is an amplification of the lower recording.

**FIGURE 3**

Ventricular fiber. A = Control in Tyrode solution. B = 1 min following barium (1 mM). C = 2 min, D = 3 min, E = 4 min. A single stimulus initiated a train of activity. The impaled cell is not the dominant pacemaker. The propagated impulse is eventually blocked. F = 60 min after reintroduction of control Tyrode. In each case, the upper tracing is an amplification of the lower recording.

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Depolarization was followed by a slow diastolic depolarization with a presystolic upsweep at the threshold potential. In concentrations below 0.75 mM, barium did not induce automaticity. Concentrations of 0.2 to 0.5 mM did, however, induce an early diastolic hyperpolarization followed by a gradual depolarization to the resting membrane potential following electrically stimulated beats. Before the onset of automaticity, the resting membrane potential decreased (toward zero) by 11 to 57% depending on the concentration of barium chloride (Fig. 4). Coincident with the observed depolarization, the rate of depolarization slowed and overshoot decreased. The duration of the stimulated action potentials increased, primarily due to the prolongation of the plateau phase. This prolongation was reversible with rapid rates of stimulation (Fig. 5).

Following the appearance of automaticity, continued exposure of the preparation to the same concentration of barium produced progressive positive chronotropic effects (Fig. 6). The increase in rate was apparently related to two factors: (1) the increase in the slope of the diastolic depolarization, and (2) a decrease in the maximal repolarization potential observed during the early diastolic period. The threshold potential did not change appreciably.

All of the changes induced by barium were reversible by the reintroduction of Tyrode solution (Fig. 3). The complete reversal of the barium effects, however, required a much greater period of time than that required to produce them (Fig. 3).

Figure 7 shows the effects of cooling a preparation exhibiting barium-induced automatic rhythm from 35 to 25°C. Initially the slope of the diastolic depolarization decreases with concomitant slowing of the rate of activity and the duration of the action potential increases owing to prolongation of the plateau phase. Following the last action potential, a diminutive response is noted which represents
either a local response or an electronic spread from adjacent cells. The cell became completely quiescent at 25°C, with the resting membrane potential remaining near the previous threshold potential.

Rapid electrical stimulation applied to preparations exhibiting barium-induced automatic rhythm produced a postdrive depression or complete suppression of the pacemaker activity (Figs. 8 and 9). A slight degree of hyperpolarization appeared during the imposed drive which promptly returned to the predrive value. Following the rapid stimulation, the slope of diastolic depolarization decreased resulting in the slowing of the rate of activity (Fig. 8). In addition, frequently the threshold potential was slightly increased (Fig. 8). A minimal decrease in action potential amplitude appeared during the drive. Similar rates of stimulation applied to ventricular strips in control Tyrode solution did not produce hyperpolarization.
**Discussion**

Considering the numerous reports ascribing to barium the capability of producing automatic activity in various excitable tissues, it is not surprising that a similar effect is found in mammalian ventricular fibers. In this study, pacemaker-like activity was regularly produced in canine ventricular preparations by barium chloride in concentrations above 0.75 mM. In each instance, slow diastolic depolarization appeared which was followed by a presystolic upsweep at the threshold potential typical of a pacemaker cell. We considered the possibility that this apparent pacemaker activity induced in the ventricular fibers may have been illusory—that the slow diastolic depolarization may have merged with a slow upsweep of an impulse conducted from an occult Purkinje fiber pacemaker creating an illusion of a pacemaker potential similar to that recently described in Purkinje fiber preparations (15). This is unlikely in view of the lack of activity following the introduction of epinephrine into the tissue chamber as well as the absence of typical Purkinje fibers in serial sections of some of the preparations.

The barium-treated preparations often required an electrical stimulus to initiate repetitive activity. Similar behavior has been described for latent pacemaker cells found in the lower portion of the sino-atrial node (16). The reason for the required stimulus is not clear, but may be due to the inactivation of the sodium-carrier system, which is expected to occur in Purkinje fibers when the membrane potential is sharply reduced (17). It is possible that an analogous situation occurred in the barium-treated preparations, i.e. the reduction in the resting membrane potential produced inactivation of the sodium-carrier system which tended to prevent spontaneous excitation. The introduction of an electrical stimulus, by causing a “dip” in the direction of hyperpolarization and reactivation of the Na-carrier mechanism would then initiate activity. This would be perpetuated by the diastolic depolarization that followed each action potential.

Another possible mechanism for the repetitive activity following a stimulated beat may be based on the reentry mechanism. Reentry, in combination with a slow diastolic depolarization, may simulate a pacemaker activity. The present data do not exclude the possibility of repetitive reentry in some instances. On several occasions, however, automaticity developed spontaneously, preceded by subthreshold oscillatory potentials. Here, at least, true pacemaker activity was present.

The observed changes in rise time of the action potential and the decrease in overshoot are probably the consequence of the loss of resting potential and need not be considered as evidence for a primary influence of barium.
ions on the sodium-carrier mechanism. Barium may, therefore, differ from other bivalent cations such as calcium.

The mechanism for the genesis of the barium-induced pacemaker activity is obscure. Dudel and Trautwein have proposed that membrane changes in natural pacemaker fibers are the result of the interaction of two factors: (1) a high membrane conductance for sodium, and (2) a decrease in potassium conductance during diastole producing a slow diastolic depolarization until threshold for excitation is reached (18). That barium may produce similar changes in ionic conductance in the ventricular fibers is suggested by its action in decreasing the resting potential and increasing the duration of the action potential. These changes could be the result of either an increase in sodium conductance or a decrease in potassium conductance, singly or together. The finding by Lehmukuhl and Sperelakis of an increase in the membrane resistance of cultured chick embryo cardiac cells when exposed to barium is consistent with a decrease in potassium conductance (14). Recent studies on frog sartorius muscle have shown the occurrence of spontaneous excitation and decrease in potassium conductance following the administration of barium chloride in similar concentrations suggesting that barium ions occupy the sites available to the passage of potassium (19).

The rapid cooling of the barium-treated ventricular preparations produced similar effects as seen in natural pacemaker cells (20), namely, lengthening of the action potential, initial slowing and eventual suppression of automaticity.

Rapid electrical drive superimposed on the barium-induced automatic rhythm produced inhibitory changes similar to those described for rapidly driven natural pacemaker cells (16). This postdriven suppression has been ascribed, in part at least, to the release of acetylcholine from neighboring nerve fibers (16, 21, 22). Although the release of acetylcholine from atrial pacemaker tissues by sub- and suprathreshold stimuli has been demonstrated, acetylcholine release was not regularly observed in mammalian ventricular preparations in the studies by Vincenzi and West (22). This dichotomous response was believed due to the presence of fewer cholinergic nerves in the ventricular muscle as compared to the atrium. This would suggest that the postdriven hyperpolarization and suppression of automaticity seen in the barium-treated ventricular preparations were not a result of acetylcholine release, but were due to changes in membrane permeability to potassium, or other ions. Even in the sino-atrial nodal tissues of the cat, which contain a high density of nervous tissue, Lu et al. showed that acetylcholine release was not the sole mechanism for postdrive depression since atropine was only partially effective in preventing its appearance (16). A late postdrive acceleration of firing was not seen as has been described for sino-atrial nodal pacemaker cells (16).

References


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