Effect of Quinidine and Temperature on Sodium Uptake and Contraction Frequency of Cultured Rat Myocardial Cells

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SUMMARY The effects of quinidine and temperature on Na influx and contraction frequency of synchronously contracting rat myocardial cells in monolayer cultures were studied. Quinidine (10^{-6} M to 10^{-2} M) produced a prompt reduction in Na influx, maximum after 30 seconds of exposure, and dose-dependent along a sigmoid log dose-response curve. At 37°C, Na influx (µmol/10^10 cells per sec) decreased from 30.19 to 24.70 (P < 0.001) and 10.49 (P < 0.001) on exposure to quinidine, 10^{-4} and 10^{-2} M, respectively. Simultaneously the contraction frequency decreased from a control of 120/min to 105/min and 48/min with 10^{-4} M and 5 x 10^{-4} M quinidine. At higher concentrations spontaneous contractions ceased. The effects on Na influx and contraction were reversible by washing the cells free of the drug (30 seconds). A temperature-dependent decrease in the Na influx between 37°C and 22°C also induced a decrease in contraction frequency. Between 25°C and 35°C the Q_{10} values for Na influx and contraction frequency were 2.41 and 2.44, respectively. Under all conditions tested there was a constant linear relationship (r = 0.98) between Na influx and contraction frequency for all values of Na influx greater than 11.82 µmol/10^{11} cells per sec. Na influx and contraction frequency were insensitive to tetrodotoxin (10^{-5} g/ml) but very sensitive to verapamil and to changes in extracellular Na. Quinidine affected only the verapamil-sensitive Na influx. The results indicate a close relationship between verapamil-sensitive inward Na movement and automaticity in these cells and demonstrate that the quinidine-induced changes in automaticity are closely linked to the effect on Na influx.

ELECTROPHYSIOLOGICAL studies have shown that quinidine decreases automaticity of cardiac tissue, primarily decreasing the rate of depolarization during phase 4 of the cardiac action potential. Several studies also have shown a marked effect of quinidine on myocardial sodium exchange. In rabbit atria quinidine reduced the passive movement of sodium into the cell, a finding similar to that reported for cat papillary muscle. McCall used cultured human cells and demonstrated a dose-dependent effect of quinidine on passive sodium influx and membrane permeability to that ion. Studies such as these have prompted the conclusion that the action of quinidine and quinidine-like agents is related, in some way, to an alteration in membrane permeability to sodium and potassium, but direct evidence, especially with regard to sodium, still is lacking.

The present study was performed to explore the relationship of the effects of quinidine and temperature on Na uptake to their effect on automaticity in cultured rat myocardial cells. These cells, grown until a confluent monolayer is formed, show spontaneous, synchronous contractions in vitro that are representative of intrinsic automaticity of the tissue. Many of the electrophysiological features of these cells indicate that their action potentials are slow channel-dependent* and that they are similar to cardiac pacemaker tissue in various situations. As has been pointed out, since these cells also possess the advantages provided by other cultured cells for ion flux studies in that, even when grown as a monolayer, cultured cells may be considered as single cells. Radioisotope tracers can be quickly and completely removed from the extracellular fluid, making possible the detection of small changes in
Intracellular Na and K concentrations. This in turn allows accurate measurement of absolute membrane fluxes of these ions without the presence of a fixed extracellular compartment to complicate diffusion into, or out from, the cell.

Methods

Myocardial cell cultures from the hearts of 1- to 2-day-old rats were prepared by the method of Harary and Farley,11 with modifications12 designed to improve the percentage of myocardial cells present. The cells then were grown for 4 days, by which time a satisfactory monolayer of some 1 x 10^6 cells per 6-cm Petri dish had formed. Prior to carrying out the study, randomly selected plates from each batch were examined under phase contrast microscopy, to determine (1) the percentage of cells showing visible contractile shortening (as a measure of the percentage myocardial cells present) and (2) whether the contractions were synchronous. Only those cultures in which at least 80% of the cells showed synchronous contractile shortening were used for the study.

Because the pH of growth medium depends on a controlled atmosphere of 5% CO_2 in air, its stability could not be maintained during the flux experiments. For this reason all experiments were performed with the cells in Krebs' solution containing (mM) Na^+, 137.09; K^+, 5.65; Ca^{2+}, 2.5; Mg^{2+}, 1.17; Cl^-, 147.16; PO_{4}^{2-}, 0.58; SO_{4}^{2-}, 1.17; glucose, 10.98; plus phenol red, 0.0002%; and calf serum, 5%. The buffer used in this solution was tris(hydroxymethyl)aminomethane (Tris) (16 mM)-citric acid (5 mM). Regular spontaneous contractions of the cells could be maintained in this Krebs' solution for up to 24 hours. After transfer of the cells from Eagle's Minimal Essential Medium to Krebs' solution an equilibration period of 3 hours was allowed before measurements were made.

Preliminary experiments were conducted to determine the rate of Na exchange in these cells, by measuring the rate of 24 Na efflux from cells previously loaded with the isotope. This measurement indicated (Fig. 1) that 24 Na loss from the cells, at 37°C, could be described by a single exponential with a half-time of 1.5 minutes. Since the cells were in steady state with respect to Na exchange, the rate constant for Na influx must equal that for the Na efflux.

The methods used to measure Na influx have been described in detail elsewhere.16, 17 Briefly, Na influx was measured by exposing the cells to 24 Na, in Krebs' solution, for periods of time, short compared to the half-time of Na exchange, and calculated from the equation:

\[ \text{Na}_{\text{in}} = \frac{\text{d}[	ext{Na}]/\text{dt}}{10^6/n} \]

where \( \text{Na}_{\text{in}} \) is the influx in \( \mu \text{mol}/10^6 \text{ cells per sec} \), \( \text{d}[	ext{Na}] \) is the amount of the ion entering the cell (calculated from intracellular radioactivity and the specific activity of the Na-soak solution) in time, dt, and n is the cell number (as a fraction of \( 10^6 \) cells, per plate). Since the time, dt, is short compared to the total exchange time for Na it is assumed that there is negligible efflux of tracer in that time. All Na influx measurements were made over a maximum time of \( 24 \) Na exposure of 30 seconds. The plates were gently agitated on a controlled temperature warming plate at 37°C throughout the 30-second exposure to \( 24 \) Na, to ensure adequate mixing of the solution and stability of temperature. Blank plates, containing no cells, were included with each experiment to determine background activity on the plates.

When the effect of the immediate application of quinidine on the Na influx was measured, the drug was included in the radioactive influx solution. In situations in which the cells were exposed to the drug for longer than 30 seconds, with subsequent influx determination, they were exposed to the drug in a nonradioactive solution which was replaced by one containing the same concentration of the drug, plus \( 24 \) Na for the influx determination over a further 30-second period. When the effect of temperature on the Na influx was measured the cells were first washed for 30 seconds with nonradioactive Krebs' solution that had been warmed to the desired temperature in a water bath; this solution then was replaced by \( 24 \) Na-Krebs at the same temperature for 30 seconds.

Cell counts, expressed as a fraction of \( 10^6 \) cells per plate, were obtained for each experiment from randomly selected plates that had been treated in every way like the experimental plates. After washing to remove extracellular radioactivity, however, the monolayers were trypsinized to provide a cell suspension that could be counted in a hemocytometer.

Contraction frequency was measured by visual examination under phase contrast microscopy, counting the number of contractions in a given time, and the results were expressed as contractions per minute. Throughout the period of measurement of contraction frequency the cells were supported on a heated microscope stage adjusted so that the solution in the Petri dish (monitored continuously via a thermistor probe) was maintained at the desired temperature.

\( 24 \) Na was obtained from New England Nuclear Corporation. All isotope counting was carried out with a Packard Tri-Carb liquid scintillation spectrometer. \( 24 \) Na was counted without added scintillant.18 All experiments were carried out

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Figure 1: 24 Na efflux from cultured myocardial cells. Points represent previously accumulated cellular 24 Na (log scale) before and at various times during the efflux period. Nonradioactive solution was continually replaced at a rate of 60 ml/min. Increasing this to 100 ml/min did not increase rate of 24 Na loss, indicating that washing at 60 ml/min was not rate-limiting for 24 Na efflux. 24 Na loss is described by a single exponential with rate constant of 0.008 sec^{-1}. Each point is mean ± SEM (n = 5).
with quinidine sulfate and the concentrations shown refer to the molar concentrations of the sulfate salt.

Results

EFFECT OF QUINIDINE ON SODIUM INFLUX

At 37°C, each of the concentrations of quinidine sulfate produced a prompt inhibition of the Na influx. This was dose-dependent along a sigmoid log dose-response curve (Fig. 2). Compared to the control value of 30.19 ± 0.92 (SE of mean) μmol/10^11 cells per sec, the Na influx was significantly (P < 0.001) lowered by each concentration of quinidine tested. The effect of quinidine on the Na influx (Fig. 2) represents the effects of the drug on total sodium influx, which, under the experimental conditions used, shows no evidence of an active component.

The time course of the action of quinidine (10^-4 M) on the Na influx is illustrated in Figure 3. In the presence of quinidine the Na influx rapidly declined to new steady levels within 30 seconds after exposure to the drug. Although no spontaneous recovery of the Na influx was seen in the presence of quinidine, the effect was easily reversible by simply washing the cells (six times at 37°C) with a quinidine-free solution (Fig. 3).

These results indicate that quinidine very rapidly effects cellular Na uptake, presumably because of a high affinity for binding to Na channels, but that this effect, and therefore also the binding of the drug to the cell membrane, are easily reversed. These results are similar to the apparent kinetics of quinidine binding and release demonstrated for another strain of cultured cells and, as such, appear to represent a drug-specific rather than a cell-specific phenomenon.

EFFECT OF QUINIDINE ON CONTRACTION FREQUENCY

During the course of these experiments there was a considerable decrease in the spontaneous contraction frequency of the cells. As in the case of the Na influx, this conformed to a progressive dose-dependent effect of the drug (Fig. 4). At 37°C, in the absence of quinidine, the frequency of contraction was 120.33 ± 1.74 (SE of mean) per minute. Increasing concentrations of quinidine significantly (P < 0.001) decreased the spontaneous activity (Fig. 4) until, at concentrations in excess of 5 x 10^-4 M, all spontaneous contractions were abolished.

In the presence of quinidine there appears to be close conformity between the Na influx and the contraction frequency (Fig. 3). The results also suggest that the effect of quinidine on Na influx is closely related to its effect on the frequency of contraction of these cells. This relationship was examined further in a series of paired observations on Na influx and contraction frequency in the presence of each of the concentrations of quinidine, up to and including 5 x 10^-4 M. These observations indicated a close linear correlation between Na influx and contraction frequency in the presence of quinidine. The relationship is described by the equation y = 3.99x + 1.35 (r = 0.98, P < 0.001), where y = contractions per minute and x = Na influx, for all values of Na influx in excess of 10.5 μmol/10^11 cells per sec. Although values of Na influx lower than this were obtained in the presence of quinidine, the concentrations of the drug required to produce these lower values resulted in total abolition of spontaneous contraction of the cells, and were therefore not applicable to the above correlation.

EFFECT OF TEMPERATURE ON Na INFLUX

The contraction frequency was extremely temperature-sensitive, a finding noted by several other observers. It was believed therefore, that the relationship between Na influx and contraction frequency could, in addition, be explored by measuring both the Na influx and frequency of spontaneous contraction over a range of temperatures from 37°C to 22°C.

A progressive decrease in temperature resulted in a progressive decline in Na influx (Fig. 5). Na influx (μmol/10^11 cells per sec) followed a sigmoid curve, decreasing from a control value of 30.51 ± 0.93 (SE of mean) at 37°C to 10.51 ± 0.41 at 22°C. Although a progressive
reduction in temperature below 22°C was not carried out, from the curve (Fig. 5) it appears that the Na influx is reaching or approaching a minimum value at that temperature, because the curve is beginning to level off between 25°C and 22°C.

Although in the present experiments (Fig. 5) the relationship between the Na influx and temperature was not linear, it was possible to estimate the Q10 for the Na influx between 25°C and 35°C. A value of 2.41 was obtained.

EFFECT OF TEMPERATURE ON CONTRACTION FREQUENCY

A progressive decrease in temperature from 37°C to 22°C led to a concomitant decrease in the contraction frequency of the cells within the monolayers (Fig. 6). There was a gradual decline in contraction frequency from 37°C to 31°C, with a more rapid decline below this point, until a temperature of 25°C (Fig. 6) was obtained. Below this temperature, regular synchronous contractions of the cell ceased, although occasional, slow, irregular contractions of individual cells still could be seen. The Q10 for rate of spontaneous contraction by the cells between 25°C and 35°C was 2.44, a value very similar to that previously reported for both cultured rat cells and chick heart cells.

It was apparent, therefore, that over the range tested a progressive decrease in temperature led to a simultaneous decline in both the Na influx and spontaneous contraction frequency of the myocardial cells. The estimated Q10 values for the two parameters, moreover, were also very close, being 2.41 and 2.44, respectively. With temperature changes or with quinidine there was a close linear relationship (P < 0.001) between Na influx and contraction frequency. It was also of interest to note that the regression equation calculated in this case (y = 4.05x + 1.64; r = 0.98) was almost identical to that previously calculated for the quinidine effect.

EFFECT OF QUINIDINE AND TEMPERATURE REDUCTION ON Na INFLUX AND CONTRACTION FREQUENCY

Quinidine and temperature reduction produced a prompt effect on Na influx and, simultaneously, on the intrinsic rate of contraction. The possibility that the two effects could be additive was investigated by repeating the initial experiments with quinidine on Na influx and contraction frequency, this time at 30°C (Figs. 2 and 4). In the presence of each of the concentrations of quinidine, from 10^-8 M to 10^-3 M, a decrease in temperature from 37°C to 30°C significantly (P < 0.001) increased the effects of each concentration of the drug (Figs. 2 and 4) on both Na influx and contraction frequency.

The overall relationship between Na influx and contraction frequency in the present studies was obtained from the mean values for Na influx, together with the corresponding mean values for contraction frequency, over the range of experimental conditions under which spontaneous contractions were observed. It was found (Fig. 7) that there was a
close linear correlation between Na influx and contraction frequency (P < 0.001) in cultured myocardial cells.

Another test of the relationship between Na influx and contraction frequency was carried out by determining Na influx and spontaneous contraction frequency of cells in a Krebs' solution containing 154 mM Na (normal Krebs') and one containing 120 mM Na. Reducing extracellular Na concentration from 154 to 120 mM by replacement with choline chloride produced a prompt reduction in the Na influx (µmol/10^11 cells per sec) from 31.35 ± 0.65 (SE of mean) to 24.52 ± 0.43 (P < 0.001) and simultaneously reduced the spontaneous contraction frequency from 120/min to 103/min. Since one of the initial effects of a reduction in extracellular Na must be to reduce Na influx, this observation suggests that, in these cells, Na influx, or at least a portion thereof, is important in the maintenance of intrinsic contraction rates.

Verapamil specifically blocks slow responses, reportedly because of blockade of an inward Ca^{2+} current. More recently, however, it has been shown that in addition to blocking the Ca^{2+} current the drug also blocks the slow Na^{+} currents in embryonic chick myocardial cells. Although the cells used for the present experiments were insensitive to tetrodotoxin (demonstrating continued contractile activity in the presence of concentrations of up to 10^{-6} g/ml) they were extremely sensitive to verapamil even in low concentrations. In these cells verapamil produced a dose-dependent decrease in contraction frequency and Na influx, both of which were maximal within 30 seconds after addition of the drug. All contractile shortening disappeared in concentrations of verapamil in excess of 2 x 10^{-7} M, and a maximum inhibition of the Na influx was achieved with verapamil in concentrations between 10^{-8} and 10^{-6} M (a more complete discussion of these results will be presented separately). On addition of verapamil (10^{-4} M) all visible contractile responses ceased and the Na influx (µmol/10^11 cells per sec) decreased from a control value of 30.49 ± 0.52 (SE of mean) to 8.56 ± 0.47 (P < 0.001) within 30 seconds. In cells pretreated with verapamil, quinidine did not, in concentrations up to and including 10^{-5} M, produce any further decrease in the Na influx, indicating that in this preparation quinidine affected only that portion of the Na influx which also is verapamil-sensitive.

The present studies did not permit a distinction between the action of verapamil on the electrical activity as opposed to an electromechanical uncoupling. It therefore can be stated only that the verapamil-sensitive Na influx represents Na uptake associated with the contractile behavior of the cells. This may reflect either changes in the underlying electrical activity or changes in the mechanical response, or both. The studies, however, do indicate that the quinidine-induced changes in Na influx reported here are indicative only of changes in the contraction-related Na uptake.

**Discussion**

Previous authors have emphasized the value of using monolayer cultured cells as a preparation in which to study membrane ion exchange. The advantages of this type of preparation are equally applicable to cultures of spontaneously contracting myocardial cells. The preparation used for the present experiments consisted of cultures of a high degree of purity. The monolayers contained at least 80% myoblasts or myocardial cells, and the ion flux determinations, reflecting largely the fluxes in the majority, would refer primarily to the myoblast/myocardial cells. All experiments were performed when the cultures were between 4 and 5 days old, at which time the cells were relatively insensitive to tetrodotoxin; a concentration considerably in excess of 10^{-6} g/ml was necessary before any demonstrable effect on contraction frequency could be observed. This finding is similar to that reported for cultured myocardial cells by other workers and indicates that at this early stage in culture these cells are probably not fast Na channel-dependent.

The half-time of the Na exchange in the cells in the present experiments, carried out at 37°C, is of the order of 1.5 minutes. If one assumes an intracellular Na concentration of 10–12 mM and a volume to surface area ratio of approximately 1.5 x 10^{-4} cm (calculated from the data of Jongsma and van Rijn) for rat myocardial cells in culture, this rate of Na extrusion would approximate an absolute Na efflux of 14 pmol/cm² per sec. Since, under control conditions, the cells are in a steady state with respect to intracellular Na, this Na efflux must be equal in magnitude to the Na influx. From the present data for control cells at 37°C, beating at a rate of 120/min, some 70% of the total Na influx (9.8 pmol/cm² per sec) is verapamil-sensitive and associated with the contractile activity of the tissue. If all of this "extra" Na influx occurs in association with the action potential (duration, approximately 120 msec), then the influx per beat above baseline is of the order of 41 pmol/cm². It is of interest that this value, although only approximately 60% of that reported by Langer for excitation-associated Na influx, is almost identical to that measured by Conn and Wood in whole isolated dog heart at rates of 110/min at 38°C. This similarity between the calculated
excitation-associated Na flux in the present study, and that observed by Conn and Wood, suggests some degree of similarity between cultured myocardial cells and other mammalian cardiac tissue. The present study demonstrates a very prompt and significant effect on quinidine on both the Na influx and contraction frequency of the cultured cells. The time courses of these effects (Fig. 3) are interesting from the point of view of both the rapidity of their onset and ease of reversibility. The inhibition of Na uptake in the present studies is seen much earlier than that in adult mammalian myocardium, for example, cat papillary muscle. Several factors may account for this observation. First, since there is no fixed extracellular compartment the drug will have immediate access to all of the cells, without the delay usually involved in diffusion throughout the extracellular compartment. Second, these cells are an immature population from neonatal myocardium. The rapid effect of quinidine may therefore be a function of the cells themselves, reflecting increased vulnerability of immature membrane systems to quinidine. The time course is, however, very similar to the time course of the action of quinidine on the Na influx of another strain of functionally very different cultured cells; this would suggest that perhaps the rapid access of the drug to the cells is the more important factor. The ease of reversibility of the quinidine effects indicates very loose binding of the drug to the cell membrane, allowing it to be washed out quickly in the absence of a significant extracellular compartment.

Both the contraction frequency and Na influx of these cells are very sensitive to temperature. The results of our studies of the sensitivity of contraction frequency to temperature are very similar to those of other workers, both in terms of the configuration of the temperature-response curve and the overall Q10 for contraction frequency between 25°C and 35°C. In the present study, however, it was possible to equate these changes with changes in the Na influx, the relationship between the two adhering closely to a linear regression. 

This study demonstrates a very close parallel between contraction frequency and Na influx in cultured heart cells, both in the presence of quinidine and in the presence of a reduced temperature. Moreover, the relationship between contraction frequency and Na influx is the same for both conditions. The studies with verapamil indicate that approximately 70% of the Na influx of the preparation is linked to contractile activity, and that only this portion of the Na influx is modified by quinidine. Despite the close correlation between the effects of quinidine on both Na influx and contraction frequency, it was not possible to define accurately a cause and effect relationship. The fact that decreasing extracellular Na abruptly decreases the contraction frequency of these cells at least suggests an influence of an inward Na flux on automaticity. Therefore, although not conclusive, these observations raise the possibility that quinidine may affect automaticity via a direct effect on cellular Na influx.

References

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