Influence of Lithium Ions on the Transmembrane Potential and Cation Content of Cardiac Cells

E. E. CARMELIET

From the Department of Physiology, University of Leuven, Leuven, Belgium

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

ABSTRACT The effect of lithium ions on cardiac cells was investigated by recording the changes in transmembrane potential and by following the movement of Li, Na, and K across the cell membrane. Isolated preparations of calf Purkinje fibers and cat ventricular muscles were used. Potentials were measured by intracellular microelectrodes; ion transport was estimated by flame photometric analysis and by using the radioactive isotopes of Na and K. It was shown (*a*) that Li ions can replace Na ions in the mechanism generating the cardiac action potential but that they also cause a marked depolarization and pronounced changes in action potential configuration; (*b*) that the resting permeability to Li ions is high and that these ions accumulate in the cell interior as if they were not actively pumped outwards. In Li-Tyrode $[K]_i$ decreases markedly while the K permeability seems to be increased. In a kinetic study of net K and Na fluxes, the outward movement of each ion was found to be proportional to the second power of its intracellular concentration. The effect on the transmembrane potential is explained in terms of changes in ion movement and intracellular ion concentration.

INTRODUCTION

In order to learn more about the fundamental problem of permeability in excitable membranes, it is of interest to investigate in some detail the similarity of action of different ions on the membrane potential. Attention has been focused on Rb and Cs as K substitutes, and on Li as a Na substitute. It has been shown for a variety of tissues that Li ions permeate the cell membrane barriers and that they can replace Na as the depolarizing agent during the upstroke of an action potential (for references, see Schou, 1957). The permeability coefficient for Li entry is about the same as for Na in frog sartorius (Keynes and Swan, 1959), in unmyelinated nerve fibers (Armett and Ritchie, 1963), and in red cells (Maizels, 1954). An efficient active outward movement, however, seems to be lacking in these cells.

Earlier work on cardiac tissue was merely concerned with the influence of Li ions on the spontaneous activity of the heart (Dulière, 1927) and the electrocardiographic disturbances after oral or parenteral lithium uptake (Radomski *et al.*, 1950; Butcher *et al.*, 1953; McKusick, 1954). The only work with intracellular microelectrodes was done on the frog ventricle (Stein *et al.*, 1955).

All these results indicate that Li inhibited spontaneous activity and caused changes in the electrical activity of heart muscle. Li ions seem thus to have quite different effects than Na ions and one might ask therefore if Li ions could carry enough inward current to generate an action potential in the case of heart tissue.

It was felt worth while therefore to restudy in some detail the similarities and differences between Na and Li with the aid of intracellular recording and the direct measurement of cation movement. It will be shown: (*a*) that Li ions can replace Na ions as a depolarizing agent during the upstroke of an action potential but that they also cause a marked depolarization and pronounced changes in action potential configuration, (*b*) that the resting permeability to Li ions is high and that these ions accumulate in the cell interior while $[K]_i$ decreases; they also seem to increase the permeability to K ions. In a kinetic study of K and Na fluxes, the outward movement of each ion was found to be proportional to the second power of its intracellular concentration. An explanation of the potential changes will be presented in terms of changes in ion movement and intracellular ion concentration.

Preliminary accounts of this work have appeared (Carmeliet, 1962 *a* and *b*).

METHODS

Preparation The experiments were performed on calf Purkinje fibers and cat papillary or trabecular muscles.

Calf hearts were obtained at the local slaughterhouse. The heart was removed within 10 min. after the animal had been shot; it was opened and rinsed with cooled Tyrode solution. Purkinje preparations were taken out of the left ventricle and were carried to the laboratory in Tyrode solution at 4°C. During a period of at least 1 hr. the preparations were allowed to heal over and to recover from injury in Tyrode at 37°C, aerated with 95 per cent O_2, 5 per cent CO_2.

Cats, weighing about 2.5 kg, were anesthetized with ether; papillary or trabecular muscles less than 0.5 mm in diameter were dissected out of the right ventricle and immediately put into Tyrode solution at 37°C.

Solutions The Tyrode solution had the following composition (mM): Na 149.8, K 5.4, Ca 1.8, Mg 0.5, Cl 147.7, HCO_3 11.9, H_2PO_4 0.32, glucose 1 gm per liter. In order to obtain inexcitability NaCl was replaced by choline chloride (Hoffman-La Roche) on a mole for mole basis; atropine sulfate was added (10 mg per liter) to avoid the choline effects. Li-Tyrode was prepared by replacing NaCl and $NaHCO_3$ by equivalent amounts of LiCl and $LiHCO_3$. Lithium bicarbonate was prepared in solution by neutralizing Li_2CO_3 by the proper amount of HCl under continuous aera-

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

tion with 95 per cent O_2 and 5 per cent CO_2. The pH of the final Tyrode was found to be from 7.2 to 7.5. The Li salts were analytical grade reagents and were obtained from three different sources: Union Chimique Belge, Merck & Company, Inc., and Fisher Scientific Co.

Electrical Recording Transmembrane potentials were recorded through glass microelectrodes filled with 3 M KCl solution (resistance between 10 and 20 MΩ). Potential differences were measured between an intracellular and an extracellular microelectrode, using a cathode follower and a differential preamplifier (see Carmeliet, 1961). The preparation could be stimulated by square wave pulses through a pair of AgCl electrodes.

Chemical Analysis Only cat ventricular preparations were used for chemical analysis of Na, K, and Li. After being isolated from the right ventricle, they were put in a vial containing 30 ml of the bathing solution at 37°C; a mixture of 95 per cent O_2, 5 per cent CO_2 was bubbled through the solution and allowed, besides aeration, for a good exchange with the extracellular space. The preparations were not stimulated and did not contract spontaneously.

At the end of the experimental period, the muscles were taken from the bath and gently blotted between two sheets of ashless filter paper (Whatman, No. 42). Put into platinum crucibles their wet weight was evaluated using a Mettler balance. Overnight the preparations were dried to constant weight in an oven at 95°C. After measuring the dry weight, they were ashed in an oven at 500°C during 24 hrs. The ashes were dissolved in deionized doubly distilled water and analyzed in duplicate for K, Na, and Li by flame photometry (Beckman, model B). The wet weight of the individual muscles ranged from 1 to 5 mg. Sometimes two to three muscles were pooled in order to stay sufficiently above the limits of detection of the method used.

Intracellular concentrations were calculated as follows, using K as an example

$$[K]_i = (K_t - V_e \cdot [K]_o)/(V - V_e)$$

where $[K]_i$ = the intracellular K concentration (mmoles per liter fiber water)
$[K]_o$ = the K concentration of the medium
K_t = total K content of the muscle in mmoles per kg wet tissue
V_e = the extracellular space, assumed to be 0.3 liter per kg wet tissue
V = the volume of the total muscle water in liters.

Measurements of K^{42} and Na^{24} Fluxes Radioactive K and Na were obtained from the Centre d'Etude de l'Energie Nucléaire, Mol, Belgium, in the form of carbonate or bicarbonate. The corresponding chloride salts were formed by adding the proper amount of 0.1 N HCl. After checking complete neutralization, water and other constituents of Tyrode solution were added. The activity of the final solution was not higher than 10 μc/ml.

The preparation was mounted on a stainless steel bar by means of two silk threads which were fixed to the connective sheath of the Purkinje bundle or to the tendon of the papillary muscle. It was placed at the bottom of a test tube filled with 5 ml of bathing solution and set in the thermostated bath at 37°C. Through a fine polyethyl-

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

ene tube a mixture of 95 per cent O_2, 5 per cent CO_2 was bubbled through the solution.

To measure K* influx the preparation was dipped for a timed period in a test tube filled with 5 ml active solution. At the end of the influx period it was rinsed for 5 sec. in a large volume of inactive Tyrode; the preparation was quickly removed, cut between the silk sutures, and gently blotted between two sheets of filter paper. The total activity was estimated by a well-type scintillation counter.

To measure K* or Na* efflux, the preparation, loaded with radioactive ions, was passed through a series of test tubes in which it remained for a predetermined period. The radioactive ions that had left the preparation during that time could thus be measured by counting the activity of the test tubes. In such a way the rate of efflux could be measured. At the end of the experiment, or at any time during the experiment, the total activity in the preparation could be determined by placing the test tube, containing the preparation, in the well-type counter. By adding the amounts of K* or Na* which left the preparation during the individual periods to the quantity of activity present at the end of the experiment, it was possible to know the activity in the preparation at any time from the beginning of the efflux period.

RESULTS

Part I. Influence of Li on the Transmembrane Potential

SPONTANEOUS ACTIVITY

According to earlier work (Dulière, 1927; Stein *et al.*, 1955) partial or complete substitution of Na by Li in the perfusion solution will decrease the frequency of the spontaneous heart beat and eventually cause complete arrest in diastole. In order to find the mechanism of arrest of spontaneous activity the effect of Li ions was studied on a calf Purkinje preparation. At an external potassium concentration of 2.7 mM, Purkinje fibers are spontaneously active. In the experiment illustrated in Fig. 1 a microelectrode was inserted near to a pacemaker site and the electrical recording shows the characteristic slow diastolic depolarization. Within the first minute after all Na ions were replaced by Li ions, the rate of diastolic depolarization slowed while the maximum resting potential remained constant. In other experiments there was sometimes a transient small hyperpolarization of about 3 mv. Due to a shift of the pacemaker site, which normally occurred under these conditions, we were unable to determine an eventual change in threshold potential. After perfusion for 3 min. with Li-Tyrode, the diastolic depolarization rate was so low that only an abortive action potential was generated that smoothed out into a stable potential of about −85 mv. Electrical stimulation still was possible. Afterwards a depolarization started whose time course will be described in a subsequent section. The major factor causing arrest of spontaneous activity is thus the decrease in rate of slow diastolic depolarization.

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

PERSISTENCE OF ELECTRICAL ACTIVITY IN Li-TYRODE

Although it was worth while to learn about the mechanism of heart arrest in Li-Tyrode, the main aim of the present work was to see whether Li ions could replace Na ions in the generative mechanism of the action potential. In order to study the persistence of electrical activity in Li-Tyrode, Purkinje and ventricular muscle preparations were first rendered inexcitable by bathing them in a sodium-free solution, choline ions replacing all Na ions. Under these experimental conditions all electrical activity disappeared after about 30 min.

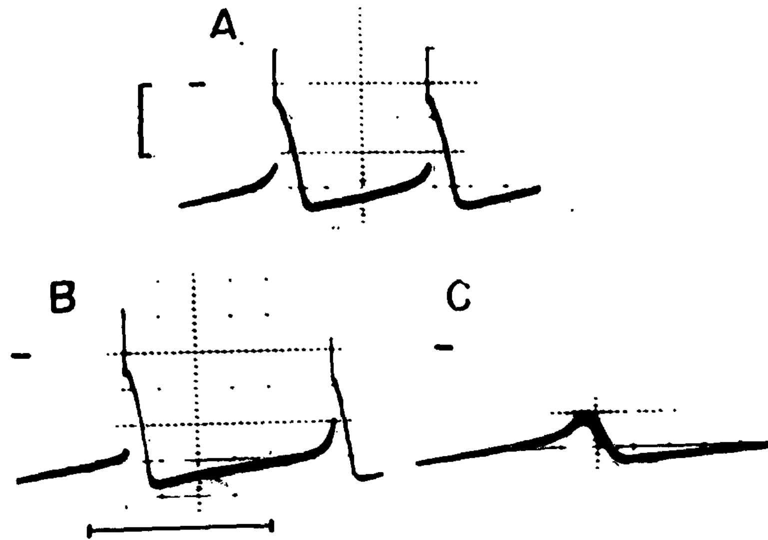

FIGURE 1. Influence of Li-Tyrode on spontaneous activity in a calf Purkinje fiber. A, Na-Tyrode; B and C, after 2 and 3 min. respectively in Li-Tyrode. Note slowing of diastolic depolarization rate and shift in pacemaker site. The external K concentration was 2.7 mM. Voltage calibration, 50 mv. Time calibration, 1 sec. Zero potential level indicated by small bar.

To make sure that no sodium ions were left in the extracellular space the perfusion with choline-Tyrode was prolonged over a period of 1 hr. Subsequently perfusion with Li-Tyrode was started. Within 1 to 2 min. electrical activity reappeared upon stimulation. The first action potentials were of low amplitude and short duration but grew larger and longer as the perfusion with Li-Tyrode continued.

These results indicate clearly that Li ions can move inwards during a depolarization at a rate comparable to that of Na ions. As noted before Li ions caused secondary effects that made their behavior different from that of Na ions.

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

RESTING AND ACTION POTENTIAL IN Li-TYRODE

Cat Ventricular Muscle

Depolarization Very thin ventricular muscles, having a diameter of less than 0.5 mm and a length of 5 mm, were dissected from the cat right ventricle. After a period of more than 1 hr. in Na-Tyrode, the membrane resting and action potentials were recorded from different areas of the preparation to make sure that the whole preparation had recovered from the dissection.

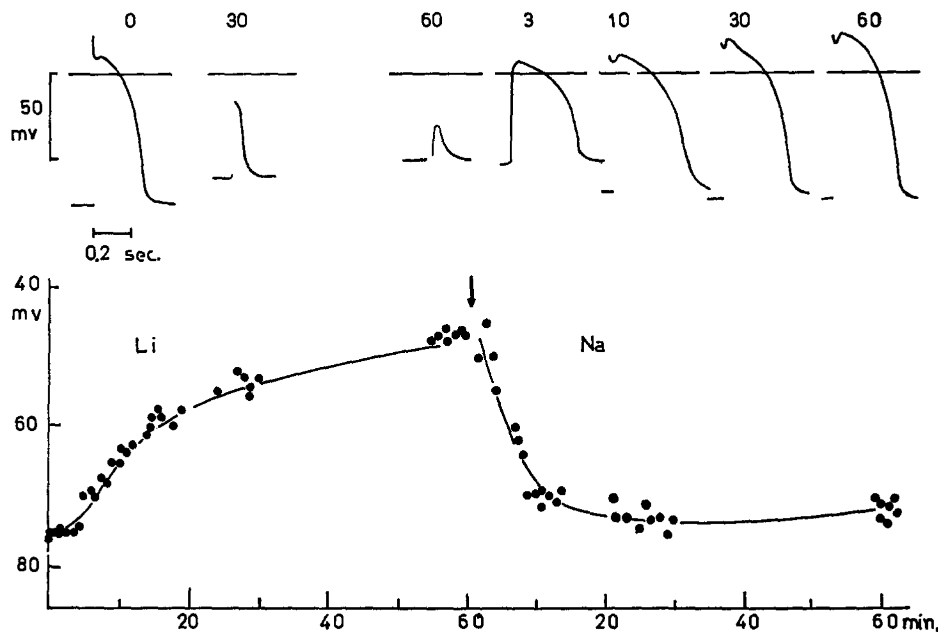

FIGURE 2. Change in resting and action potential of a cat papillary muscle during soaking-in period in Li-Tyrode and during recovery in Na-Tyrode. The numbers above the action potentials indicate the time in minutes at which the potentials were recorded.

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

When the Li-Tyrode perfusion was started, the time course of the decrease in resting potential was followed by inserting the microelectrode in different spots over the preparation. The punctures were limited to two or three superficial cell layers and could be performed at a rate of approximately one per minute.

In Fig. 2 the changes in membrane resting potential of a typical experiment are shown. Three different phases may be distinguished. (*a*) During the first 3 min. there was practically no change in resting potential, although there was a shortening of the action potential at that time. As a rule the preparation was stimulated only at 5 min. intervals by a few test stimuli. (*b*) During the

subsequent 15 min. the membrane became depolarized to −60 mv in a more or less exponential way. (*c*) Thereafter the rate of depolarization slowed and the decline in membrane potential was roughly linear as a function of time.

In five such experiments the mean resting potential fell from −74.9 mv ± 0.35 (20) in normal Tyrode to −60.5 mv ± 1.30 (21) after 20 min. in Li-Tyrode, to −55.3 mv ± 1.03 (19) after 30 min., and to −48.64 mv ± 0.71 (22) after 60 min. perfusion. The mean values of the resting potential are given, followed by the standard error and number of actual measurement in parentheses.

When the external K concentration was 2.7 mM instead of 5.4 mM, the control value of the membrane potential in the Na-Tyrode was higher, about −84 mv; during the first few minutes in Li-Tyrode there often was a hyperpolarization of a few millivolts. The rate of depolarization was greater during the second phase but no statistically significant difference was found in the final value after 1 hr. in Li-Tyrode.

Action potential Before any change in resting potential occurred in Li-Tyrode, the repolarization rate during the action potential rose, resulting in a shorter plateau and a more pronounced spike. As the fiber became depolarized, the shortening was more prominent and at the same time the amplitude of the upstroke decreased. Between 30 and 60 min. in Li-Tyrode, excitability decreased markedly. Action potentials still were propagated, but the amplitude and the rate of rise of the upstroke became very small. After more than 1 hr., propagation often failed in different fibers (Fig. 2).

Part of the decrease in amplitude is due to the depolarization (Weidmann, 1955). When the membrane was hyperpolarized by passing electric current through the membrane, the amplitude and rate of rise of the upstroke could be increased, but never to the initial value in the Na-Tyrode. Decreasing the extracellular K and thereby increasing the resting potential had a similar effect. A second factor responsible for the fall in action potential amplitude can be found in the increased rate of repolarization. By lowering the temperature or substituting sulfate for chloride, the action potential became longer and of greater amplitude. Other possible interfering factors will be discussed later.

Purkinje Fibers

Depolarization The influence of Li substitution on the resting membrane potential of Purkinje fibers was essentially the same as in the case of cat ventricular muscle. Quantitatively the absolute values of the membrane potential were different. In normal Tyrode, with 5.4 mM K, the resting potential was −84.6 mv ± 0.56 (6). After 30 min. in Li-Tyrode the potential became −62.6 mv ± 1.96 (6) and −59.2 mv ± 1.09 (6) after 1 hr. Compared to

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

cat ventricular muscle the depolarization was slower after the initial fast depolarization.

Action potential In a general way the modifications of the Purkinje action potential were less pronounced and took a longer time to develop than in cat ventricular muscle. Excitability was maintained for more than 90 min. when the precaution was taken to stimulate the preparation at a frequency lower than 30 per min.; the action potentials were of short duration but showed a typical upstroke and repolarization phase.

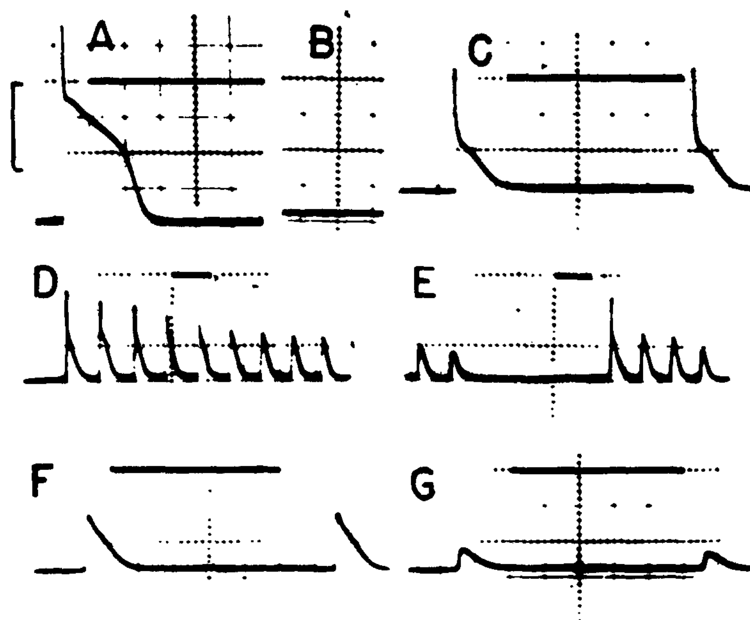

FIGURE 3. Influence of Li-Tyrode on resting and action potentials in a calf Purkinje fiber. A, Na-Tyrode; B, choline-Tyrode; C, 25 min. in Li-Tyrode; D and E, 40 min. in Li-Tyrode, influence of increase in stimulation frequency; F, 45 min. and G, 50 min. in Li-Tyrode. Voltage calibration, 50 mv. Time calibration, 1 sec. Zero potential level indicated by time calibration bar. Note difference of time scale in D and E.

A typical experiment is illustrated in Fig. 3. The preparation was stimulated at a frequency of 42 per min. (cycle length 1.4 sec.). Before the perfusion with Li-Tyrode, the preparation was bathed during 30 min. in choline-Tyrode until there was complete inexcitability (Fig. 3B). A typical action potential obtained after 25 min. in Li-Tyrode is shown in Fig. 3C. Its amplitude and duration were markedly smaller than values obtained in normal Na-Tyrode (Fig. 3A); there was a pronounced spike and the plateau level was nearer to the resting potential.

The decrease in action potential amplitude was largely dependent on the rate of stimulation. In the experiment of Fig. 3 the effect of an increase in

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

frequency was very marked after 40 min. perfusion with Li-Tyrode. For a short time the cycle length of the electrical stimulation was reduced from 1.4 sec. to 0.9 sec. As can be seen in Fig. 3D and E the amplitude of the action potential dropped to 30 per cent of its initial value in 12 sec. without any simultaneous change in resting potential. A rest of less than 5 sec. was sufficient to reestablish practically the full height of the action potential. However, due to the high rate of stimulation some disturbance persisted; this was manifested by a faster decrease in action potential amplitude during the second series of stimuli (Fig. 3E). Thereafter the frequency was again lowered to 42 per min. In Fig. 3F and G action potentials obtained after 45 and 50 min. in Li-Tyrode are shown. Although the drop in resting potential was slower at this stage, the amplitude of the action potential continued to fall.

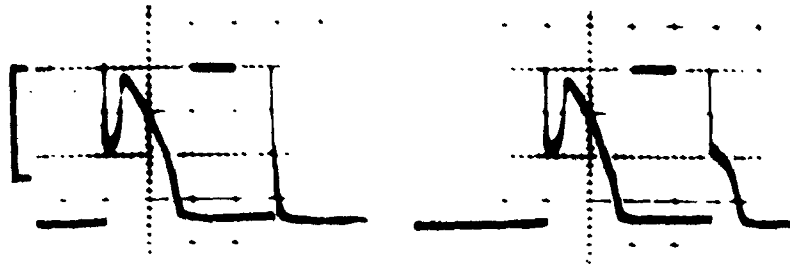

FIGURE 4. Three different types of action potential in Li-Tyrode. Calf Purkinje fiber after 20 min. in Li-Tyrode. Electrical stimulation. Voltage calibration, 50 mv. Time calibration, 1 sec.

When the preparation was stimulated at a frequency above one per sec. from the start of the perfusion with Li ions, some peculiar changes in action potential configuration were often observed. Fig. 4 shows three different types of electrical response recorded during the same impalement: "normal plateau" action potentials alternated during many minutes with very short "spike" action potentials or with action potentials showing a hump during the plateau. Although these modifications were not seen in every experiment, they were most frequently encountered during the first half-hour of Li perfusion. In many cases they could be evoked by interpolating a short rest of a few seconds in a normal series of stimuli; when stimulation was restarted, the first action potential as a rule was very short and the second showed a hump during the plateau.

RECOVERY IN NORMAL Na-TYRODE

Cat Ventricular Muscle

In the presence of 5.4 mM K recovery of the resting potential was very fast. As can be seen from Fig. 2 the potential increased from −47 to −70 mv in

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

10 min. If the diffusion rate of lithium ions in the extracellular space is of the same order of magnitude as that measured for Na* (Boulpaep, 1962), this time would correspond to the time necessary to deplete the extracellular medium of 90 per cent of its Li ions. On continuing the perfusion with Na-Tyrode the resting potential further increased but more slowly. For five experiments the mean value of the resting potential was 70.9 mv $\pm$ 0.34 (15), after 15 to 20 min. in recovery solution. In some experiments there was an indication that the potential after 1 hr. of recovery was a few millivolts lower than that at 20 minutes but the difference was not statistically significant. The mean value for five experiments after 1 hr. was 71.8 mv $\pm$ 0.70 (15).

Action potentials, which were of low amplitude and short duration after 1 hr. in Li-Tyrode, became large and of long duration during the first minutes of recovery in Na-Tyrode (Fig. 2). They were characterized by a very slow rate of depolarization during the upstroke and absence of a distinct spike; the total amplitude corresponded approximately to the plateau height in normal Tyrode. As the membrane potential increased to its normal value, the rate of rise during the upstroke increased and the spike reappeared. After 1 hr. in recovery Tyrode the amplitude of the action potential was practically normal but a hump in the plateau often persisted.

Calf Purkinje Fibers

Compared to cat ventricular muscle, recovery of the resting and action potential was slower in calf Purkinje fibers. The course of recovery of the resting potential was S-shaped. During the first 5 min. there was hardly any change in the resting potential. Thereafter the potential started to increase and recovery was fast during the subsequent 15 min. After 20 min. the mean membrane potential for five experiments was -76.60 mv $\pm$ 0.56 (5). From 20 min. on the increase in resting potential again was slower.

Pictures of the resting and action potentials of a typical experiment are shown in Fig. 5. Very peculiar action potentials were observed during the first 10 min. of recovery in Na-Tyrode (Fig. 5A–D). The most striking aspects of these action potentials were: (*a*) a very slow rate of depolarization, most often consisting of two distinct phases, (*b*) an extremely long duration of a few seconds. In most cases the depolarization phase consisted of a fast spike potential, attaining only a few millivolts, which was then followed by a slow depolarization creeping towards the plateau level; the time necessary to attain this level was 0.4 to 0.8 sec. (see Fig. 5A and B). As the resting potential increased with time, the amplitude of the fast depolarization phase increased. At the same time the rate of depolarization during the second phase became greater. When one compares the amplitude of the fast spike with the rate of the second slow depolarization in each action potential of the series A to D,

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

it is obvious that there is a correlation between the two parameters; the higher the spike, the greater the rate of depolarization during the hump. Independent of a change in resting potential the amplitude of the spike also was influenced by the previous diastolic period. When the stimulus arrived earlier than 2 sec. after the end of the repolarization, there was a marked decrease in amplitude of the fast upstroke (Fig. 5A).

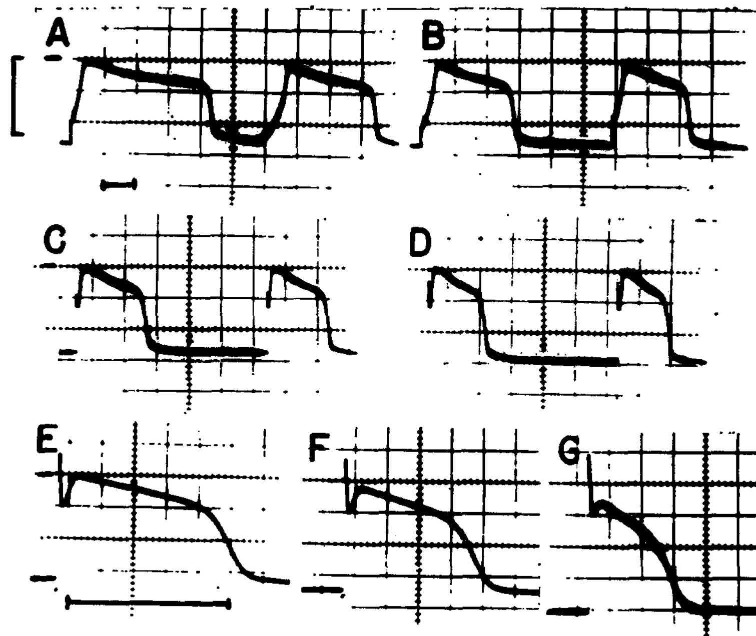

FIGURE 5. Recovery of resting and action potentials in Na-Tyrode after a previous period of 1 hr. in Li-Tyrode. Continuation of experiment of Fig. 3. A, B, C, and D after 5 min., 7 min., 10 min. and 12 min. respectively in Na-Tyrode. E, 20 min., F, 40 min., and G, 60 min. in Na-Tyrode. Voltage calibration, 50 mv. Time calibration, 1 sec. Note difference of time scale in A, B, C, D as compared to E, F, G.

At the start of recovery, action potentials lasted for several seconds and were followed by a small negative afterpotential, instead of the normal positive afterpotential. Gradually the repolarization rate increased, thereby reducing the total duration of the action potential. Between 40 and 60 min. of recovery in Na-Tyrode, action potentials with a practically normal upstroke and spike were recorded (Fig. 5E and F). A hump in the plateau still persisted for some time but decreased steadily. After recovery for more than 1 hr. the total duration of the action potential became normal and the potential during diastole was stable (Fig. 5G).

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

RECOVERY IN THE ABSENCE OF EXTRACELLULAR K IONS

In the course of this investigation a working hypothesis was developed which explained the electrical phenomena in terms of ion shifts through the membrane (see Discussion). In order to find out to what extent the recovery of the membrane potential in Na-Tyrode was related to an increase in $[K]_i$, the restoration of the resting potential was studied in the absence of extracellular potassium.

A cat ventricular muscle was first bathed during 50 min. in Li-Tyrode (5.4 mM K) and then changed to a K-free Li-Tyrode for 10 min. The result

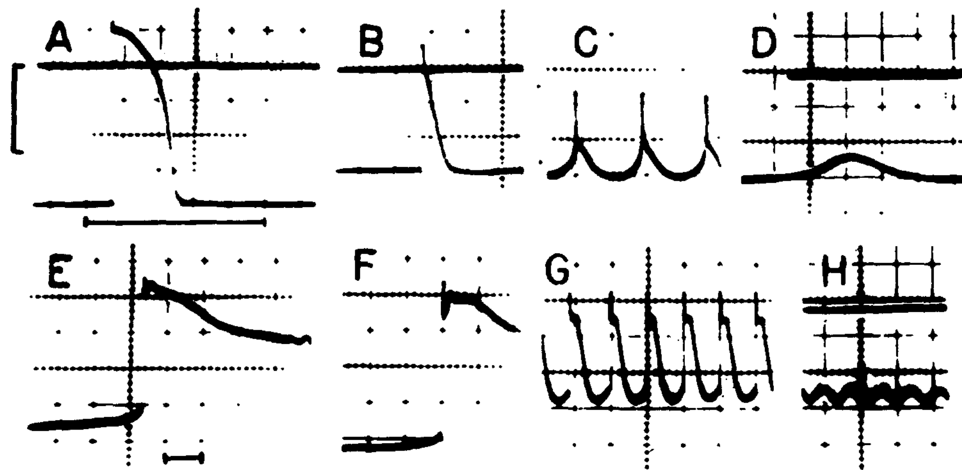

FIGURE 6. Recovery in a K-free Tyrode. A, normal Tyrode, B, 30 min. in Li-Tyrode (5.4 mM K), C and D after 5 and 10 min. in K-free Li-Tyrode, E, F, G, and H after 4 min., 5 min., 8 min., and 10 min. recovery in K-free Na-Tyrode. Voltage calibration, 50 mv. Time calibration, 1 sec. Note difference in time scale for upper and lower row.

was a slight hyperpolarization of about 7 mv (Fig. 6C–D), probably due to the increase in K gradient. Transient spontaneous oscillations of a type seen in Purkinje fibers in a K-free Na-Tyrode (Carmeliet, 1961) were observed. After the potential became stable, recovery was followed in a K-free Na-Tyrode. In less than 5 min. the membrane repolarized to −90 mv (Fig. 6E–F). This hyperpolarization was faster and larger than the change in potential during recovery in potassium-containing Tyrode (see Fig. 2). However, the potential was unstable; a slow depolarization resulted spontaneously in a propagated action potential, with a plateau lasting for several seconds. With time these long action potentials became shorter and the maximum resting potential decreased (Fig. 6G). The frequency of spontaneous firing increased and finally, after 10 min., the potential returned to the level of −40 mv

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

(Fig. 6H). This result demonstrates clearly that a hyperpolarization during recovery from Li-Tyrode is possible in the absence of external K. The repolarization in a K-containing recovery medium is therefore a complex phenomenon which is dependent on K ions only in that a stable membrane potential is produced.

Part II. Influence of Li on Cation Movement

In order to make a comparison between the electrical effects of Li ions and their influence on ion movement through the membrane, two different experimental approaches were used. The first one was to analyze cat papillary muscle for Li, K, and Na content after variable periods of perfusion with Li-Tyrode and during recovery in Na-Tyrode. The second method consisted of measuring unidirectional fluxes of labeled K and Na under the same experimental conditions.

CHEMICAL ANALYSIS The following general scheme was adopted for this series of experiments. After isolation from the right ventricle, papillary and trabecular muscles were allowed to recover from dissection during 1 hr. in normal Tyrode. At the end of this period they were transferred to the Li-Tyrode for 1 hr. The soaking-in period in Li-Tyrode was followed by a recovery period in Na-Tyrode which lasted up to 2 hrs. The whole experiment was subdivided into half-hour periods, at the end of which muscles were removed for analysis.

In most experiments all Na ions in the perfusion fluid were replaced by Li ions and the concentrations of K, Ca, and Mg held constant. A few experiments were performed in a K-free Li-Tyrode or with a partial replacement of Na by Li ions. In this latter case the soaking-in period was extended up to 4 hrs.

Soaking-in period Figures for K, Na, and Li content per kilo wet tissue, obtained at different experimental periods, are assembled in Table I. All Na ions were replaced by Li ions in the bathing solution. The values for the intracellular concentration of these ions in Table II were calculated from the individual ion and water content of each muscle, assuming an extracellular space of 30 ml per 100 gm wet weight. The extracellular space was not measured in these experiments, but, as the mean water content of the muscles was similar to that obtained by Page (1962) for the same species, his value of the mannitol space was thought to be applicable to our results. If one had taken the inulin space instead of the mannitol space, this would have changed the actual values of the concentrations, but not the general trend found for the ion shifts.

As can be seen, the cardiac cell lost K and Na very fast in Li-Tyrode and this loss was compensated by a nearly equivalent entry of Li ions. The increase

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

of the intracellular Li concentration during the first half-hour was smaller than during the second half-hour period. This might reflect a diffusion delay in the extracellular space, the exchange of Li for Na not being instantaneous. Because it was assumed that after half an hour all extracellular Na was replaced by Li, the entry of Li might have been underestimated. Such an assumption is open to criticism and might have caused, in the same way, an

TABLE I

ION CONTENT IN WET TISSUE.
CAT VENTRICULAR PREPARATION

	K	Na	Li	H_2O
	mmoles/kg	*mmoles/kg*	*mmoles/kg*	*per cent*
Na-Tyrode	70.17±1.68 (16)*	62.38±2.62 (16)		75.85±1.22 (16)
Li 0.5 hr.	56.87±1.76 (9)	7.78±1.30 (9)	64.40±2.00 (9)	77.96±0.46 (9)
Li 1.0 hr.	34.85±2.55 (10)	3.51±0.56 (10)	101.20±2.33 (10)	76.59±0.92 (10)
Na 0.5 hr.	49.36±3.62 (9)	54.47±4.13 (9)	30.48±1.46 (9)	76.22±0.62 (9)
Na 1.0 hr.	54.43±1.80 (19)	62.74±3.30 (19)	19.78±0.90 (19)	77.98±0.77 (19)
Na 2.0 hrs.	59.55±2.25 (5)	65.21±2.21 (5)	11.49±0.85 (5)	75.91±0.96 (5)

* Values in parentheses denote number of muscles used; variability of data given as ± standard error.

TABLE II

INTRACELLULAR ION CONCENTRATION

Extracellular space, 30 per cent wet weight

	K	Na	Li	Σ
	mmoles/liter intracellular water	*mmoles/liter intracellular water*	*mmoles/liter intracellular water*	*mmoles/liter intracellular water*
Na-Tyrode	151.28±5.81 (16)*	37.52±5.57 (16)		188.80±5.85
Li 0.5 hr.	115.11±3.39 (9)	16.13±2.73 (9)	40.23±3.95 (9)	171.47±4.86
Li 1.0 hr.	71.02±4.92 (10)	7.42±1.03 (10)	121.21±5.74 (10)	199.65±4.79
Na 0.5 hr.	102.98±7.08 (9)	22.69±8.70 (9)	65.93±2.93 (9)	191.60±8.47
Na 1.0 hr.	110.37±3.73 (19)	38.86±6.38 (19)	41.34±1.92 (19)	190.57±8.49
Na 2.0 hrs.	126.49±6.18 (5)	44.05±4.44 (5)	25.07±1.91 (5)	195.61±5.02

* Values in parentheses denote numbers of muscles used; variability of data is given as ± standard error. The intracellular ion concentration was calculated for each muscle from the individual ion and water content, supposing an extracellular space of 30 per cent wet weight.

overestimation of the intracellular Na concentration. Compared to normal conditions, the sum of the calculated values for intracellular ion concentration was smaller after the first half-hour in Li-Tyrode but greater after 1 hr. Applying the variance ratio test, one finds, however, that the differences are not statistically significant. $P > 0.05$; F:2.577 for the comparison of data obtained in normal Tyrode and after 0.5 hour in Li-Tyrode; $P > 0.10$; F:2.385 for the comparison normal Tyrode–1.0 hr. Li-Tyrode.

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

Taking the muscle density as 1.05 gm/ml, the extracellular space as 30 ml per 100 gm wet weight, and the mean fiber diameter as 10 μ, 1 kg of cat ventricular muscle contains 652 ml of fibers with a total surface of 2.61 $\times$ 10^6cm^2. From the concentration changes after 1 hr. in Li-Tyrode the mean net Li influx was calculated to be 56.26 mmoles/kg hr., which corresponds to 5.99 pmoles/cm^2 sec. The net K efflux was 35.32 mmoles/kg hr., corresponding to 3.76 pmoles/cm^2 sec. After correcting for the extracellular Na in Na-Tyrode, the net Na efflux was 13.93 mmoles/kg hr. or 1.48 pmoles/cm^2 sec. These values must be considered as mean values over the whole period of 1 hr. It is clear from Table II, and from the radioactive Na efflux curves to be given later, that the Na flux was higher during the first half-hour and smaller during the second half-hour. For potassium it is impossible to deduce from the experimental results whether the net outward movement of the ion followed an

TABLE III
INTRACELLULAR LI CONCENTRATION IN CAT VENTRICULAR PREPARATIONS, BATHED IN 15 mM Li, 135 mM Na SOLUTION

1 hr.	2 hrs.	3 hrs.	4 hrs.
mmoles/liter intracellular water	*mmoles/liter intracellular water*	*mmoles/liter intracellular water*	*mmoles/liter intracellular water*
14.69	22.08	22.06	29.15
20.11	18.00	23.08	38.31
		27.23	33.64

exponential time course. This aspect will be analyzed in more detail after the results with radioactive K and Na have been presented.

The results so far give convincing evidence that Li is handled by the cell in a different way than Na. The most plausible explanation is that Li is not actively pumped out but behaves as a passive ion. Other evidence for the passive behavior of Li was found when the inward movement of Li was followed in preparations bathed in a substituted Tyrode solution containing 15 mM Li and 135 mM Na. In Table III the intracellular Li concentrations of individual muscles are given for different durations of perfusion with this solution. As can be seen the intracellular Li concentration became equal to the extracellular level after 1 hr. and was more than double the value after 4 hrs.

Recovery period When the preparation that had been bathed for 1 hour in Li-Tyrode was transferred to Na-Tyrode for recovery, the reversed ionic shifts were observed. The largest changes in concentration occurred during the first half-hour (Table II). Li left the fibers very fast and was re-

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

placed by K and Na. The movement of Li and Na may be assumed to be passive, but the net entry of K must be largely active. The presence of a large amount of Li in the cell does not seem, therefore, to inhibit active K transport. The recovery of $[K]_i$, although rapid during the first half-hour, was still incomplete after 2 hrs., while $[Na]_i$ attained normal values after 1 hr. in recovery Tyrode. The incomplete recovery of $[K]_i$ is due mainly to some 25 mmoles of Li per liter intracellular water remaining in the cell.

When the values of $[Li]_i$ are plotted semilogarithmically, it can be seen that Li efflux did not follow an exponential time course. Estimates of mean rate constants for the different periods give 1.218 $hr.^{-1}$, 0.934 $hr.^{-1}$, and 0.501 $hr.^{-1}$. Li efflux was thus not directly proportional to the internal Li concentration. This might be due to a competition between Li and Na for common sites. It will be seen later, however, that K and Na net outflux also deviates from the single exponential law.

EXPERIMENTS WITH RADIOACTIVE K^{42} Before analyzing K flux into its components of influx and efflux it is worth while to report the results of a few experiments that were actually designed as exploratory experiments before chemical analysis was attempted. They provide a direct demonstration in the same preparation of a substantial K loss in Li-Tyrode and a reentry in recovery Tyrode.

Cat ventricular muscles and calf Purkinje preparations were loaded with K^{42} until complete equilibrium was attained (more than 4 hrs. in K^{42}, Na-Tyrode). After a wash of 6 min. in inactive Tyrode, allowing most of the extracellular K^* to be removed, the total radioactivity present in the preparation was measured by putting the test tube with the preparation into the well of the scintillation counter. To make sure that a full equilibrium with respect to K^* exchange was obtained, the preparation went again into the radioactive Tyrode for a supplementary hour. When no further increase in K^{42} content was found after this control period, the total activity then was a measure of the exchangeable internal K content.

The effect of Li on the intracellular K concentration was then investigated by putting the preparation into a Li-Tyrode containing radioactive K at the same specific activity as in the Na-Tyrode used for loading. Under these conditions a change in total radioactivity of the preparation would indicate a change in intracellular K content. Fig. 7 gives an example of such an experiment. In six cat ventricular muscles the intracellular K^* content declined with a rate constant of 0.843 $hr.^{-1}$ (range 0.60 to 1.15 $hr.^{-1}$). This value can be compared with the value of 0.76 $hr.^{-1}$, estimated from direct chemical analysis. In seven calf Purkinje preparations the rate constants ranged from 0.27 to 0.43 $hr.^{-1}$ (mean 0.36 $hr.^{-1}$). The slower net outflow of K ions in these latter preparations is most likely due to the larger diameter of the cells. From

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

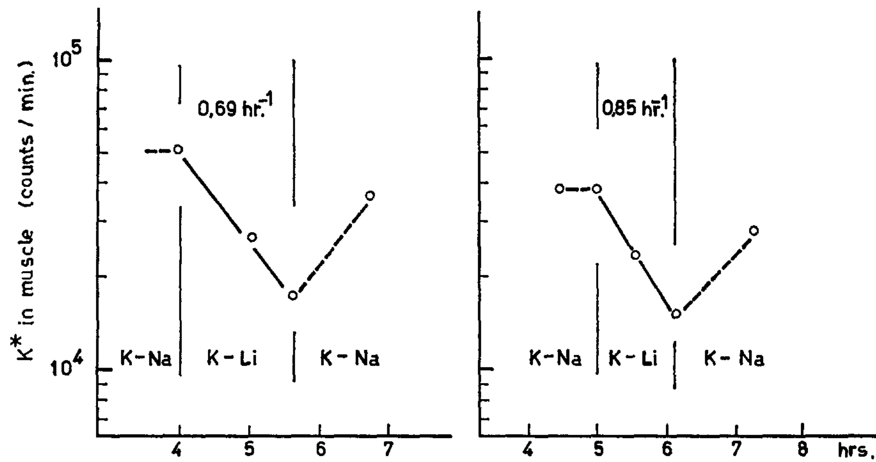

FIGURE 7. Influence of Li-Tyrode and Na-recovery Tyrode on K* content of two cat ventricular preparations. The specific activity of K* was the same in both solutions.

TABLE IV

RATE CONSTANT (HR.$^{-1}$) OF K* EFFLUX IN CAT VENTRICULAR PREPARATION

Experiment	5.4 mM K_o		Experiment	K-Free		
					Li-Tyrode	
	Na-Tyrode	Li-Tyrode		Na-Tyrode	10 to 60 min.	100 to 140 min.
m 91	1.094	1.341	m 93	0.266	1.434	0.375
m 92	0.693	1.155	m 94	0.249	1.299	0.363
m 103	0.630	1.260	m 95	0.217	1.386	0.385
m 104	0.924	1.341	m 101	0.242	0.866	0.365
m 105	0.799	1.155	m 102	0.196	0.885	0.318
m 106	0.770	1.094				
Mean	0.818±0.069*	1.225±0.043		0.234±0.038	1.174±0.124	0.361±0.036

* Standard error.

Fig. 7 it is equally clear that recovery in Na-Tyrode brought about a substantial reentry of K into the intracellular compartment.

Unidirectional K fluxes at 5.4 mM $[K]_o$ The next step was an attempt to explain the decrease in $[K]_i$ in terms of changes in K efflux and K influx.

K efflux increased markedly when the preparation was bathed in Li-Tyrode. The mean rate constant for six cat papillary muscles in 5.4 mM K rose from 0.818 hr.$^{-1}$ to 1.225 hr.$^{-1}$ (Table IV). The maximum increase occurred with a delay of 5 to 15 min. (see for example Fig. 8A and Fig. 9A and B). The observed delay probably reflects the time necessary for complete exchange of

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

Li for Na ions in the extracellular medium. The effect of Li on K efflux was not followed over periods longer than 1 hr. so that an eventual change of rate constant over longer times could not be established.

On replacing the preparation in Na-Tyrode, K efflux dropped to a lower value than that obtained during the control period before being exposed to Li ions. This is understandable if the large decrease in $[K]_i$ occurring under these conditions and the subsequent dilution of radioactive K by the influx of K are taken into account. Another factor causing the large drop in K efflux

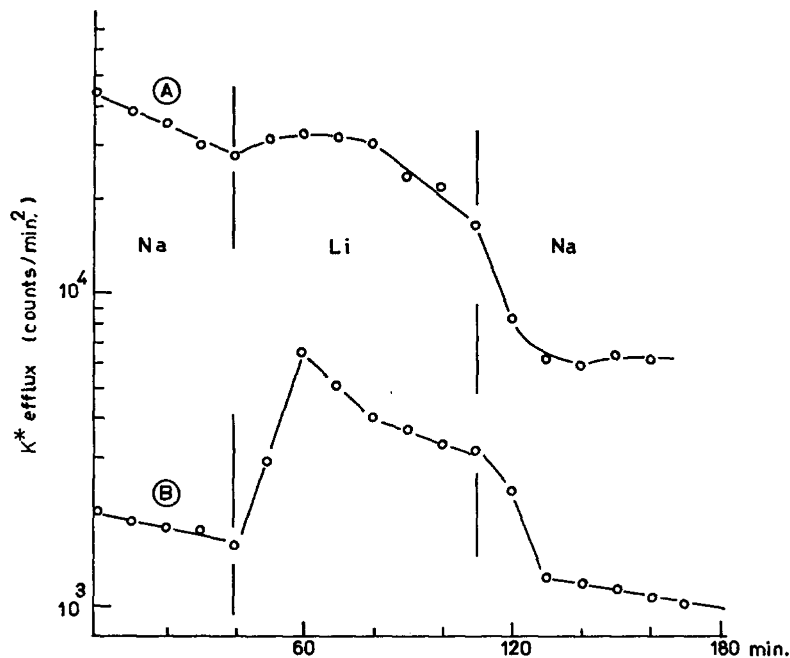

FIGURE 8. Influence of Li ions on K* efflux in calf Purkinje fibers; in A, K_o was 5.4 mM; in B, a K-free solution was used throughout.

could be an interference of K influx with K efflux, the net flux of K being directed inward. After 30 min. in recovery Na-Tyrode the K efflux tended to increase again as can be seen in Fig. 9A and B. This can be understood if the rise in $[K]_i$ has a larger effect on K efflux than the concomitant dilution of radioactive K ions.

From the observed change in K efflux and the drop in intracellular K concentration it is possible to predict a substantial inhibition of K influx. A rate constant of 0.818 hr.$^{-1}$ for K efflux corresponds to an outward movement of 84.5 mmoles K in 1 hr.; in steady-state conditions K influx will compensate for K outflux. A change of the rate constant to 1.225 hr.$^{-1}$ will

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

cause an outward movement of 106.8 mmoles K in 1 hr. If there were no change in K influx, this would lead to a net loss of 22.32 mmoles K. The actual decrease in $[K]_i$ was 80 mM after 1 hr. Therefore K influx must have been smaller than in normal Tyrode.

K influx in Li-Tyrode was measured in three cat ventricular preparations and compared with two determinations in normal Na-Tyrode. All muscles remained for 25 min. in the labeled solution, at the end of which they were

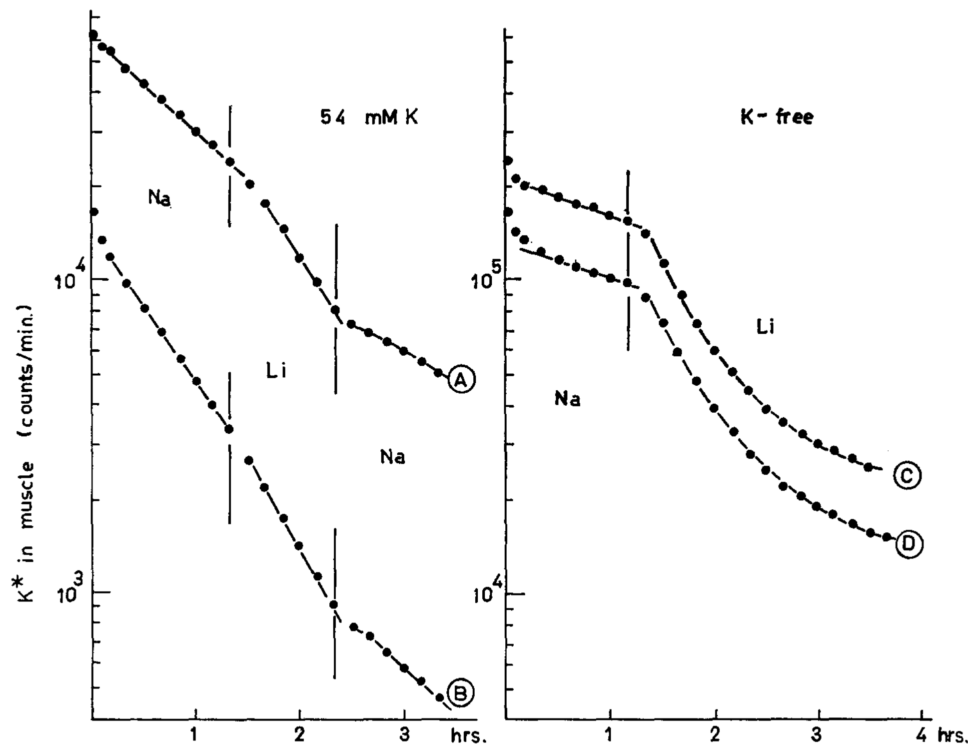

FIGURE 9. Influence of Li ions on K* efflux in cat ventricular preparations. A and B, K_o was 5.4 mM, in C and D, K-free solutions. For values of rate constants see Table IV.

gently blotted with filter paper and their activity measured. A correction was applied for the activity due to K* in the extracellular space (assumed to be 30 per cent of the wet weight). Expressed as counts per minute per milligram wet weight K* influx for the three preparations in Li-Tyrode was 16.8, 19.8, and 33.6 per cent of the average value in Na-Tyrode. As the uptake period was not negligible compared to the half-time for K* exchange, a correction has to be made for the concomitant efflux of radioactive ions. The corrected values were 20.7, 22.1, and 39.6 per cent respectively. Possible reasons for this fall in K influx will be analyzed in the Discussion.

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

K efflux in K-free solutions* In order to explain the electrical phenomena observed in K-free Li solutions, K efflux was investigated under the same experimental conditions. In Fig. 8B, K efflux is shown for a Purkinje preparation. The maximum increase in K efflux was obtained 20 min. after Na was replaced by Li and amounted to five times the value in Na-Tyrode. The same relative change in rate constant was found in cat papillary muscles (Table IV and Fig. 9C and D).

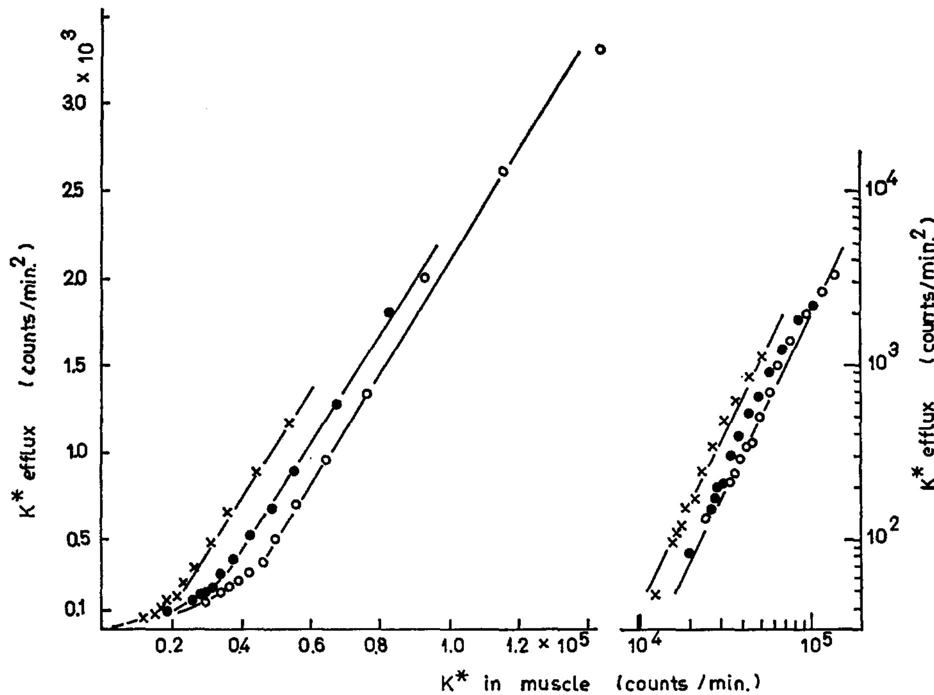

FIGURE 10. K* efflux as a function of $[K]_i^*$ for three different cat ventricular preparations in K-free Li-Tyrode. On the left $[K]_i^*$ and K* efflux on normal scales; on the right on logarithmic scales. The straight lines on the right half have been drawn with a slope of 2.

When the experiment in K-free Li-Tyrode was continued for a longer time, K efflux did not follow an exponential time course but there was a continuous fall in the rate constant. During the first hour the rate constant was 1.174 hr.$^{-1}$; after 2 hrs. it fell to 0.361 hr.$^{-1}$ (Table IV). As there was no extracellular K and hence no K influx, the decrease of labeled K content was a direct measure of the decline in intracellular K concentration. If the K efflux were directly proportional to the internal K concentration, K* content would have fallen in an exponential way. In the same way, according to the equation $-d[K]_i^*/dt = k\,[K]_i^*$, one should have found a straight line with intercept at zero,

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

when K* efflux was plotted as a function of K* content. As can be seen from Fig. 10, K efflux varied directly with $[K]_i$ over quite a large range; for lower values of $[K]_i$ however, the "proportionality" factor decreased. K efflux is thus not proportional to the first power of $[K]_i$. Applying the general formula for reaction rate $-dc/dt = kc^n$, and plotting log K efflux as a function of log $[K]_i$, a straight line relationship was found, the slope being equal to two (Fig. 10). K efflux seems thus to be directly proportional to the second power of

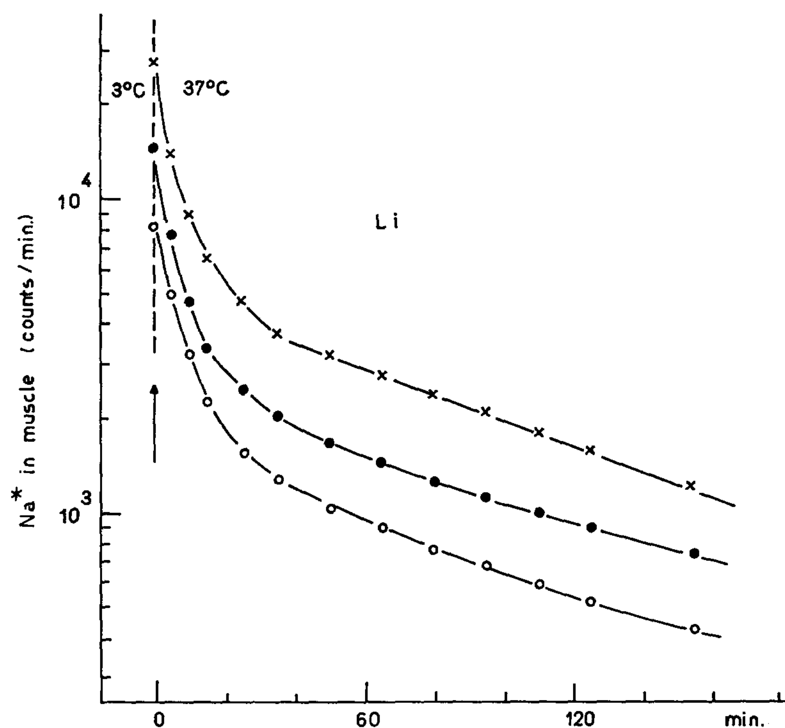

FIGURE 11. Na* efflux of three cat ventricular preparations in Li-Tyrode at 37°C. Previous to this period the preparation had been bathed during 30 min. in inactive Li-Tyrode at 3°C. See text for further explanation.

the intracellular K concentration. It must be stressed that this relationship was found during a large net K efflux in the presence of Li and one may ask whether the same exists in Na-Tyrode. Taking the experimental results obtained in K-free Na-Tyrode, a similar relationship can be found but was not analyzed in detail because the range of change in intracellular $[K]_i$ was too small.

NA EFFLUX When Na efflux in mammalian heart muscle is studied at 37°C, it is very difficult to distinguish between the washout of the extracellular space and the exchange of the intracellular compartment. Both phases can be

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

isolated if Na efflux is started at a low temperature (3°C) (Boulpaep, 1962). Low temperature will slow down all exchange of Na ions but Na efflux from the cell interior will be much more affected than extracellular diffusion. The general procedure in our experiments was as follows: The preparation was loaded with Na* in Na-Tyrode for 2 hrs. The washout was started at 3°C in Li-Tyrode during half an hour and was then continued at 37°C. For some experiments the temperature was raised only to 23°C.

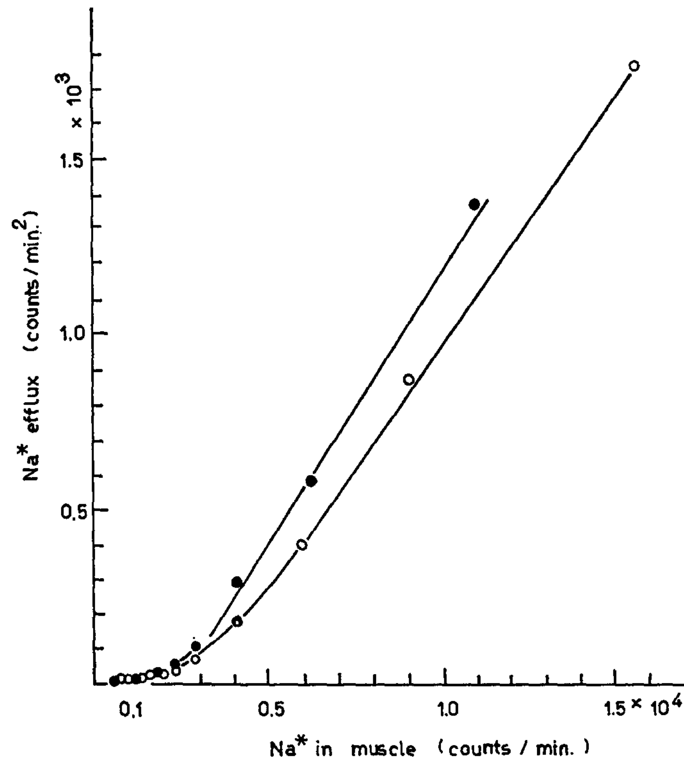

FIGURE 12. Na* efflux as a function of $[Na]_i^*$ for two different cat ventricular preparations in Li-Tyrode.

In Fig. 11 three examples are given of the Na efflux in Li-Tyrode at 37°C, after the extracellular space had been washed free of Na* at 3°C during 30 min. The effluxes at low temperature are not shown.

Na* outflow was very fast and most of the Na left the cell during the first hour. As there were no Na ions in the extracellular medium, and hence no Na influx, the decrease in Na* measured directly the decrease of intracellular Na concentration. From the graphs it is obvious that $[Na]_i$ did not fall in an exponential way. The rate constant decreased from 6.58 hr.$^{-1}$ (mean of five experiments) during the first 10 min. to 0.545 hr.$^{-1}$ after 60 min. When Na*

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

efflux was plotted against $[Na]_i^*$ (Fig. 12) a graph was obtained similar to that for K* in a K-free Li solution. On double logarithmic scale, however, a straight line relationship between the two variables was found with a slope equal to two (Fig. 13). This indicates that Na efflux over the range studied was directly proportional to the second power of $[Na]_i$.

Na exchange diffusion In skeletal muscle Na efflux drops to half its value in Li or choline-Ringer; this result was interpreted as evidence for the

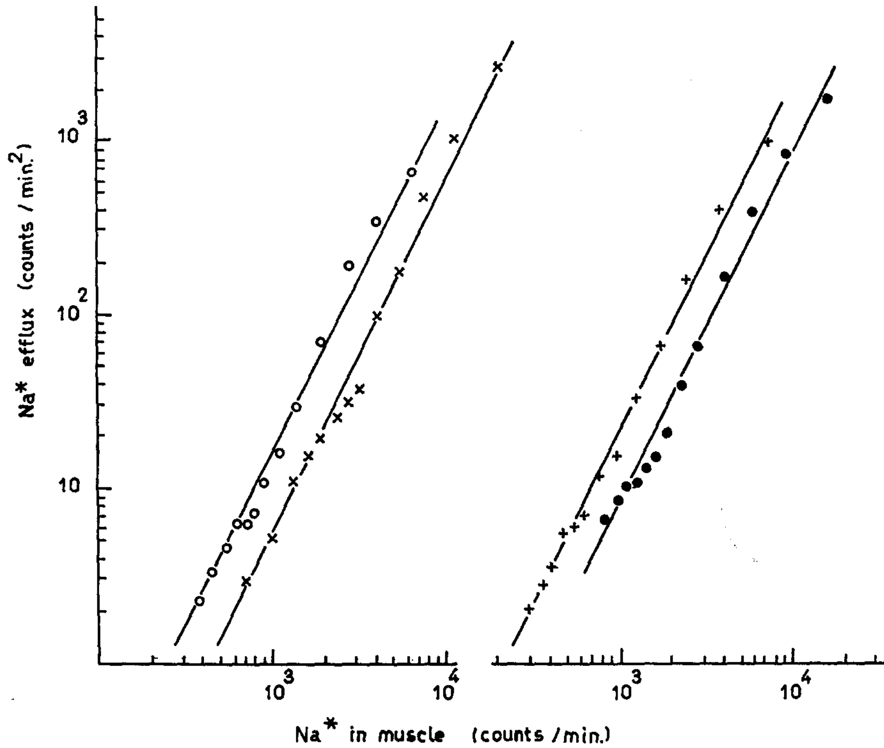

FIGURE 13. Na* efflux as a function of $[Na]_i^*$ on double logarithmic scale. Cat ventricular preparations. The straight lines have been drawn with a slope of two. All points of the four experiments lay in a narrow range but were drawn apart to avoid crowding. The filled and open circles are from the same experiments as in Fig. 12.

existence of an exchange diffusion which was responsible for 50 per cent of the Na outflux (Keynes and Swan, 1959). If there was an exchange diffusion in heart muscle a decrease in Na efflux also would be expected during perfusion with Li-Tyrode, and an increase when returning to normal Na-Tyrode after Li perfusion.

As can be seen in Fig. 14, Na* efflux in heart muscle increased immediately, when a fiber previously bathed in Li-Tyrode was transferred to Na-Tyrode. Such response is in accord with the hypothesis of an exchange diffusion mecha-

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

nism. However, when one returned to the Li-Tyrode the expected decrease in Na efflux did not occur, but in three out of four experiments Na efflux increased (see Fig. 14B). This increase occurred with some delay, in contrast to the immediate rise observed in Na-Tyrode. One of the factors responsible for the increase of Na efflux in Li-Tyrode might be the large net outflow of Na ions under these conditions, obscuring the decrease due to the absence of an

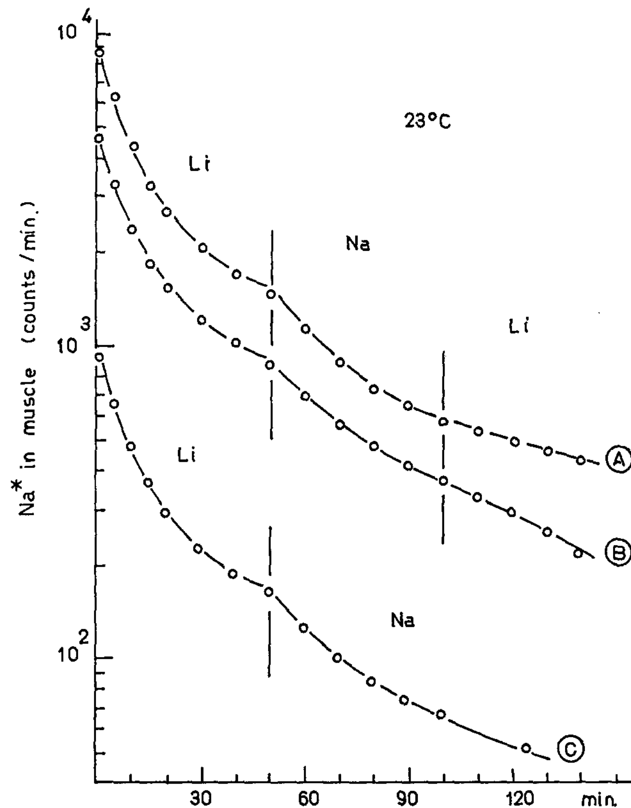

FIGURE 14. Influence of Li- and Na-Tyrode on Na* efflux in cat papillary muscles. The preparations had been washed during 30 min. in Li-Tyrode at 3°C before Na* efflux was followed at 23°C. See text for further explanation.

Na exchange diffusion. For a similar reason the interpretation proposed for the increased Na efflux in Na-Tyrode after Li perfusion as being due to an Na exchange diffusion, becomes less firm. The existence of a large net inward movement of Na ions under these conditions will have a double effect: on the one hand it will decrease the specific activity of the intracellular Na ions and hence reduce the apparent Na efflux, but on the other hand, it will increase $[Na]_i$ and thus increase Na efflux. This last factor will be more important because Na efflux was found to be proportional to the second power

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

of $[Na]_i$. In view of such a complex situation, the reported results cannot be considered as definitive proof of the existence of Na exchange diffusion in heart muscle.

DISCUSSION

The reappearance of action potentials in Li-Tyrode, after the preparation was rendered inexcitable in choline-Tyrode, indicates that Li ions can replace Na ions in their role during the upstroke of an action potential. Cardiac tissue therefore is not an exception to the general rule. It is quite obvious, however, that Li produces other effects which make it different from Na. These side effects consist of (*a*) a large depolarization and characteristic changes in action potential configuration; (*b*) a prominent influx of Li ions, so that $[Li]_i$ increased to 120 mM after 1 hr.; (*c*) a pronounced decrease in $[K]_i$, due to an increased K efflux and a decreased K influx. It is of interest to see how far

TABLE V

Experimental solution	E_m Observed mv	E_K mv	E_m calculated for $b = 0.0256$
Na-Tyrode	−74.85	−89.01	−74.85
Li 0.5 hr.	−55.32	−81.72	−67.72
Li 1.0 hr.	−48.64	−68.81	−55.70

Comparison of the observed resting potential with the theoretical equilibrium potential for K (Nernst equation) and the calculated diffusion potential using the Goldman equation. The experimental data to calculate E_K and E_m were taken from Table II.

the changes in ion movement and intracellular K concentration can be made responsible for the changes in transmembrane potential.

Depolarization The largest possible extent to which a fall in $[K]_i$ contributes to the depolarization can be estimated from the change in the calculated equilibrium potential for K, using the Nernst equation, or from the change in the calculated membrane potential, using the Goldman equation (see Hodgkin and Katz, 1949). Assuming an equilibrium distribution for chloride and an equal permeability for Li and Na, this equation yields the following expression for the membrane potential in Li-Tyrode

$$E_m = -\frac{RT}{F} \ln \frac{[K]_i + b[Na + Li]_i}{[K]_o + b[Li]_o}$$

in which b represents the permeability for Na or Li relative to that for K. From Table V one can see that neither the calculated change in E_K, nor the estimated E_m, with b constant and equal to 0.0256, can fully explain the ex-

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

tent or the time course of the depolarization. This would indicate that the assumptions underlying the above equation are false, *e.g.* the chloride ion not being passively distributed, or that the ratio P_{Li}/P_K has largely increased, an assumption which is not contradictory with the observed Li inward movement.

Evidence for a high permeability of heart muscle cells to Li ions was provided by the demonstration of a large net entry of Li ions. The net inward movement of Li was found equal to the sum of both Na and net K outward movement. Expressed as a change in intracellular content and calculated over a period of 1 hr. Li influx amounted to 0.937 mmole Li per kg wet weight per min. In frog sartorius this value was only 0.028 to 0.055 mmole per kg min. (Keynes and Swan, 1959). The difference becomes less but is still considerable when the smaller diameter of cardiac cells is taken into account. Keynes and Swan calculated a flux of 1.2 to 2.2 $\times$ 10^{-12} moles per cm^2 sec. for frog sartorius membrane. In cat papillary muscles this value was 5.99 $\times$ 10^{-12} moles per cm^2 sec.

A high permeability to Li and a decrease in $[K]_i$ will each cause a depolarization. The first factor is expected to provoke a fast depolarization (order of seconds) as is seen, for example, if the extracellular K concentration is increased; the second factor will cause a slower depolarization (order of minutes).

Different observations, however, remain unexplained. The depolarization occurred with a delay and during that time the rate of slow diastolic depolarization decreased and the action potential shortened. These effects can be explained if the increase in K efflux observed with the preparation in Li-Tyrode is taken into account. The effect of Li on K efflux is assumed to indicate an increase in P_K, and not to be the consequence of the depolarization. K efflux, in fact, rose before there was any depolarization. Furthermore, it is possible under suitable conditions to demonstrate an increased K efflux in the presence of Li ions, accompanied by a hyperpolarization. It is known that Purkinje fibers depolarize to -45 mv in a K-free Na solution (Weidmann, 1956) and that this depolarization is due to a decrease in P_K (Carmeliet, 1961). When Na ions were replaced by Li ions, under these conditions, the potential returned to the -90 mv level during the first minute. Full sized spontaneous action potentials were present during 4 to 5 min. The fibers then became quiescent but could still be stimulated electrically. After the phase of hyperpolarization the resting potential decreased in a way similar to that described for Li substitution in normal Tyrode. During the hyperpolarization a fivefold increase in K efflux occurred. This rise in K efflux cannot be accounted for by the change in resting potential; an increase in intracellular negativity would decrease K efflux. The hyperpolarization seems thus to be due to a rise in K permeability.

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

The depolarization by Li ions in the presence of a normal K concentration is thus explained: (*a*) by the large influx of Li ions and (*b*) by the decrease in $[K]_i$. The increase in P_K has the effect of counterbalancing and retarding the influence of these two factors. The actual time course of the depolarization will thus depend on the relative importance of all factors considered. The question might be raised whether or not the depolarization could be caused by impurities (*e.g.*, traces of heavy metals) in the Li salts used. The answer is indirect: (*a*) the depolarization was fully and quickly reversible, (*b*) the Li salts used did not cause an immediate depolarization in frog sartorius.

Compared to other tissues, the depolarization of cardiac cells was large and fast. A fall in resting potential exists also in nerve preparations (Crescitelli, 1952; Gallego and Lorente de Nó, 1952; Cerf, 1955; Böhm and Straub, 1962). In frog sartorius the potential was constant up to 30 min. in Li-Ringer (Keynes and Swan, 1959), but a substantial depolarization might be predicted from the observed ion shifts after many hours in cold Li-Ringer. The difference in the case of cardiac muscle could be due to an animal species factor, to the higher temperature, or to the difference in fiber diameter.

The depolarization in Li-Tyrode was explained by the influx of Li ions and the decrease in $[K]_i$. During recovery in Na-Tyrode, the membrane potential will now hyperpolarize and return to its normal value because of the increase in $[K]_i$ and the outward movement of Li ions, Li efflux being larger than Na influx. The importance of the Li efflux in causing an increase in resting potential was demonstrated by the large and fast hyperpolarization in a K-free Na-Tyrode. In a K-free Tyrode $[K]_i$ could not increase, but only decrease and the only factor responsible for the hyperpolarization was the outflow of Li ions.

Action Potential The increase in K permeability seems to be the main factor in explaining the shortening of the action potential duration in Li-Tyrode. A faster inactivation of a hypothetical Li carrier would affect the action potential in the same way. An increase in K permeability explains also the slowing of the rate of diastolic depolarization.

The decrease in action potential amplitude was found to be due partly to the depolarization and partly to the increased rate of repolarization. A third factor is certainly the steadily increasing Li concentration inside the cell, thereby lowering the equilibrium potential for Li towards the zero potential level.

The amplitude of the action potential also was very sensitive to an increase in frequency. A possible explanation might be found in: (*a*) a fast increase of $[Li]_i$ upon stimulation and (*b*) a rapid decrease in availability of carriers for Li inward movement. Difficult to explain is the finding that the amplitude was restored to its initial value after a rest of a few seconds. One explanation

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

would require an intensive active outward movement of Li ions, which is highly improbable. The second proposal, however, would imply that the reactivation of a hypothetical Li carrier in a partially depolarized fiber is much slower than the reactivation of Na carrier in normal Tyrode (Weidmann, 1955).

The possibility of having spikes followed by a slow, distinct depolarization was thought by other authors (Hoshiko and Sperelakis, 1962) to be indicative of junctional transmission at the intercalated dics. Although such a hypothesis might well explain our results, an attempt will be made to explain these modifications in terms of permeability changes of a homogeneous membrane. According to a widely accepted view, the fast repolarization during the spike of a normal cardiac action potential is due to a substantial decrease in sodium conductance; *i.e.*, inactivation of the Na carrier system. As the Na conductance does not fall to zero and the K conductance decreases simultaneously, the membrane stays depolarized during the plateau. It has been pointed out by Noble (1962) that the plateau may show a hump if the fall in K conductance is not immediate but develops with a certain delay. The more pronounced spikes and the presence of "humps" during the plateau, observed in Li-Tyrode, may then tentatively be explained by: (*a*) a faster and greater fall in Li conductance after the crest of the action potential and (*b*) a large delay in the fall of K conductance.

The slow depolarizations, following a small spike potential observed during recovery in Na-Tyrode, are explained in the same way as being due to a delayed fall in K conductance. The extremely slow upstrokes of the action potential, during the first minutes in recovery Tyrode, are most probably due to a competition between Na moving in and Li moving out of the cell. This phenomenon was most pronounced in Purkinje fibers that had been stimulated at high frequency and were thus most loaded with Li. Of special interest is the observation of a positive relationship between spike amplitude and rate of subsequent slow depolarization. This would indicate a faster and larger fall in K conductance when the membrane is depolarized to a larger extent. A fall in membrane conductance, proportional to the decrease in membrane potential between certain limits, was found in Na-free choline-Tyrode (Hutter and Noble, 1960; Carmeliet, 1961). The long lasting plateau during early recovery can be understood if one takes into account that K efflux must be small, due to the low $[K]_i$.

Active Li Efflux? A direct check of the existence of an active Li outward movement in Li-Tyrode could not be made. Under all conditions examined, there was a steadily increasing intracellular Li concentration while $[K]_i$ decreased.

The most plausible explanation for these results is to assume that Li is not actively pumped out, or is pumped only at a very low rate. Of particular in-

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

terest, with respect to this question, is the observation that $[Li]_i$ increased to double its extracellular concentration when the preparation was perfused with a solution containing only 15 mM Li. An increase of intracellular Li above the serum level also was described by Schou (1958) in different tissue cells of the rat.

The outflow of Li in a Na recovery medium was very large at the beginning but the intracellular concentration of Li was still 25 mM after 2 hrs. of recovery. If one compares Li efflux into Na-Tyrode with Na efflux into Li-Tyrode at equal intracellular concentrations, Li efflux comes out to be eight times smaller than Na efflux. If Li transport, under these conditions, is mediated through the same mechanism as for active Na outward movement this would indicate a low efficiency of the transport mechanism for Li.

Efflux versus Intracellular Ion Concentration

The simplest hypothesis about the efflux of an ion is to assume that it is directly proportional to its internal concentration. For Na and K efflux in Li-Tyrode, we have seen that the situation is more complex. The net movement of these ions was proportional to the second power of their intracellular concentration. A similar situation might exist for Li outward movement because the rate constant for Li efflux estimated from the chemical analysis was not constant but decreased over the 2 hrs. studied. It might be added that in frog sartorius, too, Na efflux was not directly proportional to $[Na]_i$ but was proportional to the third power of the internal Na concentration (Keynes and Swan, 1959; Mullins and Frumento, 1963).

Some evidence exists for part of the Na efflux being due to exchange diffusion. The results, however, did not permit an estimate of the extent to which such a mechanism contributes because of the masking effects of large net Na movements.

Dr. Carmeliet is an associate of the National Fund for Scientific Research of Belgium.
Received for publication, June 27, 1963.

REFERENCES

1. ARMETT, C. J., and RITCHIE, J. M., 1963, On the permeability of mammalian non-myelinated fibres to sodium and to lithium ions, *J. Physiol.*, **165**, 130.
2. BÖHM, H. W., and STRAUB, R. W., 1962, Der Effekt von Lithium-Ionen auf das Ruhepotential von markhaltigen Nervenfasern, *Arch. Ges. Physiol.*, **274**, 468.
3. BOULPAEP, E., 1962, Transport du sodium[24] dans le muscle cardiaque, *Arch. internat. physiol. et biochem.*, **70**, 113.
4. BUTCHER, W. A., WAKIM, K. G., ESSEX, H. E., and BURCHELL, H. B., 1953, The effect of lithium chloride on the electro-cardiogram of the isolated perfused heart, *Am. Heart J.*, **45**, 855.
5. CARMELIET, E. E., 1961, Chloride and Potassium Permeability in Cardiac Purkinje Fibres, Bruxelles, Presses Académiques Européennes Société Coopérative.

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024

6. CARMELIET, E. E., 1962 *a*, Effect of lithium ions on the membrane potential in cardiac Purkinje and muscle fibres, *Arch. internat. physiol. et biochem.*, **70**, 296.
7. CARMELIET, E. E., 1962 *b*, Potassium, sodium and lithium transport in cardiac muscle, *Arch. internat. physiol. et biochem.*, **70**, 422.
8. CERF, J., 1955, Relation entre l'effet dépolarisant du lithium et son action dépressive sur le nerf de grenouille, *Arch. internat. pharmacodyn.*, **103**, 281.
9. CRESCITELLI, F., 1952, Some features in responses of different nerve fiber types to a deficiency of sodium, *Am. J. Physiol.*, **169**, 1.
10. DULIÈRE, W., 1927, Le lithium dans le liquide de perfusion du coeur de grenouille, *Compt. rend. Soc. biol.*, **97**, 193.
11. GALLEGO, A., and LORENTO DE NÓ, R., 1952, On the effect of ammonium and lithium ions upon frog nerve deprived of sodium, *J. Gen. Physiol.*, **35**, 227.
12. HODGKIN, A. L., and KATZ, B., 1949, The effect of sodium ions on the electrical activity of the giant axon of the squid, *J. Physiol.*, **108**, 37.
13. HOSHIKO, T., and SPERELAKIS, N., 1962, Components of the cardiac action potential, *Am. J. Physiol.*, **203**, 258.
14. HUTTER, O. F., and NOBLE, D., 1960, Rectifying properties of heart muscle, *Nature*, **188**, 495.
15. KEYNES, R. D., and SWAN, R. C., 1959, The permeability of frog muscle fibres to lithium ions, *J. Physiol.*, **147**, 626.
16. MAIZELS, M., 1954, Active transport in erythrocytes, *Symp. Soc. Exp. Biol.*, **8**, 202.
17. MCKUSICK, V. A., 1954, The effect of lithium on the electrocardiogram of animals and relation of this effect to the ratio of the intracellular and extracellular concentrations of potassium, *J. Clin. Inv.*, **33**, 598.
18. MULLINS, L. J., and FRUMENTO, A. S., 1963, The concentration dependence of sodium efflux from muscle, *J. Gen. Physiol.*, **46**, 629.
19. NOBLE, D., 1962, A modification of the Hodgkin-Huxley equation applicable to Purkinje fibre action and pacemaker potentials, *J. Physiol.*, **160**, 317.
20. PAGE, E., 1962, Cat heart muscle *in vitro*. III. The extracellular space, *J. Gen. Physiol.*, **46**, 201.
21. RADOMSKI, J. L., FUYAT, H. N., NELSON, A. A., AND SMITH, P. K., 1950, The toxic effects, excretion and distribution of lithium chloride, *J. Pharmacol. and Exp. Therap.* **100**, 429.
22. SCHOU, M., 1957, Biology and pharmacology of the lithium ion, *Pharmacol. Rev.*, **9**, 17.
23. SCHOU, M., 1958, Lithium studies. 3. Distribution between serum and tissues, *Acta Pharmacol. et Toxicol.*, **15**, 115.
24. STEIN, E., KLEINFELD, M., GREENE, H., and MEYERS, S., 1955, Action of lithium chloride on the isolated perfused frog heart, *Am. J. Physiol.*, **183**, 121.
25. WEIDMANN, S., 1955, The effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system, *J. Physiol.*, **127**, 213.
26. WEIDMANN, S., 1956, Elektrophysiologie der Herzmuskelfaser, Bern, Medizinischer Verlag H. Huber.

Downloaded from http://rupress.org/jgp/article-pdf/47/3/501/1838812/501.pdf by guest on 24 April 2024