

Effect of Verapamil and of Extracellular Ca and Na on Contraction Frequency of Cultured Heart Cells

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ABSTRACT Monolayer cultures of myocardial cells were prepared by trypsin dispersion of neonatal rat ventricles. The cells were cultured for 4–5 days by which time a synchronously contracting monolayer of some 1.0×10^6 cells per 6-cm diam petri dish had formed. The contraction frequency and Na influx of the cells were unaffected by tetrodotoxin (2×10^{-5} mg/ml) but both were markedly reduced by the addition of verapamil (10^{-9} M to 10^{-5} M). The effect of verapamil on both parameters occurred very rapidly. Although unresponsive to change in $[Ca]_0$ between 0.3 mM and 3.0 mM, the contraction frequency of the cells declined rapidly as the $[Ca]_0$ was reduced below 0.3 mM. On the other hand the beating rate of the cells was linearly related to $[Na]_0$ between 40 mM and 170 mM. At values $[Na]_0$ below 40 mM the cells ceased to contract. It is therefore apparent that both $[Ca]_0$ and $[Na]_0$ contribute to the maintenance of the contraction frequency of cultured myocardial cells, but the latter is by far the more important. There also appeared to be, under all conditions, a close relationship between verapamil-sensitive Na influx and contraction frequency. For the greater part this relationship was linear although at higher Na influx values it appeared to show evidence of saturation.

INTRODUCTION

It is generally accepted that, in most cardiac pacemaker tissue, automaticity results from a decreased potassium (K) conductance in the presence of a depolarizing current during diastole. Phase 4 depolarization in sinoatrial tissue (Brooks and Lu, 1972; West, 1972) has been attributed largely to an inward calcium (Ca) current, although in spontaneously firing Purkinje fibers changes in extracellular calcium concentration ($[Ca]_0$) had little or no effect on automaticity (Draper and Weidmann, 1951; Weidmann, 1955). Similarly, in cultured chick heart cells increasing $[Ca]_0$ had little influence on the contraction frequency (Sperelakis and Lehmkuhl, 1966). On the other hand, decreasing extracellular sodium concentration ($[Na]_0$) diminished or abolished pacemaker function in Purkinje fibers (Draper and Weidmann, 1951; Weidmann, 1955) leading to suggestions that Na was primarily responsible (Trautwein and Kassebaum, 1961) or that both Na and Ca might be implicated (Imanishi, 1971; Katzung, 1975). The nature of the ionic basis for the depolarizing current involved in automaticity has not therefore been completely defined, although it is possible that the ion

involved may depend on the tissue under study, thereby accounting for conflicting results obtained from differing preparations.

Myocardial cells cultured from the hearts of neonatal rats exhibit regular and rhythmic contractions *in vitro* which must reflect the intrinsic automaticity of the preparation. Once a confluent monolayer is formed, contractions become synchronous throughout the tissue. It has been stated that the contraction rate of a monolayer, at any given time, reflects the automaticity of the most rapidly contracting cell (Harary and Farley, 1963 *b*; Mark and Strasser, 1966). It is probable, however, that the automaticity, or rate of diastolic depolarization, of all cells in the culture is very similar because: (*a*) of the tight electrical coupling which exists between adjacent cells; and (*b*) the excitation wave front may change from beat to beat with little or no change in contraction frequency (Jongsma et al., 1975). For this reason it is felt that alterations in ionic exchange associated with changes in intrinsic automaticity of these cells will be reflective of processes involving all of the cells of the monolayer rather than just a few isolated "pacemaker" cells. Previous studies (Sperelakis and Lehmkuhl, 1966) have indicated that the enhanced automaticity of chick heart cells in culture is due to a reduced membrane K permeability (P_K). In view of the influence of $[Na]_0$ and $[Ca]_0$ on the function of the sinoatrial node and Purkinje fibers, however, additional ionic determinants of automaticity of heart cells in culture may also exist. The present studies, therefore, were carried out to investigate the possible roles of Ca and Na in the regulation of the contraction rate of spontaneously beating cultured heart cells.

MATERIALS AND METHODS

With the hearts of 1–2-day old rats, myocardial cell cultures were prepared by the method of Harary and Farley (1963 *a*). After removal of the hearts from the animal, however, the hearts were trimmed of all atrial tissue and only the ventricles used for culture preparation to ensure, as nearly as possible, a homogeneous cell population. After disaggregation of the hearts to single cells, by repeated trypsinizations, the modifications of Blondel et al. (1971) to improve the percentage of myocardial cells present in the cultures were employed. This step permitted the preparation of cultures containing at least 80% myocardial cells, a percentage similar to that obtained by both Blondel et al. (1971) and Langer and Frank (1975). After this, the cells were incubated at 37°C in growth medium (MEM) containing 10% calf serum for 4 days, by which time a satisfactory monolayer of some 1×10^6 cells per 6-cm diam Petri dish had formed. Before each study, randomly selected plates from each batch were examined under phase-contrast microscopy to determine the percentage of cells showing visible contractile shortening, and to ensure that contractions were synchronous.

Since the pH of MEM depends on a controlled atmosphere of 5% CO₂ in air, its stability could not be maintained during experiments in which plates were continually being removed from the incubator. Accordingly, all experiments were carried out with the cells in a balanced salt solution containing (mM): Na⁺ 136.80; K⁺ 5.35; Ca²⁺ 2.25 (ionized Ca²⁺ 1.90); Mg²⁺ 1.03; Cl⁻ 148.22; PO₄³⁻ 0.43; glucose 11.10; plus calf serum 5% and phenol red 0.0002%. Regular spontaneous contractions of the cells can be maintained in this solution for at least 24 h. The cells were allowed to equilibrate in this solution for a period of 3 h before any measurements were made, although no change in the contraction frequency of the cells was noted during this period.

The low Na solutions were prepared by osmotic replacement of NaCl with choline

chloride. A direct effect of the choline chloride on the cells, however, was excluded by performing a duplicate set of experiments in which glucose was used as the NaCl replacement. This gave results essentially identical to those obtained with choline chloride, indicating that the results obtained were a reflection of the low Na rather than of the Na-replacing agent. Differing levels of ionized Ca were obtained by adding or subtracting appropriate quantities of anhydrous CaCl₂ when the salt solution was prepared, the ionized Ca levels being verified with an Orion biochemical ionized calcium analyzer model SS-20 (Orion Research Inc., Cambridge, Mass.). Since the contribution to the total osmolality of the solution from CaCl₂ is small, no attempt at osmotic correction for Ca was made. Verapamil HCl was freely soluble in the balanced salt solution, at the desired concentrations, without the necessity of using a specific solvent. The pH of all solutions was 7.2.

Na influx was measured by methods detailed previously (Lamb and MacKinnon, 1971; Lamb and McCall, 1972). The cells were exposed to ²⁴Na, in balanced salt solution, for a period of time, short compared to the total Na exchange time, and the influx was calculated from the equation:

$$M_{in} = d[C_i]/dt \cdot 10^5/n,$$

where M_{in} is the influx in $\mu\text{M}/10^{11}$ cells/s, $d[C_i]$ is the amount of ion entering the cell (calculated from intracellular radioactivity and the specific activity of the soak solution) in time dt , and n is the cell number (as a fraction of 10^6 cells) per plate. All measurements of Na influx were made over a 15-s exposure to ²⁴Na (half-time of Na exchange in these cells = 1.5 min [McCall, unpublished observation]). Since this is very short compared to total Na exchange time, it can be assumed that there is negligible efflux of tracer in that time (Lamb, 1971), and no correction need be applied. The plates of cells were gently agitated on a controlled temperature warming plate at 37°C during the influx period, to ensure adequate mixing and stability of temperature throughout the experiment. Blank plates, containing no cells, were included with each experiment to determine background activity on the plates. Where the effect of a drug on Na influx was measured, the drug was included in the radioactive influx solution.

The contraction rate of the cells in culture was recorded by visual examination of the cells under phase contrast microscopy and counting the number of contractions in a given time, and was expressed as contractions per minute. Throughout the rate determinations the cultures were kept at a constant 37°C on the microscope stage by using a Sage Air Curtain incubator (Sage Instruments Div., Orion Research Inc., Cambridge, Mass.).

The ²⁴Na used was obtained from New England nuclear, Waltham, Mass., and the tetrodotoxin from Sigma Chemicals, St. Louis, Mo. Dr. Arnold Schwartz, Department of Cell Biophysics, Baylor College of Medicine, Houston, Tex., kindly supplied the verapamil used in the present experiments. All isotope counting was carried out with a Packard Tricarb Liquid Scintillation Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) the ²⁴Na being counted without added scintillant.

RESULTS

Effect of Tetrodotoxin

Initial studies were carried out in an attempt to assess how much, if at all, the contraction rate of these cells was influenced by tetrodotoxin (TTX)-sensitive currents. The cells were exposed to TTX, in balanced salt solution, in concentrations from 10^{-8} to 2×10^{-5} g/ml for periods of time from 30 s to 30 min. None of the concentrations of TTX used produced measurable effects on either the

contraction frequency or the Na influx in these cells. It should be pointed out, however, that the present study did not include electrophysiological recordings from the cells. These data, therefore, although suggestive, do not absolutely preclude the existence of TTX-sensitive Na channels in the preparation. The concentration (2×10^{-5} g/ml) used, however, was previously found sufficient to abolish excitation in Purkinje fibers (Dudel et al., 1967), decreasing the beating rate of cardiac cell strands (Lieberman et al., 1975), and several orders of magnitude greater than that required to abolish electrical activity in whole chick embryo hearts (De Haan et al., 1975). It is therefore felt that the present results are strongly suggestive of a relative insensitivity of the cells to TTX.

Effect of Verapamil on Contraction Rate

Although relatively insensitive to TTX, the contraction rate of the cells was extremely sensitive to the addition of verapamil. This effect of verapamil was dose-dependent (Fig. 1a) and had an almost immediate onset. A maximum effect of each concentration of verapamil on the rate of beating was observed within 15 s of addition of the drug, thereafter showing no further time-dependent effect nor any tendency to spontaneous recovery in the continued presence of the drug. With verapamil the beat rate (contractions per minute) decreased from a control of 122.10 ± 2.37 (SEM) to 104.50 ± 1.20 (SEM) ($P < 0.001$) and to 36.10 ± 2.43 (SEM) ($P < 0.001$) in 10^{-9} M and 2×10^{-7} M, respectively. All spontaneous contractions of the cells were abolished by concentrations of verapamil of 5×10^{-7} M and greater. From the data shown in Fig. 1a the half-maximal inhibitory dose (ID_{50}) for verapamil on contraction frequency was of the order of 7.5×10^{-8} M.

Effect of Verapamil on Na Influx

Verapamil, in addition to having a pronounced effect on the contraction rate of these cells, markedly affected the Na influx. As in the case of the rate effect, the verapamil-induced reduction in Na influx was immediate upon addition of the drug and showed no further time-dependent changes in magnitude between 15 s and 1 h. With increasing concentrations of verapamil there was a progressive decrease in Na influx, dose-dependent along a sigmoid log dose-response curve (Fig. 1b). The Na influx ($\mu\text{M}/10^{11}$ cells/s) declined from a control value of 30.08 ± 1.10 (SEM) to 26.41 ± 0.68 (SEM) ($P < 0.01$) and to 8.44 ± 0.25 (SEM) ($P < 0.001$) in 10^{-9} M and 10^{-5} M verapamil, respectively. Maximal inhibitory effect on the Na influx occurs with verapamil in concentrations from 10^{-6} M to 10^{-5} M with an ID_{50} at 5×10^{-8} M, very similar to the ID_{50} for the verapamil-induced rate effect.

The results indicate that approximately 72% of the Na influx in these cells is abolished immediately upon the addition of verapamil, in concentrations of 10^{-6} M or greater, to the bathing solution. It was therefore possible to divide the Na influx, under various conditions, into its verapamil-sensitive and verapamil-insensitive components, by measuring the flux in the absence and in the presence of 10^{-5} M verapamil.

The relationship between the rate and Na-influx effects of verapamil was of

some interest. It was found that there existed close linear relationships between total Na influx and contraction rate, and between verapamil-sensitive influx and contraction rate. For total Na influx the relationship could be described by the equation $y = 5.89x - 50.0$ ($r = 0.99$; $P < 0.001$), and for verapamil-sensitive influx by the equation $y = 5.77x - 0.20$ ($r = 0.99$; $P < 0.001$) (in each case $y =$ contraction frequency and $x =$ Na influx).

Na influx changes associated with changes in the contraction rate of the cells may be a reflection of changes in either the electrical or the mechanical activity of the preparation. The latter possibility deserves serious consideration in view of the findings of McLean et al. (1974), in cultured chick heart cells, that verapamil produced electromechanical uncoupling within 3–5 min, rather than a

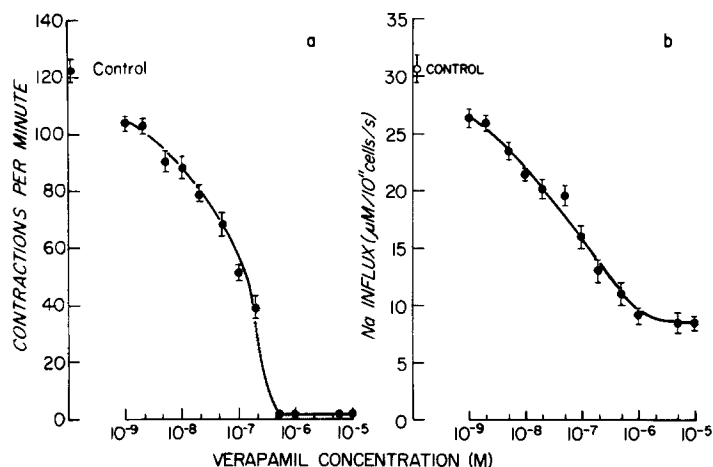


FIGURE 1. The effect of the immediate application of various concentrations of verapamil (log scale) on (a) the spontaneous contraction frequency and (b) Na influx of monolayers of myocardial cells. Points are mean \pm SEM ($n = 20$ for contraction frequency measurements; $n = 10$ for flux measurements). Verapamil effect on rate significant, $P < 0.001$ at all concentrations shown. For Na influx effect, $P < 0.01$ at 10^{-9} M and $P < 0.001$ at 5×10^{-9} M and greater.

deterioration of slow Na current. It was of interest, however, that in the present studies, lanthanum chloride (5×10^{-4} M) which has been shown to uncouple excitation from contraction in mammalian heart muscle (Sanborn and Langer, 1970) and cultured heart cells (Langer and Frank, 1972), resulted in complete cessation of all contractile activity but had no appreciable effect on Na influx.

Verapamil blocks slow current responses, initially considered to be due to specific blockage of an inward Ca current (Kohlhardt et al., 1972). More recently, however, the drug has been shown to influence slow Na currents, as well as Ca currents (Shigenobu et al., 1974) in embryonic chick myocardial cells. The possible implications of this latter observation to the present study were investigated by assessing the effect of both $[\text{Ca}]_0$ and $[\text{Na}]_0$ on the contraction rate of the cells.

Effect of $[Ca]_0$ on Contraction Rate

The monolayers of myocardial cells were exposed to various concentrations of ionized Ca in balanced salt solution. Changes in $[Ca]_0$ between 0.3 mM and 3.0 mM had no influence on the contraction rate of the preparation (Fig. 2). As the $[Ca]_0$, however, was reduced below 0.3 mM the spontaneous rate declined rapidly from the control level. This (Fig. 2) would indicate that extracellular Ca is required to maintain a normal beating rate, but such that half-maximal activation occurs at a $[Ca]_0$ of 0.15 mM, the maximal effect occurring with 0.3 mM $[Ca]_0$. It therefore requires a very low $[Ca]_0$ to influence the contraction rate of cells and that changes in $[Ca]_0$ between 0.3 mM and 3.0 mM do not appreciably influence the automaticity of the preparation.

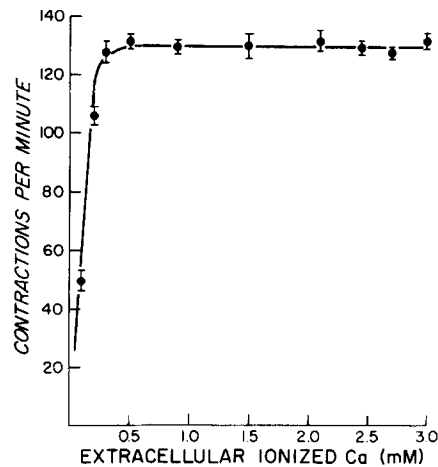


FIGURE 2. Spontaneous contraction frequency of cells at different levels of $[Ca]_0$. Each point is mean \pm SEM of six observations.

Effect of $[Na]_0$ on Contraction Rate

In contrast to the effect of changes in $[Ca]_0$, even small changes in $[Na]_0$ had a significant effect on the cells' contraction rate (Fig. 3). When $[Na]_0$ was decreased below 40 mM all spontaneous contractions ceased. The relationship between $[Na]_0$ and contraction frequency could be described by a single linear regression ($r = 0.99$; $P < 0.001$) as shown in Fig. 3. Despite the close adherence to the calculated linear regression, however, the data points shown (Fig. 3) suggest that above 150 mM $[Na]_0$ the rate of beating is reaching a plateau with respect to $[Na]_0$. The results indicate a close relationship between extracellular Na and spontaneous contractile activity of heart cells in culture. The effect of $[Na]_0$ on contraction rate was immediate upon addition of the low Na solutions, and promptly reversed by returning the cells to the control solution.

Effect of $[Na]_0$ on Na Influx

As would be expected, reducing $[Na]_0$ reduces the Na influx (Fig. 4) immediately upon addition of the low Na solutions to the cells. The Na influx was measured

in the absence and presence of verapamil (10^{-5} M) at each of the $[Na]_0$ values shown (Fig. 4), allowing separation of the flux into its verapamil-sensitive and nonsensitive components. It is of interest (Fig. 4) that for values of $[Na]_0$ of 25 mM and below no verapamil-sensitive Na influx could be recorded. There were,

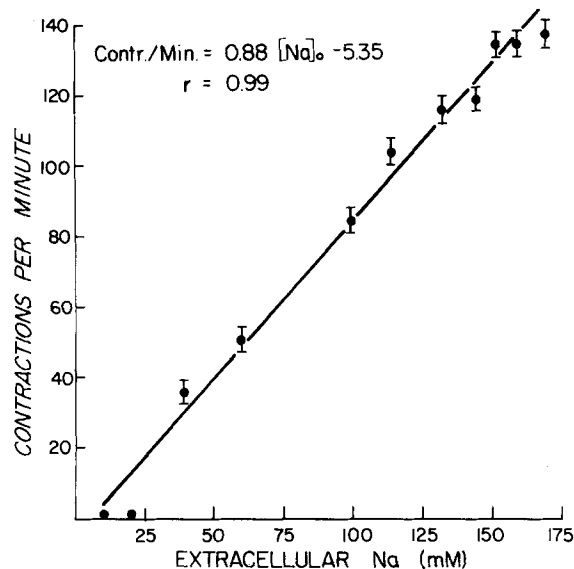


FIGURE 3. Spontaneous contraction frequency of cells at different levels of $[Na]_0$. Each point is mean \pm SEM of 10 observations. Line shown is calculated regression line ($P < 0.001$) together with corresponding regression equation.

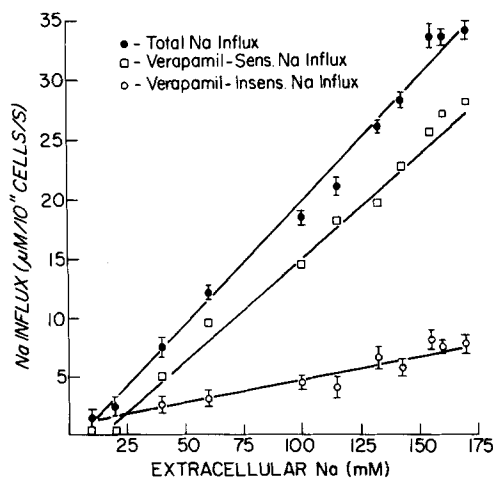


FIGURE 4. Effect of $[Na]_0$ on Na influx, myocardial cells. At each $[Na]_0$ value, influx measured in presence and absence of verapamil (10^{-5} M), allowing derivation of verapamil-sensitive and insensitive components. Each point is mean \pm SEM of 10 observations. Lines are calculated regression lines which are described by the following equations. For total Na influx, $y = 0.21x - 1.20$ ($r = 0.99$, $P < 0.001$); for verapamil-sensitive Na influx, $y = 0.18x - 2.69$ ($r = 0.99$, $P < 0.001$); for verapamil-insensitive Na influx, $y = 0.04x + 1.09$ ($r = 0.94$, $P < 0.001$).

however, close linear correlations between $[Na]_0$ and total, verapamil-sensitive and verapamil-insensitive Na fluxes throughout the range of $[Na]_0$ values tested (for each $r = 0.94$ or greater; $P < 0.001$). With the exception of the verapamil-sensitive Na influx, the calculated regression lines in each case pass very close to the intersect of ordinate and abscissa.

The overall relationship between Na influx and contraction rate at different levels of $[Na]_0$ did not conform to a single linear regression. For values of total Na influx ($\mu M/10^{11}$ cells/s) between 0 and 26.2 the relationship to contraction rate followed a linear progression described by the equation $y = 4.80x - 5.26$ ($r = 0.98$; $P < 0.001$). At values greater than $26 \mu M/10^{11}$ cells/s the relationship deviated considerably from the previous line, the contraction frequency reaching a plateau with respect to Na influx.

An almost identical relationship between verapamil-sensitive Na influx and rate, with variation in $[Na]_0$, existed. The slope of the linear regression ($y = 5.92x + 0.52$; $r = 0.99$), however, was different from that pertaining to total Na influx, and the upper limit of the linear portion in this case was $19.5 \mu M/10^{11}$ cells/s Na influx.

Since changing $[Na]_0$ has an immediate effect on contraction frequency, the present data indicate a highly significant interdependence between rate of Na entry and contraction frequency in cultured heart cells. The overall relationship between the verapamil-sensitive Na influx for both verapamil effects and $[Na]_0$ effects is shown in Fig. 5. This indicates that as the verapamil-sensitive Na influx increases toward $20 \mu M/10^{11}$ cells/s, it correlates linearly with an increase in the beating rate of the cells. Further increases above $20 \mu M/10^{11}$ cells/s result in a much lower progression of contraction rate, the points (Fig. 5) being strongly suggestive of saturation with a maximal influence on rate occurring somewhere between 25 and $30 \mu M/10^{11}$ cells/s. From Fig. 5 it can also be seen that the half-maximal effect occurs with a verapamil-sensitive Na influx of around $14 \mu M/10^{11}$ cells/s. This would correspond to a verapamil concentration of around 2×10^{-8} M (cf. Fig. 1*a* and a value for $[Na]_0$ around 100 mM (cf. Fig. 3).

Subsequent studies using ^{42}K and ^{45}Ca as tracers for intracellular K and Ca, respectively, demonstrated that (*a*) decreasing $[Na]_0$ had no measurable effect on intracellular K concentration over a 1-h period, and that (*b*) a measurable increase in intracellular Ca could only be detected after 10 min exposure to the low $[Na]_0$ (50 mM; 75 mM; 100 mM) solutions. These findings would indicate that the immediate effects of decreasing $[Na]_0$ are primarily due to the $[Na]_0$ itself, rather than being mediated via secondary changes in intracellular K or Ca.

DISCUSSION

In addition to possessing the general advantages inherent in the use of cultured cells for ion flux studies (Cheneval et al., 1972) heart cells in culture permit an evaluation of the action of cardioactive drugs at a cellular level, free from any neurogenic or hemodynamic influences. For this reason such cells have become popular as an investigational preparation, despite the significant electrophysiologic differences between these cells and adult mammalian heart (Sperelakis, 1967). Because of this, it should be appreciated that caution must be exercised in

attempting to extrapolate data regarding membrane function in these cells directly to that in the adult mammalian heart.

In the present studies no measurements of cellular electrical activity were made, and it was therefore impossible to define specific membrane ion currents directly. The contraction frequencies of the cells were unaffected by TTX in concentrations up to 2×10^{-5} g/ml, the concentration which would be expected to abolish spontaneous electrical activity in Purkinje fibers (Dudel et al., 1967). Although not conclusive, these data do suggest that fast Na currents are of lesser

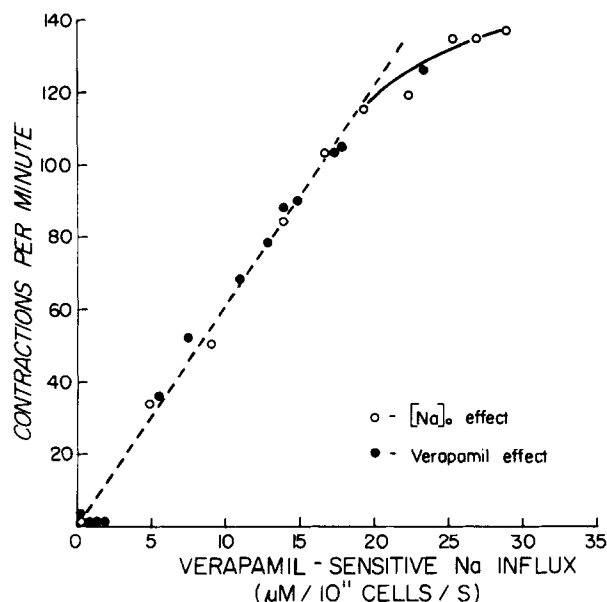


FIGURE 5. Overall relationship between verapamil-sensitive Na influx and contraction frequency from both verapamil (solid symbols) and $[\text{Na}]_0$ (open symbols) studies. Mean values only shown. The broken line represents the calculated linear regression, for values of Na influx between 0 and $20 \mu\text{M}/10^{11}$ cells/s, given by the equation $y = 6.02x - 0.95$ ($r = 0.99$, $P < 0.001$). The solid line is fitted to the remaining points by eye.

importance in the maintenance of normal activity in these cells. Insensitivity of heart cells in culture to TTX, at least in young cultures, has been previously reported by other workers (Lieberman et al., 1975; De Haan et al., 1975; Sperelakis et al., 1975). This is thought to be due to a loss of fast Na channels and a gain of slow Na channels, suggestive of reversion of the cells to a younger embryonic state (Sperelakis et al., 1975).

In view of the preponderance of slow current activity in cultured heart cells (Sperelakis, 1972) the effects of verapamil on both contraction frequency and Na influx in the present experiments are of some interest. Both were markedly decreased within 15 s of exposure to verapamil. This differs from the findings of McLean et al. (1974) indicating that in cultured chick heart cells verapamil

produced electromechanical uncoupling within 3–5 min, but did not result in deterioration of the slow Na current. In our cells the effects of verapamil appeared to be much more rapid. The reason for the apparent differences between the present findings and those of McLean et al. (1974) is unclear, but may well represent a contrast of the effects of verapamil on mammalian as opposed to avian tissue. It cannot be assured, however, that the present effects did not represent electromechanical uncoupling, despite the apparent differences between the effects of verapamil and those of lanthanum in this preparation.

It should be pointed out, however, that from the electrophysiologic standpoint, the actions of neither verapamil nor lanthanum can be regarded as specifically inhibitory to an inward Ca current. Kass and Tsien (1975) demonstrated significant effects of both lanthanum and the verapamil analogue, D600, on membrane K currents. Both agents modified the K current associated with the action potential plateau. In addition, lanthanum (5×10^{-4} M) caused a shift in the pacemaker K current associated with a steepening of pacemaker depolarization (Kass and Tsien, 1975). On the other hand, D600 had no effect on pacemaker K currents in concentrations up to 9.6×10^{-6} M (Tsien, 1975; Kass and Tsien, 1975). In the present studies, no acceleration of contraction frequency was seen upon addition of lanthanum to the cultures, and the verapamil analogue D600 does not appear to influence pacemaker K current in other preparations (Kass and Tsien, 1975; Tsien, 1974). It is therefore unlikely that the effects of lanthanum or verapamil in this study represent effects on membrane K currents, although this possibility does exist and has to be considered in the overall interpretation of the data.

It has also been recently shown that racemic verapamil and D600 may have a measurable effect on fast Na currents (Bayer et al., 1975) predominantly reflecting the action of the (*d*) isomer. The concentrations necessary for this effect however were considerably in excess of those found to have a significant effect in this preparation, and therefore it is unlikely that this mechanism was operative, especially in view of the insensitivity of the preparation to TTX.

Although the actions of verapamil cannot therefore be regarded simply as resulting from specific blockade of a slow inward current, it is unlikely that the effects of the agent seen in this preparation are due to effects on either membrane K, or fast Na, currents.

Approximately 70% of the Na influx of the cells is abolished by the immediate application of 10^{-5} M verapamil. This represents $22 \mu\text{M}/10^{11}$ cells/s or, from the volume and area measurements of these cells (derived from Jongsma and Van Rijn, 1972), approximately $10 \text{ pmol}/\text{cm}^2 \cdot \text{s}^{-1}$ of verapamil-sensitive Na influx. If this were all associated with the electrical activity of the cells, beating at a rate of 120/min and having an action potential duration of 120 ms (Hyde et al., 1972), then the Na influx per beat, above base line, would be of the order of $41 \text{ pmol}/\text{cm}^2 \cdot \text{s}^{-1}$, similar to that found by Conn and Wood (1959) in whole dog heart.

The enhanced automaticity of cultured heart cells appears to depend primarily on a low membrane K conductance (Sperelakis and Lehmkuhl, 1966). The data presented here indicate a dependence also on $[\text{Ca}]_0$ and $[\text{Na}]_0$, especially the

latter. Decreasing $[Ca]_0$ below 0.3 mM has a pronounced effect on contraction frequencies of the cells. It was not possible to obtain an absolutely Ca-free salt solution containing serum, because of the Ca contribution from the serum, but in a Ca- and serum-free solution the cells ceased contracting spontaneously. As $[Ca]_0$ was increased from 0.3 mM towards 3.0 mM no further increase in contraction frequency occurred, a finding similar to that of Sperelakis and Lehmkuhl (1966).

Changing $[Na]_0$ had an immediate effect on contraction rate, suggesting a primary effect of Na ions, the time being insufficient to produce secondary effects on K or Ca exchange via an influence on the cellular content of those ions. Two possibilities therefore exist. First, decreasing $[Na]_0$ may, by influencing the Na equilibrium potential or membrane resting potential, produce changes in GK to account for the rate changes seen, or alternatively, render the cells less excitable. Second, $[Na]_0$ may primarily control an inward Na current which, coupled to a low GK , may control the automaticity of the preparation. At this time it is not possible, by the methods employed, to distinguish between these two possible mechanisms. It is of interest, however, that the relationship between verapamil-sensitive Na influx and contraction frequency is the same for both the verapamil effect and the $[Na]_0$ effect. This constant relationship, which appears to reach a plateau at the higher values of Na influx, would suggest a common mechanism of action of both verapamil and $[Na]_0$. This mechanism could well be represented by an effect on a verapamil-sensitive inward Na flux, thereby influencing the spontaneous automaticity of the preparation.

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