Ionic Interconversion of Pacemaker and Nonpacemaker Cultured Chick Heart Cells

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ABSTRACT Trypsin-dispersed cells from hearts (ventricles) of 7 to 8 day chick embryos were cultured 3 to 21 days. The cells became attached to the culture dish and assembled into monolayer communities. By means of a bridge circuit, one microelectrode was used for simultaneously passing current and recording membrane potentials (V_m). The input resistance, calculated by the measured ΔV_m for a known step of current, averaged 10 MΩ. Electrotonic depolarization of nonpacemaker cells had no effect on frequency of firing. Within 2 min after addition of Ba^{++} (5 to 10 mM) to the Tyrode bath, the cells became partially depolarized and quiescent nonpacemaker cells developed oscillations in V_m which led to action potentials. With time, the depolarization became nearly complete and the input resistance increased 2 to 10 times. During such sustained depolarizations, action potentials were no longer produced and often tiny oscillations were observed; however, large action potentials developed during hyperpolarizing pulses. Thus, the automaticity of the depolarized cell became apparent during artificial repolarization. Sr^{++} (5 to 10 mM) initially produced hyperpolarization and induced automaticity in quiescent nonpacemaker cells. Elevated $[K^+]_o$ (20 to 30 mM) suppressed automaticity of pacemaker cells and decreased R_m concomitantly. Thus, Ba^{++} probably converts nonpacemaker cells into pacemaker cells independently of its depolarizing action. Ba^{++} may induce automaticity and depolarization by decreasing g_K, and elevated $[K^+]_o$ may depress automaticity by increasing g_K. The data support the hypothesis that the level of g_K determines whether a cell shall function as a pacemaker.

Cultured heart cells represent a useful tool in the investigation of the electrophysiological properties of cardiac muscle because the cells may be grown as monolayers and the impalement of cells may be visually observed. In addition, since the cells in culture are aneural, the effects of various chemical agents can be determined independently of nerves. That is, the preparation can help determine whether the site of action of an agent in intact cardiac muscle is directly on the muscle cells. For example, we have recently reported (24) that cultured chick ventricular cells are insensitive to several autonomic

agents (including acetylcholine and norepinephrine) and to tetrodotoxin. As a control, it was shown that the same cells, however, did respond to Ba^{++}. Of the divalent cations, only Ba^{++} and Sr^{++} alter the resting potentials in some excitable cells and lead to the production of cardiaclike action potentials in the presence or absence of extracellular Na^{+}. It is of interest in this connection that Nishi and Soeda (21) found that Ba^{++} produced an initial hyperpolarization followed by a sustained depolarization in frog spinal ganglion cells. The present report is concerned with a more detailed study of the effects of alkaline earth ions on the membrane potentials of cultured chick heart cells. Pacemaker and nonpacemaker cells were interconverted by Ba^{++}, Sr^{++}, and alteration in $[K^{+}]_o$. Unexpectedly, during the sustained depolarization produced by Ba^{++}, automaticity was demonstrated by the application of repolarizing pulses.

METHODS

The tissue culture and electrophysiological techniques employed have been previously described (15, 16, 23, 24). In brief, hearts (ventricles) from 7 to 8 day chick embryos and from 6-day post-hatched chicks were treated with 0.1 % trypsin, and the dispersed cells cultured 3 to 21 days. The single cells generally reassembled into monolayer groups of cells attached to the glass at many foci. There was a large variation in size of the cells, probably depending on many factors in the culturing technique. However, many of the flattened, ribbon-shaped single cells were estimated to be 3 to 7 μ thick, 10 to 20 μ wide, and 100 to 300 μ long. When in contact with one another, neighboring cells usually contracted synchronously. The incubation medium was a modified Puck's solution containing horse serum, a synthetic nutrient medium, and antibiotics. However, in all experiments, the incubation medium was removed and replaced with Tyrode's solution (glucose-free) so that Ba^{++} could be added without precipitation. The ionic composition of the Tyrode solution in mM was: 149.3 Na^{+}, 2.7 K^{+}, 1.8 Ca^{++}, 1.0 Mg^{++}, 145.3 Cl^{-}, 11.9 HCO_3^{-}, and 0.42 $H_2PO_4^{-}$. The pH of the ungassed Tyrode solution was about 8.0. The culture bath was maintained at 35°C during the impalements by means of a nichrome wire heater (15). The capillary microelectrodes (flint glass) had tip diameters of about 0.5 μ, resistances to 15 to 50 megohms, and were filled with 3 M KCl. The reference electrode was an agar-Tyrode bridge immersed in the culture bath. Both electrodes connected to calomel half-cells. The recording microelectrode was mounted in a bridge circuit so that it could be used simultaneously to pass current and to record voltage (23). A small volume (0.05 to 0.15 ml) of a concentrated solution of $BaCl_2$, $SrCl_2$, $CaCl_2$, $MgCl_2$, or KCl was added to the known volume of solution bathing the cells (1.5 ml); the final concentration of the ion was then calculated. Since the cell bath could not be gassed because of the vibrations which would result, there was a lag of about 1 to 3 min for complete mixing. Mixing occurred probably as a result of a combination of the mechanical disturbance of the bath solution produced by the addition and natural vibrations, and diffusion. No attempt was made to compensate for the hypertonicity produced at the higher $[K^{+}]_o$ levels (and resultant elevated $[Cl^{-}]_o$ levels) or for the

relatively small dilution of the bath concentrations of the other ions. Hypertonicity produced by evaporation was compensated for by the addition of a few drops of distilled water about every 1 to 2 hr; without compensation, the osmolarity of the culture bath increased less than 10%/hr (15).

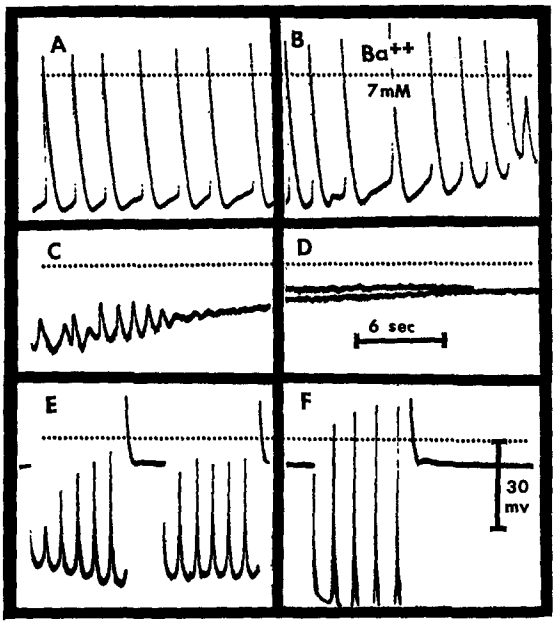

FIGURE 1. Repolarizing electrotonic pulses applied during the sustained depolarization produced by Ba^{++} initiated action potentials. Voltage and time calibrations apply to all photos; dotted lines represent the zero potential level. All photos taken from one penetration. *A*, control in normal Tyrode's medium. *B*, 1 min post $BaCl_2$ injection into culture medium; final Ba^{++} concentration 7 mM. *C*, 1.5 min post Ba^{++}. *D*, 2 min post Ba^{++}; two sweeps superimposed. *E*, at 10 min, the resting potential was 7 mv; two hyperpolarizing pulses of 1.0 na applied. *F*, pulse of 1.2 na at 10 min.

RESULTS

I. *Effect of* Ba^{++}

Addition of $BaCl_2$ to the culture bath (Tyrode's solution), to a final concentration of 5 to 10 mM, depolarized both pacemaker and nonpacemaker cultured heart cells. In one experiment the control action potentials before addition of Ba^{++} are shown in Fig. 1 *A*. The depolarization started usually within 1 min after Ba^{++} addition (Fig. 1 *B*) and reached a maximum (resting potential of -7 mv in *D*) within 4 min; the lag period appeared to be a function of mixing time. The degree of depolarization was a function of the Ba^{++} concentration; i.e., 2 mM Ba^{++} usually produced only slight depolarization, whereas 10 mM Ba^{++} rapidly depolarized the cells to nearly zero potential.

The actual depolarization attained is only approximate because of the change either in liquid junction potential of the reference electrode in the bath or in tip potential of the microelectrode following Ba^{++} addition; this change in electrode potential was monitored by a second (extracellular) microelectrode and reference electrode and was in a "depolarizing" direction. The action potential frequency increased concomitant with the initial depolarization, but during the sustained depolarization (V_m of about 0 to 15 mv), action potentials were no longer generated and often only tiny oscillations in membrane potential were observed (Fig. 1 *C–D*).

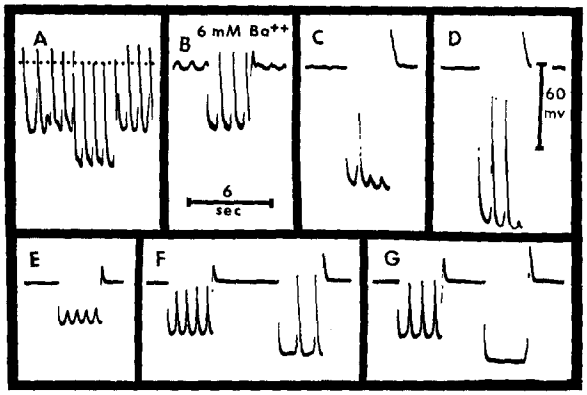

FIGURE 2. Ba^{++} transformed nonpacemaker into pacemaker cells as evidenced by the application of repolarizing pulses. Voltage and time calibrations apply to all photos. All photos taken from one impalement. *A*, control in normal Tyrode's; hyperpolarizing pulse of 1.4 na produced no change in frequency of firing. *B*, 3 min post addition of $BaCl_2$ into culture medium (6 mM); current pulse of 2.2 na. *C*, 4 min post Ba^{++}; pulse 3.6 na. *D*, 4.8 na at 5 min. *E*, 1.2 na at 7 min. *F*, pulses of 1.8 and 2.4 na at 8 min. *G*, pulses of 2.0 and 3.0 na at 9 min.

In cells almost completely depolarized by Ba^{++}, the application of repolarizing current pulses initiated large action potentials whose frequency was a function of the degree of repolarization (Fig. 1 *E–F*). The larger the current step, the lower the frequency but the larger the magnitude of the action potentials (*F* compared to *E*). Therefore, under such circumstances, the cells were clearly behaving as pacemaker cells, although this cell gave no evidence of automaticity during the sustained depolarization. An estimate of the input resistance in *F* is 46 MΩ.

Nonpacemaker driven cells were identified by the fact that their frequency of firing was not altered by polarizing currents. These cells also became converted to pacemakers following Ba^{++} addition (Fig. 2). Before Ba^{++} addition, a hyperpolarizing pulse of 1.4 na did not decrease the frequency of firing (*A*). Within 3 min after Ba^{++} addition (6 mM) the cell became depolarized (*B*). Repolarizing current pulses of 2.2 (*B*), 3.6 (*C*), and 4.8 na (*D*) again illustrate

the dependency of the frequency and magnitude of the action potentials on the magnitude of the repolarizing pulse; some oscillations failed to trigger action potentials (*C–D*). Note the low voltage natural oscillations in *B* and *C* when pulses were not applied. Later, these spontaneous oscillations completely disappeared (*E–G*); however, repolarizing pulses of 1.2 (*E*), 1.8 and 2.4 (*F*),

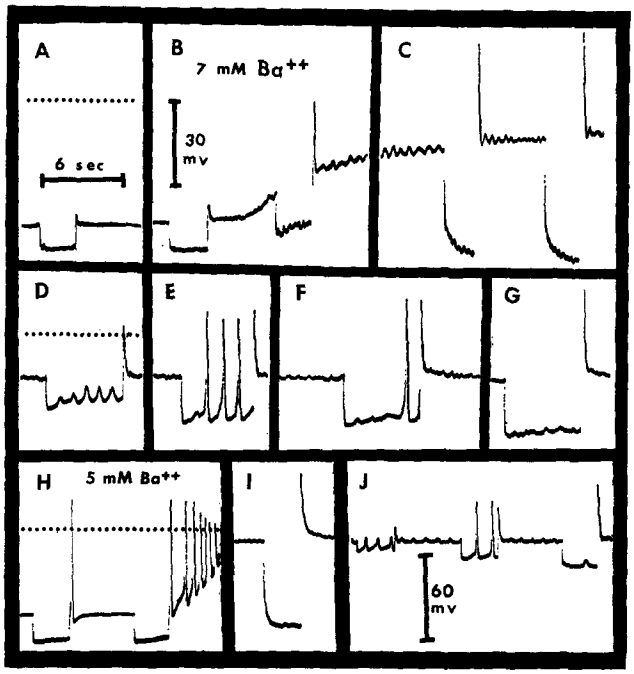

FIGURE 3. Ba^{++} rapidly increased membrane resistance concomitant with depolarization. Time calibration applies to all photos; voltage calibration in *B* applies to *A–G*, that in *H* applies to *H–J*. *A–G*, sequential photos from one impalement. *A*, control in Tyrode's; hyperpolarizing pulse of 0.5 na. *B*, 1.5 min post injection of $BaCl_2$ into culture medium; final Ba^{++} concentration 7 mM; pulses of 0.5 na. *C*, 2 min post Ba^{++}; pulses of 0.5 na. *D*, 0.12 na at 6 min. *E*, 0.18 na at 7 min. *F*, 0.20 na at 8 min. *G*, 0.24 na at 9 min. *H–J*, sequential photos from one impalement. *H*, 0.5 min post addition of $BaCl_2$ into culture medium (5 mM); hyperpolarizing pulses of 0.5 na. *I*, 1 min post Ba^{++}; pulse 0.5 na. *J*, pulses of 0.1, 0.25, and 0.4 na at 3 min.

2.0 (first pulse in *G*) still initiated membrane responses; excessive repolarizing current of 3.0 na (second pulse in *G*) did not allow firing.

Nonpacemaker quiescent cells were identified by the fact that they were naturally quiescent and did not respond to depolarizing pulses but usually did respond to anodal-break stimulation. These cells also became converted to pacemaker cells following Ba^{++} addition (Fig. 3). In the example shown in *A–G*, the hyperpolarizing pulses were not of sufficient magnitude (0.5 na) before Ba^{++} addition (*A*) to give anodal-break excitation. Shortly after Ba^{++}

addition (7 mM) (*B–C*), the cell started to depolarize and anodal-break excitations appeared even though the pulses remained constant. Concomitant with the partial depolarization, the input resistance increased drastically from about 17 MΩ to 85 MΩ; i.e., for the same current, the ΔV_m increased over fivefold. Thus, the hyperpolarization produced by the originally "subthreshold" current pulse was now sufficient to lead to anodal-break excitation. Smaller repolarizing pulses in *D* (0.12 na), *E* (0.18 na), and *F* (0.20 na) initiated action potentials whose frequency and magnitude were dependent

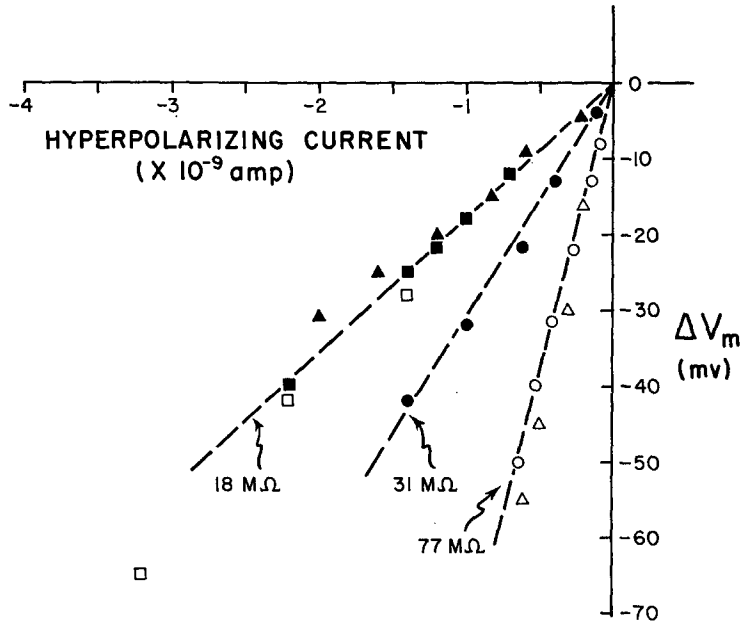

FIGURE 4. Voltage/current curves obtained from six impalements in different cultures in the presence of 5 to 10 mM Ba^{++} illustrate large input resistances and linearity. The change in membrane potential (ΔV_m) is plotted as a function of the intensity of hyperpolarizing current. Individual symbols represent successive measurements on the same cell, and the dashed lines have slopes representing the resistances as labeled.

on the applied current; the pulse of 0.24 na in *G* was too large to allow firing. A similar experiment upon another nonpacemaker quiescent cell in another culture is illustrated in Fig. 3 *H–J*. Before Ba^{++}, the cell was quiescent but exhibited anodal-break responses to hyperpolarizing pulses of 0.5 na. Shortly after Ba^{++} addition (*H*), depolarization began and spontaneous action potentials were initiated transiently until the cell became depolarized beyond the level at which the membrane could support action potentials. In *I*, taken 1 min after the Ba^{++} addition, the input resistance increased over threefold compared to that in *H*. Several minutes later the input resistance had diminished and repolarizing pulses of 0.1, 0.25, 0.4 na initiated responses whose frequency and magnitude depended upon the applied current (*J*).

It was previously reported that the steady-state voltage/current curves obtained from nonpacemaker cultured heart cells indicated that rectification was not present (16, 23); it is not known whether rectification was present in pacemaker cells. The voltage/current curves obtained from both types of cells during depolarization in 5 to 10 mM Ba^{++} also were linear for hyperpolarizing pulses, even though the input resistances were two- to eight fold greater than normal, ranging between 15 and 90 MΩ (Fig. 4).

In a few instances at high Ba^{++} concentrations (e.g., 8 to 10 mM), the sustained depolarization was analogous to a sustained plateau of a cardiac action potential. Under these circumstances, small repolarizing pulses (e.g., 0.12 na) terminated the plateau (anodal repolarization) and appeared to produce a rapid repolarization phase of an action potential. Thus, the actual repolarization was much greater than that which would be produced passively. This was then followed by a spontaneous action potential with a long duration plateau during the hyperpolarizing pulses. Occasionally, repolarization occurred spontaneously.

In several experiments, Ba^{++} produced an initial, transient hyperpolarization in the presence of ouabain (25 μg/ml).

II. *Effect of Sr^{++}*

Addition of $SrCl_2$ to the culture bath, to a final concentration of 5 to 10 mM, rapidly hyperpolarized the cells and initiated pacemaker activity in nonpacemaker, quiescent cells. For example, Fig. 5 illustrates such a hyperpolarization; since the change in electrode potential recorded by the second (extracellular) microelectrode was in a depolarizing direction, the actual hyperpolarization was probably larger. Hyperpolarizing pulses of 0.8 na did not give anodal-break responses; the bridge was slightly off balance. Graded anodal-break responses (*B*) leading to large responses (*C*) occurred a few minutes after Sr^{++} addition. There was about a twofold increase in input resistance in *C* compared to *A*. Later, the same cell fired spontaneously, as shown in *D* at twofold lower gain; the cell was transformed into a pacemaker cell as evidenced by the abolition of action potentials during hyperpolarizing pulses without the presence of driving junctional potentials. Driven, nonpacemaker cells also became hyperpolarized by Sr^{++}, the overshoot increased, the duration of the action potential decreased, and a prominent negative afterpotential appeared. The change in shape of the action potential induced by Sr^{++} included a typical plateau component evident at faster sweep speeds.

The hyperpolarization and the sinusoidal oscillations produced by Sr^{++} are further illustrated in Fig. 6. During the control period of the impalement represented in *A–F*, the cell was quiescent and failed to give anodal-break responses (*A*). Hyperpolarization and oscillations were produced after addition of Sr^{++} (*B–C*). The pulse in *B* shows a slight increase in input resistance compared to that in *A*. Action potentials accompanied by prominent nega-

tive after-potentials are superimposed on the pacemaker oscillations; i.e., the cell was a latent pacemaker. In some cases damped sinusoidal oscillations followed each action potential. However, in other cases, undamped slow sinusoidal oscillations had only small abortive spikelike responses superimposed (*D*, *J*). It is unlikely that abortive spikes would be able to sustain the oscillations; thus, the oscillations appear to be pacemaker potentials. This point is further clarified in *G* in which a series of undamped sinusoidal oscillations continued even after the spontaneous cessation of spikes, and in *H* in which the first spike is superimposed near the crest of a large spontaneous

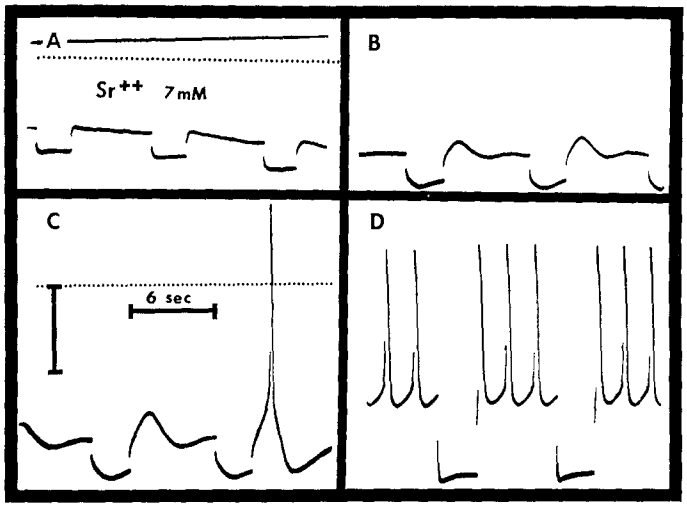

FIGURE 5. Sr^{++} produced hyperpolarization and initiated pacemaker activity. Voltage calibration in *C* represents 30 mv in *A–C* and 60 mv in *D*; time calibration applies to all photos. All photos taken from one penetration. Hyperpolarizing pulses of 0.8 na applied in *A–C* and 4.8 na in *D*. *A*, 1.5 min post $SrCl_2$ addition into the Tyrode medium (7 mM); upper trace indicates the change in electrode potential recorded by an extracellular microelectrode. *B*, 2.5 min. *C*, 3 min. *D*, reduced gain; 10 min post Sr^{++}.

oscillation. In some cases (*E*), the slow oscillations continued during the application of hyperpolarizing pulses. After long periods in Sr^{++}, some impaled cells became partially depolarized and failed to develop action potentials; only small potentials were observable (*D*, *F*, *J*). These small potentials usually became smaller with time, and they may represent junctional potentials produced nearly simultaneously with firing of action potentials in the contiguous prejunctional cell. During hyperpolarizing pulses of sufficient intensity (greater than 1.2 na), large action potentials were produced at the same frequency as that of the small potentials (*F*, *J*); depolarizing current did not lead to action potentials. It appears that action potentials developed only during hyperpolarizing pulses because the resting potential had diminished after long periods in Sr^{++} so that action potentials could not be supported.

In many cases, the slow oscillations were abolished during hyperpolarizing pulses (Fig. 7 *A–C*); sufficient hyperpolarizing current in *C* abolished one of the action potentials and revealed the presence of a driving junctional potential. During hyperpolarizing pulses, the negative after-potentials became larger (*A–C*), and in a few cases even led to repetitive firing during the pulse. In some cases, the negative after-potential followed a positive after-potential

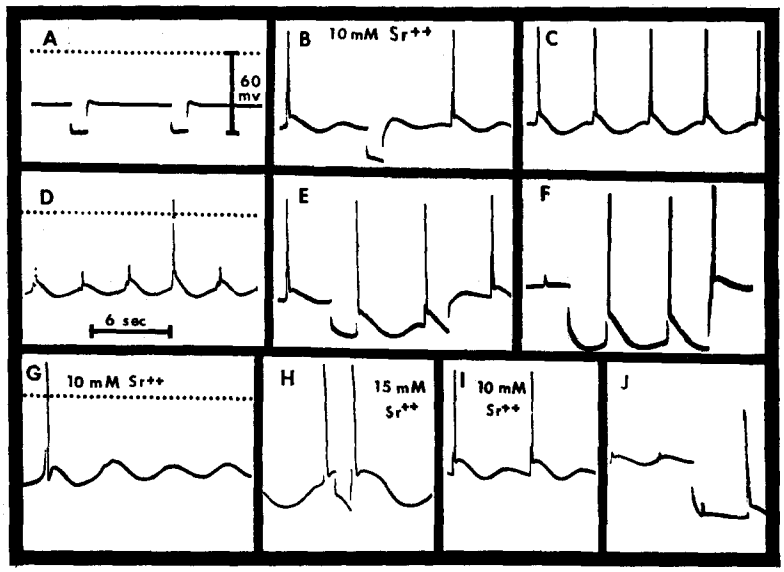

FIGURE 6. Hyperpolarization and sinusoidal oscillations induced by Sr^{++}. Voltage and time calibrations apply to all photos. *A–F*, sequential photos from one impalement. *A*, control in Tyrode's solution; hyperpolarizing pulses 3.6 na. *B*, 9 min post addition of $SrCl_2$ into the culture bath (10 mM); current pulse of 3.6 na. *C–D*, 16 and 23 min post Sr^{++}, respectively. *E–F*, 27 to 34 min; pulses of 3.6 na (*E*) and 7.6 na (*F*). *G*, from another culture 5 min after addition of 10 mM Sr^{++}. *H*, slow sinusoidal oscillations induced after several minutes in 15 mM Sr^{++} in still another culture; hyperpolarizing pulse of 2.4 na applied during crest of oscillation elicited an anodal-break response. *I–J*, from one impalement in another culture. *I*, 6 min post 10 mM Sr^{++}. *J*, hyperpolarizing pulse of 7.6 na at 10 min; bridge imbalanced.

resulting in a peculiar appearance. During depolarizing pulses (*D–F*), the slope of the pacemaker potentials and the frequency of firing increased; there was no prominent change in the negative after-potentials. A second addition of Sr^{++} to bring the total concentration to 19 mM led to larger amplitude, lower frequency sinusoidal oscillations with trains of action potentials superimposed on the crest of each oscillation (*G*); the slow oscillation initiated at least the first spike of each train. The application of long duration hyperpolarizing pulses diminished the amplitude of the slow oscillations (*H–I*). However, in *H*, the cell fired action potentials during the pulse possibly because of transmission of excitation from a contiguous cell; the second and

third spikes of the train appear to be superimposed on a large negative after-potential following the first spike. In *I*, a slightly larger pulse suddenly (as a step function) abolished the spikes, leaving only three driving junctional potentials.

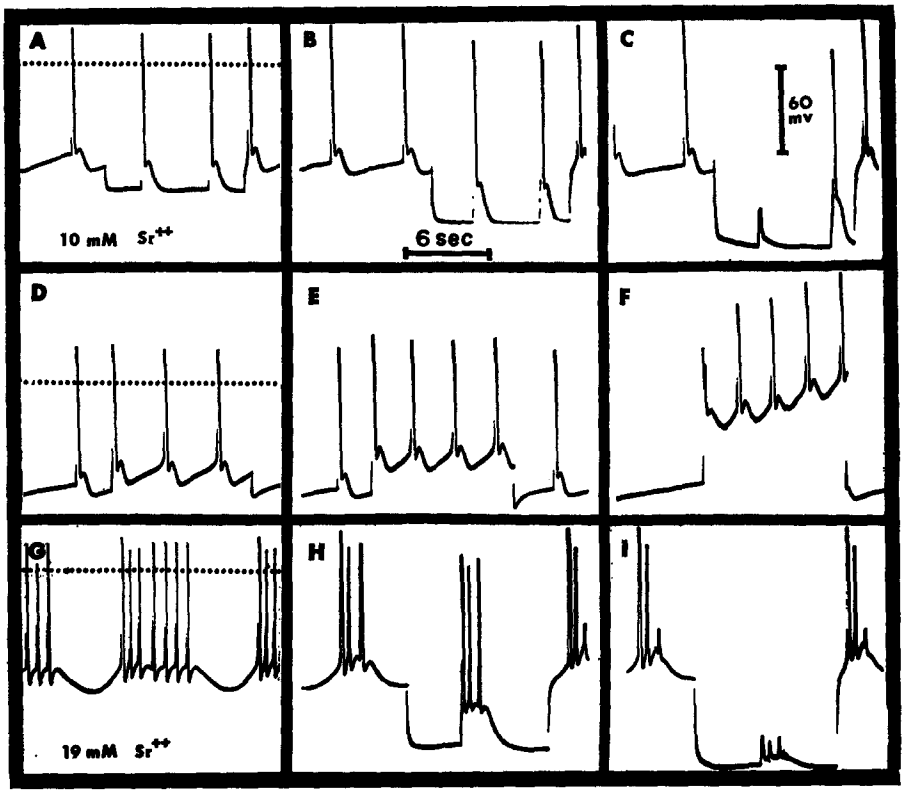

FIGURE 7. Additional characteristics of action potentials produced in the presence of Sr^{++}. All photos taken from one penetration. Voltage and time calibrations apply to all photos. *A–C*, 22 to 26 min post 10 mM Sr^{++}; hyperpolarizing pulses of 2.4 (*A*), 4.8 (*B*), and 6.0 na (*C*). *D–F*, 29 to 31 min post Sr^{++}; depolarizing pulses of 2.6 (*D*), 3.6 (*E*), and 4.8 na (*F*). *G*, 4 min post injection of a second dose of $SrCl_2$ into the culture medium; final Sr^{++} concentration 19 mM; no current applied. *H–I*, 11 to 12 min after the second dose; hyperpolarizing pulses of 4.8 (*H*) and 6.0 na (*I*).

The depolarizing action of Ba^{++} was partially antagonized by the prior presence of Sr^{++}. The depolarization was delayed and smaller in magnitude than that which would be produced in the absence of Sr^{++}; concomitant with depolarization, the large slow Sr^{++} oscillations became depressed and the frequency of firing increased. Two examples of the phenomenon are illustrated in Fig. 8 (*A–C* and *D–F*). It is evident that the cells were partially protected against the depolarizing action of high Ba^{++} (7 to 12 mM) by the presence of Sr^{++} (12 to 15 mM). In *A*, taken after 33 min in 15 mM Sr^{++}, repetitive anodal-

break responses were produced in a quiescent cell. Three min after addition of Ba^{++} (10 mM), slight depolarization and spontaneous action potentials occurred; hyperpolarizing pulses of 2.4 na abolished firing during the pulses (*B–C*). The first pulse in *B* turned off the train and the membrane became "hyperpolarized" to its resting level; the second pulse led to an anodal-break response and turned back on a sustained train of impulses. A single anodal-break response followed each pulse in *C* and led to a train of action potentials. Although not shown, the cell became further depolarized and

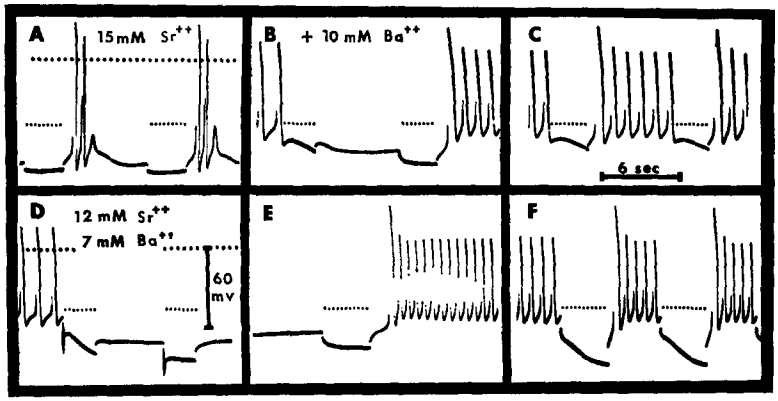

FIGURE 8. Partial antagonism of the Ba^{++} action by Sr^{++}. Voltage and time calibrations apply to all photos. The short dotted lines mark the application of pulses. Photos taken from two impalements (*A–C* and *D–F*). *A*, in Tyrode's medium containing 15 mM Sr^{++} for 33 min. *B–C*, 3 min post addition of $BaCl_2$ into culture medium (10 mM). Hyperpolarizing pulses of 2.4 na applied in *A–C*. *D–F*, in Tyrode's medium containing 12 mM Sr^{++} and 7 mM Ba^{++}; hyperpolarizing pulses of 1.2 (*D*, *E*) and 2.4 na (*F*) applied.

entirely quiescent after addition of a second dose of Ba^{++} giving a total concentration of 15 mM. However, with application of repolarizing pulses of 3.6 to 6.0 na, pacemaker activity and resultant action potentials became expressed. Thus, relatively high concentrations of Ba^{++} were required to depolarize the cells in the presence of Sr^{++}. As illustrated in another culture (*D–F*), 7 mM Ba^{++} did not depolarize in the presence of 12 mM Sr^{++}. Hyperpolarizing pulses of 1.2 na turned off the train (*D*) and later a similar pulse turned on a train of impulses (*E*). With larger pulses (2.4 na in *F*), anodal-break responses occurred each time, and the trains were not turned off except during the application of the pulse itself. This phenomenon of turning action potential trains on and off has also been produced in normal Tyrode's solution.

III. *Effect of Ca^{++}, Mg^{++}, EDTA, Zn^{++}*

Elevation of $[Ca^{++}]_o$ or of $[Mg^{++}]_o$ from normal levels of 1.8 and 1.0 mM, respectively, to levels as high as 15 mM had no significant effect over periods

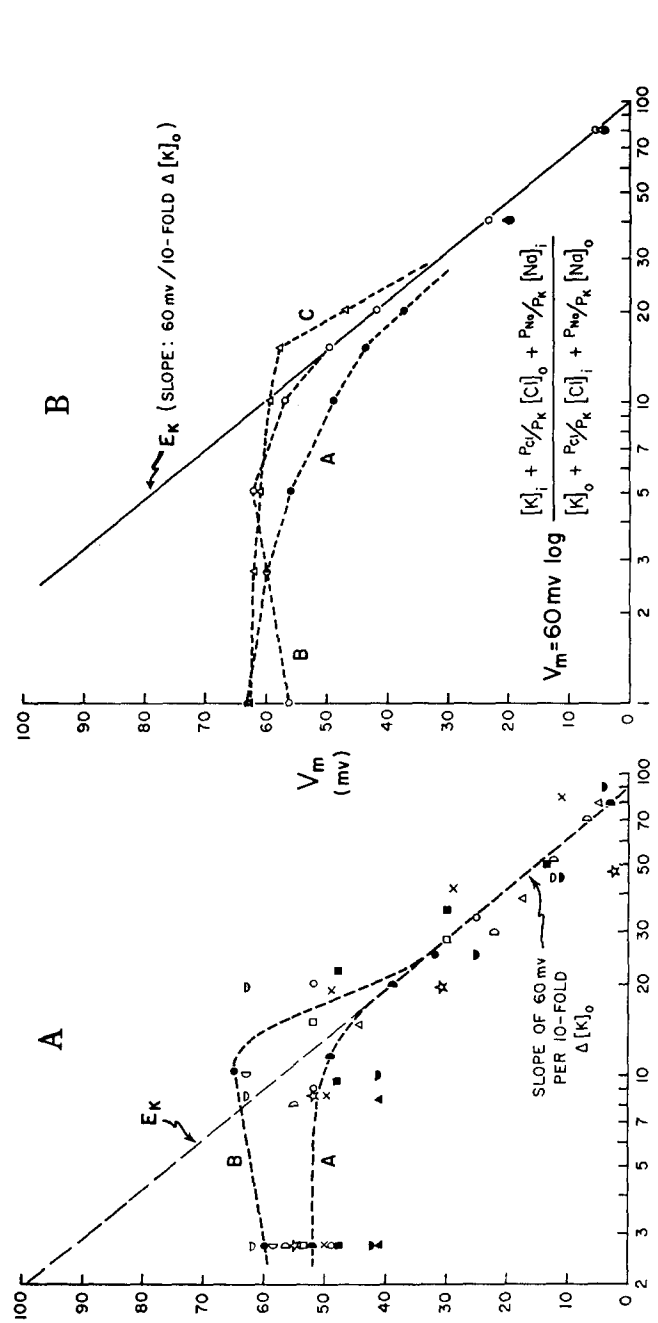

FIGURE 9. Resting membrane potential (V_m) plotted as a function of the external K^+ concentration on a logarithmic scale. A, experimental data. Individual symbols represent successive measurements on the same cell; in many instances, it was not possible to follow the resting potential of one cell over a wide range of $[K^+]_o$ because the electrode became dislodged. The line labeled E_K was drawn with a slope of 60 mv per tenfold change in $[K^+]_o$. With elevation of $[K^+]_o$ from 2.7 mM to 10 to 20 mM, the resting potentials of some cells were almost unaffected (curve *A*) whereas those of other cells became slightly hyperpolarized (curve *B*); all cells were depolarized as $[K^+]_o$ was elevated beyond 20 mM. B, three theoretical curves calculated by varying the parameters of the constant-field equation shown in order to approximate the experimental data shown in part A. The line labeled E_K was drawn with a slope of 60 mv per tenfold change in $[K^+]_o$. The following assumptions were made: (*a*) $[K^+]_i$ held constant at 100 mM, (*b*) $[Cl^-]_o$ varied with $[K^+]_o$ because KCl was added (*c*), $[Cl^-]_i$ allowed to vary passively, (*d*) $[Na^+]_o$ held constant at 149.3 mM, and (*e*) $[Na^+]_i$ held constant at 15 mM. P_{Na}/P_K and P_{Cl}/P_K were held constant at 0.05 and 0.25, respectively (curve *A*) and at 0.025 and 3.0 (curve *C*). For curve *B*, P_K was allowed to increase linearly as $[K^+]_o$ was increased; thus P_{Na}/P_K and P_{Cl}/P_K decreased from values of 0.1 and 2.0, respectively, at 1 mM to values of 0.00125 and 0.025 at 80 mM. The hypertonicity at higher $[K^+]_o$ was ignored; an obvious shrinking of the cells was not evident.

greater than 30 min on the resting and action potentials of both pacemaker and nonpacemaker cells. No significant difference could be detected either in the magnitude, shape, and frequency of the action potentials or in the slope of the pacemaker potential. Similarly, addition of 3 mM disodium EDTA had no significant effect on the electrical activity of the cells for periods up to 30 min. Since the time periods should have been adequate for chelation of much of the Ca^{++} and Mg^{++} in the bath, lowering of $[Ca^{++}]_o$ and $[Mg^{++}]_o$ does not alter the resting and action potentials. Thus, since variation of $[Ca^{++}]_o$ and $[Mg^{++}]_o$ over relatively wide ranges has no effect, it is unlikely that Ca^{++} current is involved significantly in the action potential.

In several experiments performed in a phosphate-free Tyrode solution to prevent precipitation, 1 to 5 mM Zn^{++} had no effect on membrane resistance or resting and action potentials during periods of up to 30 min.

IV. *Effect of K^+*

KCl was added to the culture bath to elevate $[K^+]_o$ above the control level of 2.7 mM; no compensation was made for the resultant hypertonicity and elevated $[Cl^-]_o$ levels. The resting potential (V_m) is plotted as a function of $[K^+]_o$ on a logarithmic scale in Fig. 9 A. In general, all cells were depolarized as $[K^+]_o$ was elevated beyond 20 mM. The slope of the line drawn through this region of the graph is 60 mv/tenfold change in $[K^+]_o$; from these data, the mean $[K^+]_i$ was estimated to be about 80 to 100 mM. However, between 2.7 and 10 to 20 mM, many cells were not depolarized (curve *A*) and several cells were actually hyperpolarized (curve *B*). In the latter cases, the maximal resting potentials and action potentials were obtained at a $[K^+]_o$ of about 10 mM. There appeared to be no correlation between this hyperpolarization phenomenon and whether the cells were pacemakers or nonpacemakers. Fig. 9 B shows three theoretical curves calculated by varying the parameters of the constant-field equation; the parameters used in the calculations are given in the figure legend.

PACEMAKER CELLS There was a distinct difference in the behavior of pacemaker and nonpacemaker cells following depolarization at higher $[K^+]_o$ levels. The typical behavior of pacemaker cells as $[K^+]_o$ was progressively elevated is shown for two cells in Fig. 10 (*A–F* and *G–I*). The control in 2.7 mM K^+ is shown in *A*; hyperpolarizing pulses abolished firing thus showing that the cell was a pacemaker. A slight hyperpolarization and increased action potential magnitude occurred after elevation of $[K^+]_o$ to 10 mM (*B*). Shortly following elevation of $[K^+]_o$ to 25 mM, the frequency of firing decreased (*C*), followed by complete abolition of spontaneous firing (*D–F*), even though the cell was capable of producing anodal-break responses (*C, D, F*). The bridge balance was altered in *D–F* because of a change in electrode resistance as a function of $[K^+]_o$. In the second example (*G-I*), the electrical

activity in 2.7 mM and in 9 mM K^+ is not illustrated; hyperpolarizing pulses abolished firing. *G* illustrates the action potentials obtained in 21 mM $[K^+]_o$. Following elevation of $[K^+]_o$ to 32 mM, there was a slight hyperpolarization concomitant with cessation of firing (*H*), followed by a gradual depolarization to −25 mv (*I*). Note that spontaneous firing was abolished by high $[K^+]_o$

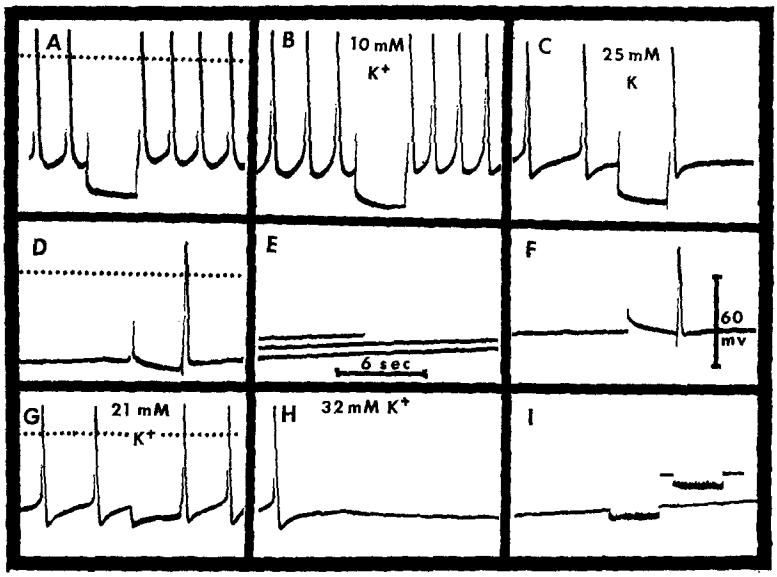

FIGURE 10. Conversion of pacemaker cells into nonpacemaker cells by K^+. Voltage and time calibrations apply to all photos. The zero potential level is marked by the dotted lines in *A*, *D*, *G*. *A–F*, photos taken from one impalement. *A*, control in Tyrode's medium; $[K^+]_o$ of 2.7 mM. *B*, 1.5 min post injection of KCl into culture medium; total K^+ concentration 10 mM. *C–F*, 4 (*C*), 5 (*D*), 6 (*E*), and 7 min (*F*) post elevation of $[K^+]_o$ to 25 mM; three sweeps superimposed in *E*. Hyperpolarizing pulses of 4.8 na applied in all photos except *E*; microelectrode resistance changed in *D* and *F*. *G–I*, from an impalement in another culture; control in 2.7 mM K^+ and activity in 9 mM K^+ not illustrated. *G*, 4 min post elevation of $[K^+]_o$ to 21 mM. *H*, 1 min post elevation of $[K^+]_o$ to 32 mM. *I*, 1.5 and 5 min (inset) later. Hyperpolarizing pulses of 2.4 na applied in *G* and *I*.

concomitant with a slight hyperpolarization; however, the subsequent depolarization did not initiate firing even though V_m passed well beyond the level of the initial threshold potential. That is, pacemaker cells were converted into nonpacemaker cells by $[K^+]_o$ levels greater than 25 mM. By comparison of the ΔV_m for the same step of current in this experiment and in many others not illustrated, it appeared that the input resistance diminished slightly in high $[K^+]_o$.

NONPACEMAKER CELLS The typical behavior of nonpacemaker cells as $[K^+]_o$ is progressively elevated is shown for two cells in different cultures in

Fig. 11 (*A–G* and *H–M*). The cells were nonpacemakers because hyperpolarizing pulses did not alter the frequency of firing; the presence of what appear to be pacemaker potentials is a necessary but not sufficient criterion for the identification of pacemaker cells because the apparent pacemaker potential may, in reality, be part of a positive after-potential. The control in 2.7 mM K^+ is shown in *A*. In 10 mM K^+ (*B*) and in 20 mM K^+ (*C*), the cell

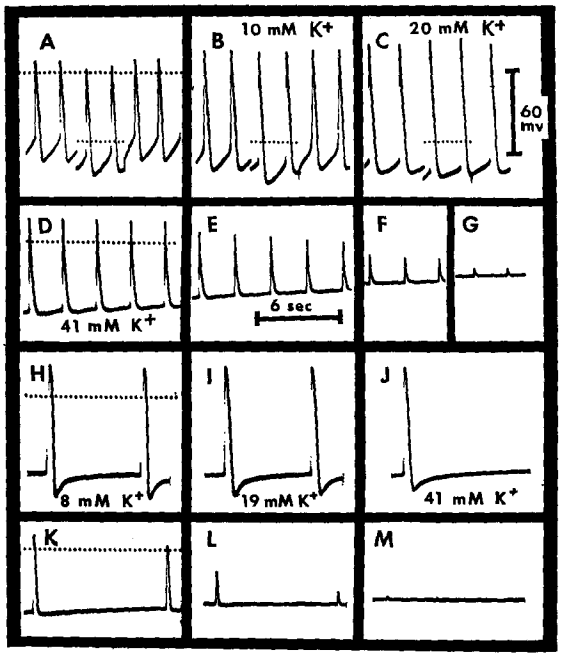

FIGURE 11. K^+ depolarization of driven nonpacemaker cells. Voltage and time calibrations apply to all photos. The zero potential level is marked by the long dotted lines in *A*, *D*, *H*, and *K*. *A–G*, sequential photos from one impalement. *A*, control in 2.7 mM K^+. *B*, 5 min in 10 mM K^+. *C*, 5 min in 20 mM K^+. *D*, 1.5 min in 41 mM K^+. *E–G*, 2 to 3 min post elevation to 41 mM K^+. Short dotted lines in *A–C* mark the application of hyperpolarizing pulses of 3.6 na. *H–M*, from an impalement in another culture; control in Tyrode's not shown. *H*, 5 min in 8 mM K^+. *I*, 5 min in 19 mM K^+. *J–M*, 1 to 2 min following elevation of K^+ to 41 mM. No current pulses applied.

became hyperpolarized and fired larger action potentials. The bridge balance altered slightly in *B–C* due to the change in electrode resistance. After elevation of $[K^+]_o$ to 41 mM, a rapid and large depolarization was produced within 3 min (*D–G*). In the second example, the control in 2.7 mM K^+, although not shown, was not different from that in 8 mM (*H*), or 19 mM K^+ (*I*). Further elevation to 41 mM produced a rapid depolarization (*J–M*). Note that in contrast to pacemaker cells, action potential-like responses were still recorded as the cells were depolarizing. These responses became progressively smaller (including less overshoot) as the cells depolarized and eventually disappeared.

In addition, the responses became briefer in duration and the positive after-potentials disappeared. Since their frequency did not alter concomitant with depolarization, the small responses most likely resulted from propagation of excitation to the impaled cell.

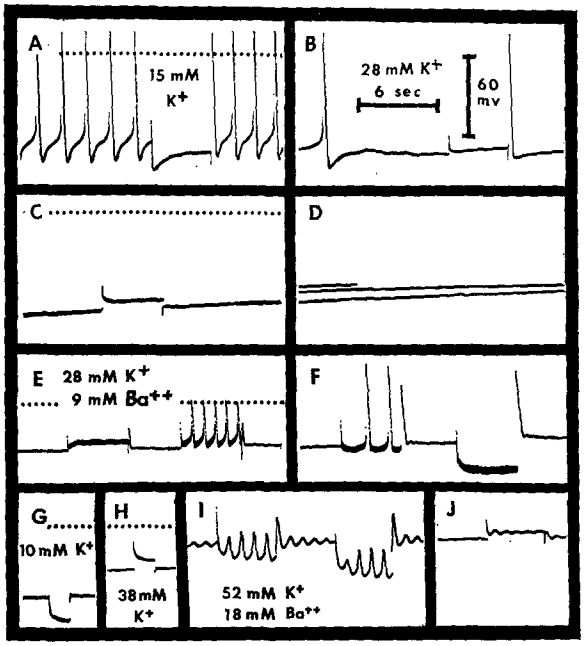

FIGURE 12. Ba^{+}-induced pacemaker activity in K^{+}-depolarized cells. Voltage and time calibrations apply to all photos. *A–F*, sequential photos taken from one impalement. *A*, in Tyrode's solution containing 15 mM K^{+}. *B*, 0.5 min after elevation of K^{+} to 28 mM. *C–D*, 1.0 to 1.5 min later; superimposed sweeps in *D*. Hyperpolarizing pulses of 4.8 na applied in *A–C*; microelectrode resistance decreased in *B* and *C* resulting in bridge imbalance. *E–F*, 3 min post addition of $BaCl_2$ (9 mM). Hyperpolarizing pulses of 2.4 and 3.6 na applied in *E*, 4.8 and 6.0 na in *F*. *G–J*, from an impalement in another culture. *G*, in Tyrode's solution containing 10 mM K^{+}. *H*, 5 min post elevation of K^{+} to 38 mM. *I–J*, 10 to 13 min post addition of $BaCl_2$ (18 mM) while in 52 mM K^{+}. Hyperpolarizing pulses of 3.6 na (*G–H*), 7.6 and 9.0 na (*I*), and 2.4 na (*J*); bridge imbalanced in *H–J*.

Ba^{++} TRANSFORMATION The addition of Ba^{++} to K^{+}-depolarized cells converted the cells to pacemakers, even though depolarization was maintained (Fig. 12). This was true of cells which were originally pacemakers (*A–F*) or nonpacemakers (*G–J*). The control in 2.7 mM K^{+} is not illustrated, but the electrical activity was not different from that in 15 mM K^{+} (*A*); the presence of pacemaker potentials and the abolition of firing by a hyperpolarizing pulse are evident. Shortly after elevation of $[K^{+}]_o$ to 28 mM, spontaneous firing ceased (*B*), and the cell gradually depolarized (*C–D*); the bridge imbalanced due to a change in electrode resistance. The pacemaker cell was converted

into a nonpacemaker. However, following addition of 9 mM Ba^{++}, repolarizing pulses now produced spontaneous action potentials (*E–F*); frequency of firing was a function of the degree of repolarizing current. Anodal-break excitation occurred after each pulse. The input resistance increased due to Ba^{++}.

In an experiment with a nonpacemaker cell (*G–J*), the resting potential in 2.7 mM K^+ was not different from that in 10 mM K^+ (*G*); the cell was quiescent and did not produce anodal-break responses to pulses of 3.6 na. The depolarization produced in 38 mM K^+ is shown in *H*; the bridge imbalanced due to a change in electrode resistance. In 18 mM Ba^{++}, in the presence of 52 mM K^+, spontaneous oscillations developed (*I*); the oscillations became larger during the application of repolarizing pulses. Later, the spontaneous oscillations disappeared but repolarizing pulses still induced oscillations (*J*). Thus, Ba^{++} induced pacemaker activity even in cells depolarized by high $[K^+]_o$.

V. *Effect of Electrotonic Current*

In contrast with K^+ depolarization in which firing is abolished, the frequency of firing of pacemaker cells is increased with electrotonic depolarization (e.g., Figs. 13 and 14) or with injury depolarization often resulting from vibration of the electrode. An impaled pacemaker cell often became spontaneously depolarized due to injury and the frequency of firing increased. A corollary phenomenon was occasionally observed in which the frequency of firing of a pacemaker cell diminished as a function of an increased resting potential produced by "sealing" of the microelectrode tip by the cell membrane. In driven nonpacemaker cells, the frequency was independent of resting potential or injury.

Electrotonic depolarization and hyperpolarization of pacemaker and nonpacemaker cultured cells were previously described (16, 23, 24). With electrotonic depolarization, damping of the magnitude of the action potentials was never observed in driven nonpacemaker cells, and the frequency of firing was unaltered. However, during pulses of depolarizing current in pacemaker cells, magnitude damping and frequency damping of the action potentials were observed in all possible combinations. In frequency damping, the successive intervals between action potentials became progressively longer. Generally, the degree of magnitude damping was a function of the current intensity; progressively larger currents gave progressively larger degrees of damping (Fig. 13 *A–D*) so that complete damping occurred in *D* following the initial response upon application of the pulse. In those cases in which frequency damping occurred, the degree of damping was an inverse function of the intensity of current, i.e., frequency damping was less pronounced at the larger depolarizations. For example, in Fig. 13 *E–J*, interval damping was most pronounced in *E–G* (lower current intensities) compared to that in

H–I (higher current intensities). Reduction of the current in *J* again produced pronounced frequency damping, with the last cycle failing to initiate an action potential. Even though the last action potential failed, the pacemaker potential cycle was terminated by something analogous to a positive after-potential. This suggests that a pacemaker potential which is generated

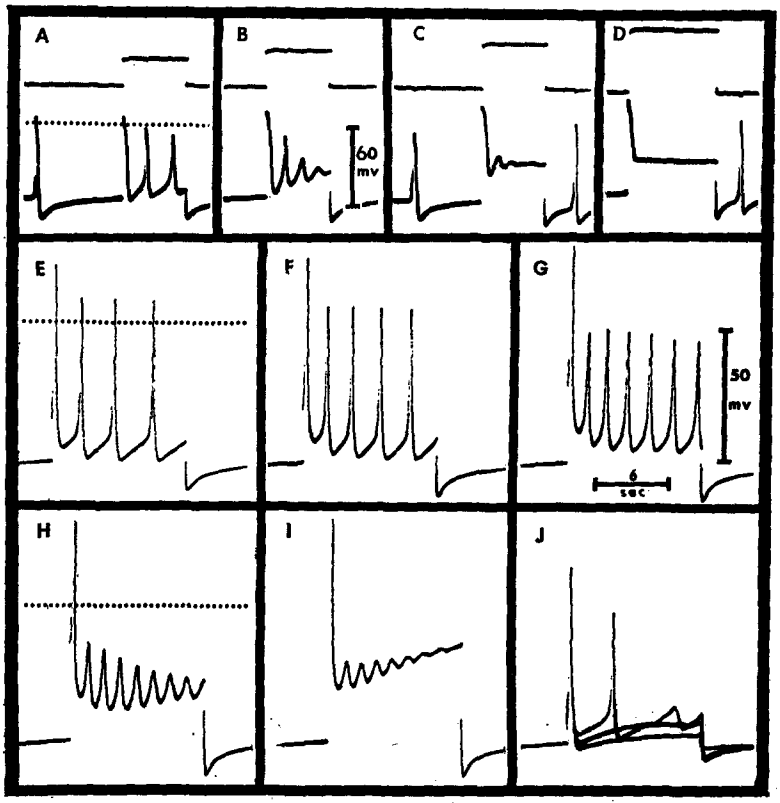

FIGURE 13. Damping of spike magnitude and frequency in pacemaker cells during application of depolarizing pulses. Time calibration applies in all photos. Voltage calibration in *B* applies to *A–D*, that in *G* applies to *E–J*. *A–D*, sequential photos from one impalement. Upper trace indicates the application of depolarizing pulses of 2.4 (*A*), 3.0 (*B*), 3.6 (*C*), and 4.8 na (*D*). *E–J*, from an impalement in another culture. Depolarizing pulses applied were, 4.8 (*E*), 6.0 (*F*), 7.4 (*G*), 9.0 (*H*), 9.6 (*I*), and 3.1, 1.4, and 0.9 na (*J*). Three sweeps superimposed in *J*.

by a decrease in g_K might be terminated by an increase in g_K, thus resetting the cycling mechanism. With further reduction in current in *J* (three sweeps superimposed), only the initial response occurred.

That the magnitude damping was not an artifact due to the bridge circuit is illustrated in Fig. 14 in an impalement with two microelectrodes at an interelectrode distance of 130 μ in a culture consisting of long, relatively thick

strands. The degree of damping at the voltage electrode was equal to that at the current-voltage electrode. Again, the degree of magnitude damping was a function of current intensity; damping was only slight in *A*, and became more pronounced in *B–D* at larger current steps. The frequency of firing on both channels in *A* was increased nearly fivefold during the pulse. The damped oscillations were in phase and of equal magnitude on both channels,

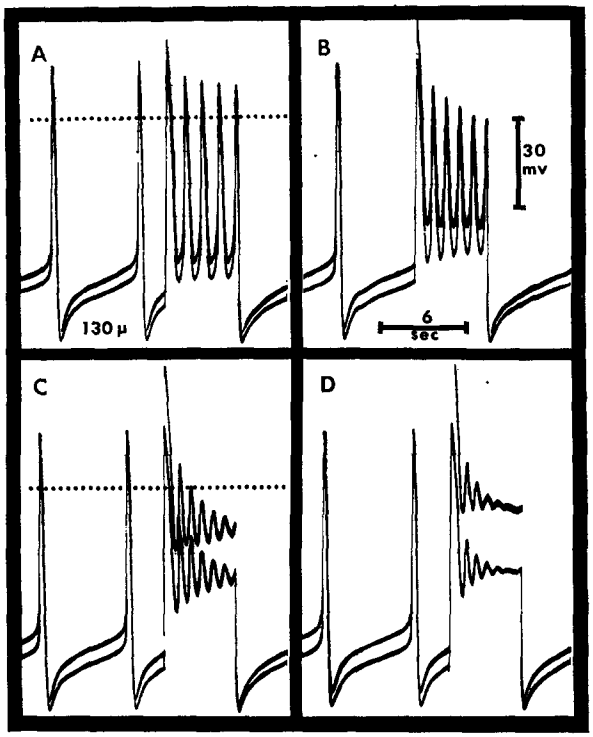

FIGURE 14. Damping of spike magnitude in a pacemaker cell during application of depolarizing pulses illustrated by impalement of two microelectrodes in one strand at an interelectrode distance of 130 μ. Voltage and time calibrations apply to all photos. Depolarizing pulses applied were 9.0 (*A*), 10.2 (*B*), 11.6 (*C*), and 12.8 na (*D*). Bridge slightly imbalanced at the larger steps of current.

suggesting that the two electrodes impaled the same cell; the input resistance was relatively low (about 3 MΩ).

"Positive off-potentials" (or postcathodal depression of excitability) were observed in cultured chick ventricular cells which were pacemakers (23); the only requirement to obtain the phenomenon was that the natural action potentials exhibit positive after-potentials. Such positive off-potentials may be seen in Figs. 13 and 14. A similar phenomenon, showing graded hyperpolarizations at the cessation of graded depolarizing pulses, was observed in cocaine-treated Purkinje fibers (33).

As stated above, driven nonpacemaker cells partially depolarized by high $[K^+]_o$ continued to develop action potentials at about the same frequency, but progressively smaller in magnitude concomitant with the progressive depolarization, until finally all electrical activity became abolished; these small potentials also became progressively shorter in duration concomitant with depolarization. Thus, depolarization by high $[K^+]_o$ resulted in short duration action potentials. However, depolarization by electrotonic current

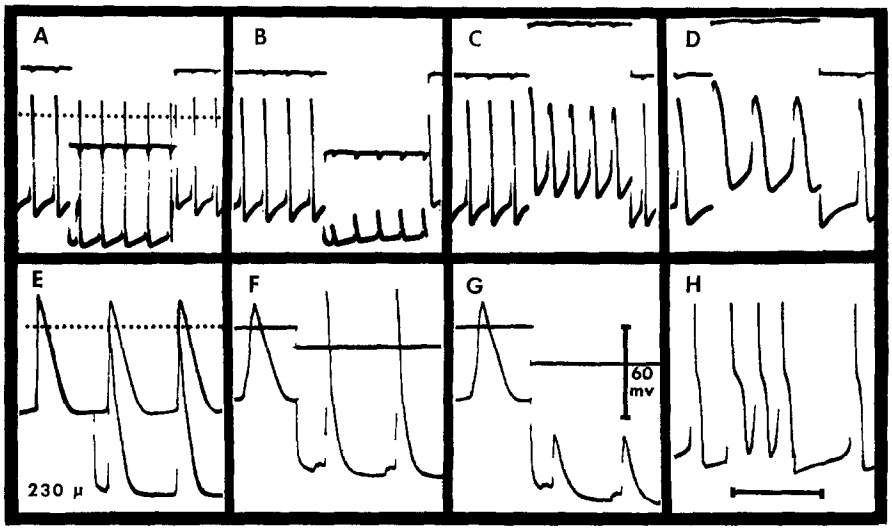

FIGURE 15. Polarizing current altered the duration of the action potentials. Voltage calibration applies to all photos; time calibration represents 6 sec in *A–C* and *H*, 3 sec in *D*, and 1.5 sec in *E–G*. Upper trace in some photos marks the application of polarizing current pulses; a downward deflection denotes hyperpolarizing current. *A–D*, sequential photos from one impalement of a driven nonpacemaker cell; application of 4.8 na (*A*), 7.6 na (*B*), 3.6 na (*C*), and 3.6 na (*D*). *E–G*, from impalement of two nonpacemaker cells in a sheet preparation by two microelectrodes at an interelectrode distance of 230 μ. Hyperpolarizing pulse of 6.0 na applied through one microelectrode in *E*; following spontaneous dislodgement of that electrode, pulses of 7.6 (*F*) and 10.6 na (*G*) were applied through the other electrode. *H*, from an impalement of a pacemaker cell; depolarizing pulse of 4.8 na applied.

produced just the opposite effect, i.e. the duration of the action potential was prolonged; hyperpolarizing current shortened the duration. This effect is shown in Fig. 15 for three cells: two driven cells (*A–D*, *E–G*) and one pacemaker cell (*H*). In *A*, a hyperpolarizing pulse increased the magnitude but shortened the duration of the action potential. More current abolished the spikes as a step function, and revealed the driving junctional potentials (*B*). Depolarizing pulses (*C*) decreased the magnitude but lengthened the duration of the action potentials; the lengthened duration is shown at faster sweep

speed in *D*. A depolarizing pulse of 3.6 na lengthened the duration about 1.5-fold (at one-half amplitude), concomitant with a depolarization of about 24 mv. In a second example of a driven cell (*E–G*), two microelectrodes were placed at an interelectrode distance of 230 μ (*E*). A hyperpolarizing pulse of 6.0 na decreased the action potential duration recorded at the site of current injection but had no effect on that recorded at the second electrode;

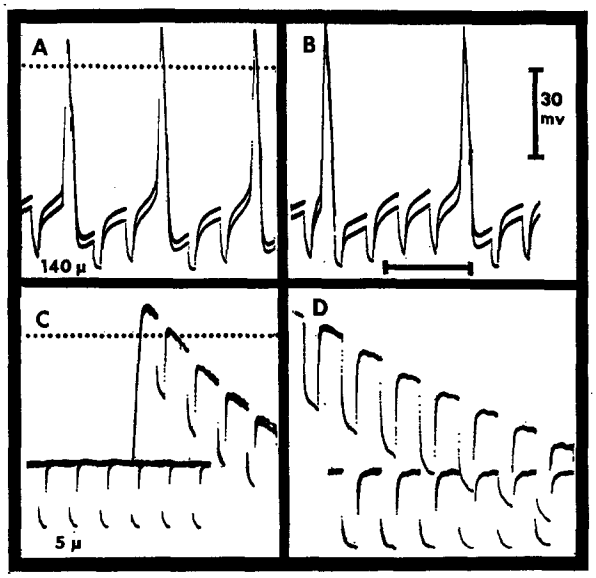

FIGURE 16. Membrane resistance during pacemaker potential and repolarization of action potential. Voltage calibration applies to all photos. Time calibration represents 3 sec in *A* and *B*, 0.3 sec in *C*, and 0.15 sec in *D*. *A–B*, sequential photos from impalement of a pacemaker cell with two electrodes at an interelectrode distance of 140 μ; hyperpolarizing pulses of 4.8 na and about 200 msec duration applied through one electrode. *C–D*, taken from impalement of a driven cell at an interelectrode distance of 5 μ; hyperpolarizing pulses of 10.6 na and about 25 msec duration applied through one electrode. Sweeps partially superimposed in *C* and *D*.

the duration was shortened about twofold, concomitant with a hyperpolarization of about 48 mv. In *F–G*, the first microelectrode became spontaneously dislodged from the cell. Hyperpolarizing current of 7.6 na applied through the second electrode showed that the action potential at this site also could be shortened (*F*). A larger step of current in *G* again abolished the spikes as a step function, leaving only the driving junctional potentials (enhanced in magnitude compared to the control without current). In a pacemaker cell (*H*), depolarizing current of 4.8 na lengthened the action potential duration; the duration was lengthened about 1.2-fold, concomitant with a depolarization of about 9 mv.

VI. *Change in R_m during the Cardiac Cycle*

The polarization resistance at one electrode (microelectrode 2) was measured during passage of constant-current pulses at a second electrode (microelectrode 1). Thus, the ΔV_m recorded by electrode 2 was independent of any bridge imbalance. As was reported previously for cultured heart cells (23), the polarization resistance is minimum during the positive after-potential and rises during the pacemaker potential. This is illustrated in Fig. 16 *A–B* at an interelectrode distance of 140 μ. In contrast, in nonpacemaker cells, there was not an increase in resistance during the rest interval between action potentials, as illustrated in *C–D* at faster sweep speeds. The resting polarization resistance was 4.7 MΩ (hyperpolarizing pulses of 10.6 na) at the interelectrode distance of 5 μ.

Although there was no plateau component of the action potential, the polarization resistance during the repolarization phase was generally about 10 to 20% larger than that at rest (cf. reference 32). For example in *C–D*, the resistance rose to 5.5 MΩ, an increase of 17%. Since in a doubly infinite cable the polarization resistance varies with the square root of R_m, a 15% increase in polarization resistance would be equivalent to a 32% increase in R_m. This increase in R_m is presumed to reflect a decrease in g_K. However, g_{Cl} (and/or g_{Na}) is undoubtedly important in determining the resting potential at physiological levels of $[K^+]_o$. If g_{Cl} were to be nearly equal to g_K, a 32% increase in R_m would then mean that g_K decreased 64% below the resting level. Although it appeared that the polarization resistance diminished during the rising phase of the action potential, it was not possible to estimate the changes with a reasonable degree of confidence.

DISCUSSION

I. *Effect of Divalent Cations*

SUSTAINED DEPOLARIZATION Depolarization of cultured heart cells by Ba^{++}, in contrast to depolarization by K^+, induces a large increase in input resistance, suggestive of a decreased g_K. Ba^{++} and K^+ have about the same crystal radii (Table I) and Ba^{++} may therefore plug K^+ channels (19, 20). The finding of prolonged action potentials in Ba^{++} is consistent with a maintained low value of g_K. The action of anodal current pulses in inducing repolarization of some Ba^{++}-depolarized cells (sustained plateau) is analogous to the normal repolarization which appears after the release of a prolonged depolarizing voltage "clamp" (cf. reference 12), and to the abrupt, sustained repolarization produced by anodal pulses applied during the cardiac plateau (34). The membrane potential of frog and toad spinal ganglion cells bathed in Ba^{++} often switched back and forth between two stable states either spon-

taneously (21) or by applying current pulses (27); Ba^{++} produced a large sustained decrease of membrane conductance, and the voltage/current curves were linear (21). In normally nonpropagating crustacean muscle fibers, large, long duration action potentials were elicited and R_m was greatly elevated in 160 mM $BaCl_2$ (9). In smooth muscle, 1 to 5 mM Ba^{++} produced rapid depolarization and cessation of spike discharge (26) or produced pla-

TABLE I

PHYSICOCHEMICAL PROPERTIES OF SELECTED IONS*

Ion	λ°	γ	Crystallographic radius	Singly hydrated radius‡	Hydrated volume	Heat of hydration	No. of water molecules	Relative shielding
			A	*A*	*A*³	(*kcal g ion*⁻¹)		
Na^+	50.10 (61.5)	0.778	0.96	3.67	150	−115	5 (4–6)	0.432
K^+	73.50 (88.2)	0.770	1.33	4.05	(150)	−90	(5) (3–6)	0.224
Cl^-	76.35 (92.2)	—	1.81	3.92	90	−59	(3) (0–6)	(0.073)
Mg^{++}	53.05	0.528	0.65	3.60	360	−501	12	2.18
Ca^{++}	59.50	0.518	0.99	3.70	310	−428	10	0.812
Sr^{++}	59.45	0.515	1.13	3.85	310	−381	10	0.624
Ba^{++}	63.63	0.508	1.35	4.08	290	−347	9–10	0.415

* Values taken from Robinson, R. A., and Stokes, R. H., Electrolyte Solutions, London, Butterworth & Co. (Publishers), Ltd., 1959, and from Bockris, J. O'M., and Conway, B. E., Modern Aspects of Electrochemistry, London, Butterworth & Co. (Publishers), Ltd., 1954.

‡ Values taken from Mullins (20).

λ°, limiting equivalent conductivity in cm^2/mho-equivalent at 25°C; the values in parentheses are for 35°C.

γ, activity coefficients for each cation as the Cl^- salt at 0.1 M and at 25°C.

Singly hydrated radius equals crystal radius + 2.72 A (diameter of water molecule) for cations with crystal radii between 0.9 to 1.7 A and crystal radiu + 2.23 A for anions. Values in parentheses are estimated values. Relative shielding was calculated from the number of water molecules per A^2 of unhydrated surface area. The order of relative shielding, from least to most shielded, is: Cl < K < Na ≃ Ba < Sr < Ca < Mg. This order is similar to that for the heats of hydration: Cl < K < Na << Ba < Sr < Ca < Mg. Thus, Cl^- sheds its hydration the easiest and Mg^{++}, the hardest. One would expect that the cations which are least shielded and which can shed their hydration the easiest could get through the cell membrane most easily if pore diameter is the limiting factor. If so, one would predict that P_K should be relatively large, and that Mg^{++} should be least active electrophysiologically.

teaus in the action potentials (3, 25), often followed by an abrupt transition into a sustained excited state (25). Ba^{++} (2 to 4 mM) partially depolarized, induced automaticity, and prolonged the action potentials of isolated cardiac fibers (2).

INITIATION OF AUTOMATICITY Initiation of automaticity in nonpacemaker cells by Ba^{++} also appears to be due to decrease in g_K. The onset of automaticity was preceded by an increase in input resistance, but automaticity preceded significant depolarization. Ba^{++} initiated automaticity

in intestinal smooth muscle without any depolarization (25). Pacemaker cells tended to have high input resistances; currents of only a fraction of a nanoampere altered the frequency and magnitude of action potentials. The difference between pacemaker and nonpacemaker cells then may lie in the level of g_K: low g_K may be characteristic of pacemaker cells. Any agent which increases g_K should inhibit the pacemaker potential and convert the cell into a nonpacemaker. For example, in cells sensitive to acetylcholine, this agent increases g_K, hyperpolarizes and inhibits pacemaker potentials. Thus, the mechanism of action of chronotropic agents may be an alteration in g_K.

The oscillations of pacemaker cells mimic known mechanical or electronic oscillators and can be empirically described by the same equations. The oscillations can be of the saw-tooth (ramp) type produced by relaxation oscillators, or they can be sinusoidal as those produced by a parallel inductance (L)–capacitance (C) circuit. The equation relating frequency of oscillation (f) to L, C, and R of the sine wave oscillator is: $f = \frac{1}{2\pi}\sqrt{1/LC - (R/2L)^2}$, in which $R/2L$ is the damping constant. Thus, $f = f_o$ (the undamped, resonant frequency) when $R = 0$. Critical damping occurs when $(R/2L)^2 = (1/LC)$. Assuming a C_m of 20 μf/cm^2 and an R_m of 500 Ωcm^2 for cultured heart cells, critical damping would occur at an L_m of 1.25 henry-cm^2; a slight decrease in R_m shifts the system away from critical damping. The biological oscillator is complicated because both R and L may vary as a function of V_m, time, and temperature. Since Ba^{++} induced oscillations concomitant with an increase in R_m, and K^+ depressed oscillations concomitant with a decrease, L_m must also be affected, otherwise an increase in R_m should lower frequency. Thus, low temperature could depress automaticity by increasing L_m. Sjodin and Mullins (22) found a large increase of L_m at low temperatures in xylene-treated squid axons; from 0.8 h-cm^2 at 18°C, L_m increased to 20 h-cm^2 at 7°C concomitant with a decreased frequency of repetitive firing. The hypothesis that low temperature inhibits a Na:K–coupled pump, thereby increasing $[K^+]_o$ in the extracellular space and increasing g_K (cf. reference 12), is unlikely for monolayer cultured heart cells because the increase of $[K^+]_o$ should be negligible in the relatively large bath.

Any phenomenon which causes a phase shift between current and voltage can produce oscillatory behavior, even though the energy storage implied by the apparent L_m is absent. Therefore, any mechanism which resists a change in membrane current will give an apparent inductance. For example, a decrease in g_K as a function of an increase in driving force (as V_m goes in a depolarizing direction) opposes a change in I_K. Thus, it is possible to obtain oscillations by allowing the resistive component to change as some function of V_m and time. Solutions of the Hodgkin-Huxley equations gave repetitive

action potentials (14, 17) and damped oscillations which were a function of the intensity of constant-current pulses (17).

The slow oscillatory pacemaker potentials produced by Sr^{++} fit nicely into the hypothesis that the level of g_K is the key parameter which determines whether a cell shall demonstrate automaticity. Since the increase in input resistance was much less pronounced than in Ba^{++}, Sr^{++} may be less effective in decreasing g_K and would not be expected to depolarize as readily. As can be seen from Fig. 9 A, the resting potential should not be very sensitive to small changes in g_K; however, a small decrease in g_K may be sufficient to initiate automaticity. Since Sr^{++} hyperpolarized, the initiation of automaticity is not necessarily dependent upon depolarization, but the oscillations may be slow because of their voltage dependency.

The action potential in Sr^{++} was larger probably because of the larger resting potential and resultant larger overshoot. It is unknown whether inwardly directed Sr^{++} current also participates during the action potential to produce a larger overshoot. However, since the action potential magnitudes were not affected by variations in $[Ca^{++}]_o$, Ca^{++} current does not appear to participate. In crustacean muscle fibers, on the other hand, the resting membrane resistance increased as $[Sr^{++}]_o$ was increased, and the effect of variation in $[Sr^{++}]_o$ suggested that this ion carried most of the inward ionic current during the action potential (9). It is unlikely that the pronounced negative after-potentials produced by Sr^{++} were generated by a decreased E_K due to a diffusion lag between a space just outside of the cell membrane and the bulk solution because the cultured cells were monolayers.

INITIAL HYPERPOLARIZATION The pronounced hyperpolarization in Sr^{++} which was maintained for many minutes perhaps was produced either by a decrease in g_{Na} or by stimulation of active extrusion of Na^+. If Ca^{++} plugged Na^+-selective channels in the membrane because of their nearly identical crystal radii, then Sr^{++} could plug both Na^+ and K^+ channels since its radius is nearly halfway between that of Ca^{++} and Ba^{++} (Table I). Thus, Sr^{++} may decrease both g_K and g_{Na}, whereas Ba^{++} may decrease only g_K. It is possible that Sr^{++} produced the initial hyperpolarization by stimulation of the active pumping of Na^+. Thus, Sr^{++} may be more effective in stimulating the pump, but Ba^{++} may be more effective in decreasing g_K. However, since Ba^{++} did produce an initial hyperpolarization of cells in the presence of ouabain, the role of the pump in the hyperpolarization is unknown. In toad spinal ganglion cells, Nishi and Soeda (21) found that an initial hyperpolarization of about 20 mv produced by 24 to 80 mM Ba^{++} reached a peak within 15 sec, gradually converting into a sustained slow depolarization. Since the hyperpolarization was not observed below 10°C, in the presence of metabolic inhibitor, or by preincubation in K^+-free solutions, but was

potentiated by microelectrophoretic injection of Na^+, the hyperpolarization was attributed to an enhanced active extrusion of Na^+.

Ca^{++}, Mg^{++}, Zn^+ It is not surprising that alteration of $[Ca^{++}]_o$ and $[Mg^{++}]_o$ had no apparent effect on the electrical properties of cultured chick ventricular cells during acute experiments. For example, it has been reported that tissues from different parts of the same heart have different sensitivities to Ca^{++} depletion (cf. references 7 and 12), and large changes in $[Ca^{++}]_o$ had no appreciable effect on the resting membrane resistance (29, 33). SA nodal cells were relatively insensitive to low $[Ca^{++}]_o$ (28). Changes in $[Mg^{++}]_o$ had almost no effect on the membrane potentials of cardiac muscle (12).

The fact that Zn^{++} did not increase membrane resistance or affect the membrane potentials of cultured heart cells may be related to the fact that the crystal radius and the hydration number of Zn^{++} are about equal to those of Mg^{++}. The membrane resistance of frog skeletal muscle, however, increased almost threefold in 0.5 mM Zn^{++} (18).

II. *Effect of K^+*

The frequency of firing of nonpacemaker cells was unaffected by polarizing current. Quiescent nonpacemaker cells usually did not respond actively to depolarization produced by electrotonic current applied intracellularly but did give anodal-break responses. The frequency of firing of pacemaker cells was increased by depolarizing current and decreased by hyperpolarizing current. Depolarization should increase frequency because the slope of the pacemaker potential is voltage-dependent and because the amount of depolarization necessary to reach the threshold potential is decreased. Depolarization due to injury often increased frequency transiently, but depolarization by elevated $[K^+]_o$ suppressed automaticity.

Spontaneous firing of pacemaker cells was generally unaffected by $[K^+]_o$ levels as large as 10 to 20 mM; further elevation of $[K^+]_o$ produced prompt cessation of firing followed by sustained depolarization. Thus, even though depolarized beyond the initial threshold potential, the cell did not fire although still excitable as evidenced by anodal-break responses. The depolarized cells were no longer pacemakers as evidenced by the lack of action potential production upon electrotonically returning V_m to the resting level. However, upon addition of Ba^{++}, the depolarized cells were reconverted to pacemakers and action potentials were produced by repolarizing pulses which returned V_m to levels capable of supporting action potentials.

The finding that some cells hyperpolarized upon raising $[K^+]_o$ from 2.7 to about 10 mM agrees with that recently reported for Purkinje fibers (1). There are two feasible explanations for this phenomenon: (*a*) There may be an electrogenic Na^+ pump which is optimally electrogenic at a $[K^+]_o$ of about

10 mM. (*b*) There may be a relatively greater effect of increased $[K^+]_o$ on g_K than on E_K; i.e., the membrane is hyperpolarized because $g_K/\Sigma g$ is increased more than E_K is decreased. Both curves *A* and *B* of Fig. 9 A can be predicted from the constant-field equation (10, 11) depending upon the values selected for the various parameters; three such theoretical curves are shown in Fig. 9 B. That P_K may increase as a function of $[K^+]_o$ is consistent with the decrease in R_m as $[K^+]_o$ was increased, and with the prompt repolarization produced by a transient increase of $[K^+]_o$ during the action potential plateau of cooled, perfused turtle ventricle (12, 34).

A number of investigators have also postulated that high $[K^+]_o$ inhibits pacemaker activity by an increased g_K (13). SA and AV nodal cells were less sensitive than were the nonspecialized fibers to the automaticity-suppressing action of elevated $[K^+]_o$ (8, cf. reference 28), and idioventricular automaticity was depressed while the sinus rate was only slightly affected (31). Increase of $[K^+]_o$ from 2.7 to 13.5 mM produced no effect in SA nodal cells, but 22 mM abolished automaticity and partially depolarized; multifocal pacemaker activity appeared at low $[K^+]_o$ levels (30). Antoni et al. (1) also observed that high $[K^+]_o$ depressed automaticity of cells in sinus node and Purkinje fibers; the pacemaker potentials were abolished by 9 mM K^+ without any depolarization, and hyperpolarizations of 1 to 8 mv were produced in 8 to 25 mM K^+. When Vassalle (30) increased $[K^+]_i$ in isolated Purkinje fibers by sucrose hypertonicity, automaticity was depressed and, conversely, automaticity was enhanced in hypotonic media; automaticity was suppressed by elevating $[K^+]_o$ from 2.7 to 5.4 mM which decreased R_m by over twofold without a change in resting potential and, conversely, lowering of $[K^+]_o$ to 2.7 mM initiated pacemaker activity. Carmeliet (4, 5, cf. reference 34) demonstrated in Purkinje fibers in K^+-free media that a decreased g_K was responsible for partial depolarization and oscillations.

III. *Summary of One Cycle of Oscillation*

The rapid repolarization phase of the action potential may be produced by a regenerative increase in g_K which permits the membrane potential to swing past its resting value towards E_K; hence, R_m is low during the positive after-potential. In low $[K^+]_o$, the resting potential can remain unaltered (since g_K is low) but the maximum potential during the positive after-potential ($-V_{max}$) can attain a larger value (larger E_K); thus, the difference between $-V_{max}$ and the resting V_m decreases as $[K^+]_o$ is increased. R_m increases to its resting value during termination of the positive after-potential. The high g_K during the positive after-potential should inhibit cycling. The positive after-potential is generally more prominent in pacemaker cells (23, 29). Termination of the positive after-potential and beginning of the pacemaker potential

are often marked by a prominent change in slope. There is a further increase in R_m during the pacemaker potential (23, 29). If threshold potential is reached before termination of the oscillation, an action potential is triggered. The action potential may then reset the train of oscillations (30), although undamped oscillations may also occur independently of action potentials.

The pacemaker potential might be due to a time- and voltage-dependent decrease in g_K (29). With electrotonic depolarization the frequency of oscillation increases, but during a given pulse the frequency sometimes progressively falls off. There may be rectification in pacemaker cells such that g_K increases with slight hyperpolarization depressing the frequency of oscillations and decreases with depolarization enhancing the frequency. At a given V_m, each cycle may be terminated by a time-dependent process; the fast repolarizing phase of each subthreshold oscillation might be due to an increase of g_K. The time-dependent component could be related to the energy availability or utilization. Metabolic schemes having appropriate feedback may oscillate; e.g., damped oscillations in DPNH levels, controlled by 3′,5′-cyclic AMP, occur in some cell-free systems (6).

At least two conditions have been postulated to be necessary for pacemaker oscillations (cf. reference 30): (*a*) a low g_K, and (*b*) a significant difference between E_K and V_m. Usually, a change in g_K would correlate well with a corresponding change in the difference between V_m and E_K; e.g., an increase in g_K would act to bring V_m closer to E_K. In K^+-depolarized cells, $E_K - V_m$ should be small, and the addition of Ba^{++} produced oscillations without much change in V_m; repolarizing pulses led to larger oscillations, even though V_m should become larger (more negative) than E_K. Sr^{++} induced pacemaker activity even though it hyperpolarized, thereby diminishing the difference between V_m and E_K.

Initiation of pacemaker potentials cannot be dependent only on V_m because: (*a*) Sr^{++} induced automaticity concomitant with hyperpolarization. (*b*) Ba^{++} converted K^+-depolarized cells into oscillating pacemakers, and large action potentials were produced during electrotonic repolarization. (*c*) Ba^{++} induced automaticity before depolarizing; furthermore, this conversion must have been independent of V_m because electrotonic depolarization of the same cells in the absence of Ba^{++} did not initiate automaticity. (*d*) Ba^{++}-depolarized cells often continued to develop small oscillations, and action potentials were produced during electrotonic repolarization. (*e*) K^+ depressed automaticity before depolarizing. Thus, there must also be a voltage-independent component to cycling.

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