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ACTIVATION OF THE ELECTROGENIC SODIUM PUMP IN GUINEA-PIG AURICLES BY INTERNAL SODIUM IONS

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SUMMARY

1. The effect of various intracellular Na concentrations ($[Na]_i$) on the membrane potential after hypothermia was studied in guinea-pig auricles.

2. For varying $[Na]_i$, the atria were cooled for 4 hr at 4–6° C in a K-poor solution with different concentrations of NaCl. The auricles were rewarmed in normal Tyrode solution at 35° C.

3. Extracellular space (ECS), intracellular Na and K concentrations ($[Na]_i$ and $[K]_i$) and membrane potential of the atria were measured before and after hypothermia.

4. The ECS, measured as inulin space, amounted to 350 ml./kg wet wt. at 35° C and to 300 ml./kg wet wt. at 4–6° C.

5. $[K]_i$ decreased during cooling and increased during rewarming the auricles. $[Na]_i$ increased during hypothermia in bathing fluids containing NaCl, but decreased in NaCl- and Na-free solutions. At the beginning of rewarming a net Na transport occurred from cells with high $[Na]_i$, while a net Na uptake took place in atria with low $[Na]_i$.

6. At the same time, the membrane potential of auricles with increased $[Na]_i$ hyperpolarized beyond the steady-state value recorded at the end of rewarming, or even beyond the calculated K^+ equilibrium potential (E_K). Afterwards, the hyperpolarization levelled off, while the E_K values increased further. The membrane potential of atria with decreased $[Na]_i$ showed no transitory hyperpolarization during rewarming.

7. The hyperpolarization beyond the steady-state value of membrane potential in rewarmed auricles was significantly correlated to the active Na efflux.

8. From these results it is concluded that the membrane potential of guinea-pig atria after hypothermia is affected by an active, electrogenic Na pump activated by intracellular Na ions.

INTRODUCTION

It was first postulated by Connelly (1959) and Straub (1961) that active Na transport contributes directly to the membrane potential of frog myelinated nerve fibres during and after tetanic stimulation. The authors suggested therefore the existence of an electrogenic Na pump in the membrane of nerve fibres, an idea previously discussed by Hodgkin & Keynes (1955). Subsequent experiments by several workers (Kernan, 1962; Cross, Keynes & Rybovà, 1965; Frumento, 1965; Mullins & Award, 1965; Adrian & Slayman, 1966; Harris & Ochs, 1966) clarified the characteristics of an active, electrogenic sodium transport in frog skeletal muscle. In recent years, evidence for electrogenic Na pumping has been obtained in numerous tissues from different animals. For example, an electrogenic Na pump has been found in some stretch receptor neurones of crayfish (Nakajima & Takahashi, 1966), in molluscan neurones (Kerkut & Thomas, 1965; Carpenter & Alving, 1968; Thomas, 1969; Carpenter, 1970; Marmor & Gorman, 1970), in non-myelinated vagus fibres of rabbit (Rang & Ritchie, 1968), in rat myometrium (Taylor, Paton & Daniel, 1969, 1970) and in guinea-pig taenia coli (Casteels, Hendrickx & Drogmans, 1970). An electrogenic Na pump does also exist in cat heart muscle as suggested by Page & Storm (1965), who studied the active Na transport in papillary muscle. Further evidence for an active, electrogenic Na extrusion in mammalian heart muscle was obtained by Tamai & Kagiyama (1968) and Glitsch (1969).

As in snail nerve cells (e.g. Kerkut & Thomas, 1965), frog skeletal muscle (e.g. Mullins & Award, 1965) and smooth muscle cells (Taylor *et al.* 1969), the electrogenic Na pump of mammalian heart muscle is activated by extracellular K ions (e.g. Tamai & Kagiyama, 1968; Glitsch, 1969). Furthermore, the effect of active Na transport on the membrane potential is augmented by an increase in the intracellular Na concentration of nerve and skeletal muscle cells (e.g. Kerkut & Thomas, 1965; Frumento, 1965). Little is known so far about the activation of the electrogenic Na pump in heart muscle by internal Na ions. The experiments described here present evidence for a correlation between the quantity of active Na extrusion which is affected by the intracellular Na concentration and the hyperpolarization of membrane potential in cardiac cells following hypothermia. This hyperpolarization may reach values beyond the calculated K^+ equilibrium potential. The results, therefore, suggest an activation of the electrogenic Na pump in guinea-pig auricles by internal sodium ions. A preliminary report of some of the results was given to the Deutsche Physiologische Gesellschaft 1970 (Glitsch, 1970).

METHODS

Principles of the method. The experiments were performed by using a modification of Kernan's method (Kernan, 1962). The method is based on the following principles: In K-poor solution, the active Na transport of the preparation is inhibited by hypothermia. At the same time, the intracellular Na concentration rises while the cell loses potassium. Rewarming in K-containing solution causes an enhanced Na extrusion from the cell and a reaccumulation of K within the cell. Hyperpolarization of the membrane potential beyond the calculated K^+ equilibrium potential (E_K) may suggest the presence of an electrogenic Na pump contributing directly to the membrane potential of the tissue during rewarming. Cooling the preparations in solutions with different Na concentrations alters the intracellular Na content. Using this method, the effect of various intracellular Na concentrations on the membrane potential was investigated during rewarming in normal Tyrode solution. Under similar conditions, the extracellular space (ECS) and the Na and K content of the preparation were measured at different intervals during the experiment. From these data, the intracellular Na ($[Na]_i$) and K($[K]_i$) concentrations and the E_K values were calculated.

Material. Isolated guinea-pig atria were used (animal weight $\sim$ 200 g).

Solutions. The compositions of the various bathing solutions used are listed in Table 1. Except for solution 7, all solutions were bubbled with carbogen (95 % O_2, 5 % CO_2; pH 7·2–7·4).

Experimental procedure. The isolated atria were first equilibrated for 30 min in normal Tyrode solution (solution 1) at 35° C, and then cooled at 4–6° C in solutions 2–7 for 4 hr. Finally, the auricles were rewarmed for 60 min in normal Tyrode solution.

Voltage recording. For measurements of the membrane potential, the preparation was fixed in a perfusion chamber of 1 ml. volume. The bathing solution flowed through this chamber at 12 ml./min. Before and after hypothermia the membrane potential of the atria was recorded intracellularly by means of the usual microelectrode technique. Recording was started 5 min after switching to normal Tyrode solution at 35° C. In control experiments, this time was found to be sufficient to obtain a steady membrane potential after changing the bathing fluid from a K-poor to a K-rich solution.

Analytical procedures. The extracellular space was measured as inulin space. For this purpose the auricles were bathed in modified Tyrode solutions containing inulin 150 mg./100 ml. The preparations were then blotted under constant pressure on filter paper and weighed. After removal of protein, the inulin concentration of the atria was determined photometrically (cf. Handelsman & Drabkin, 1954). The water content of the auricles $(H_2O)_T$ was calculated in the usual manner from the difference between wet and dry weight.

The Na and K concentrations of the tissue were measured by flame photometry after ashing the preparations and dissolving the ashes in 0·5 ml. 0·1 N-HCl and 1·5 ml. distilled water.

The intracellular cation concentration (C_i) could then be calculated from the extracellular space, the water content, the cation concentration of the preparation (C_T), and the cation concentration in the bathing fluid (C_e) by

$$C_i = \frac{C_T - C_e \times \text{ECS}}{(H_2O)_T - \text{ECS}} \text{ [m-mole/l. fibre water]} \quad (1)$$

if C_T is expressed as [m-mole/kg wet wt.], C_e as [m-mole/l.] and ECS and $(H_2O)_T$ as [l./kg wet wt.].

TABLE 1. Composition of bathing solutions (mM)

	NaCl	Choline-Cl	KCl	$CaCl_2$	$MgCl_2$	NaH_2PO_4	$NaHCO_3$	Glucose
Solution 1								
Tyrode solution	137	—	5·4	1·8	1·05	0·42	11·9	5
Solution 2								
Tyrode solution with 1·35 mM-$[K]_e$	137	—	1·35	1·8	1·05	0·42	11·9	5
Solution 3*								
Tyrode solution with 1·35 mM-$[K]_e$, 50 % (68·5 mM) NaCl	68·5	68·5	1·35	1·8	1·05	0·42	11·9	5
Solution 4								
Tyrode solution with 1·35 mM-$[K]_e$, 25 % (34·25 mM) NaCl	34·25	102·75	1·35	1·8	1·05	0·42	11·9	5
Solution 5								
Tyrode solution with 1·35 mM-$[K]_e$, 10 % (13·7 mM) NaCl	13·7	123·3	1·35	1·8	1·05	0·42	11·9	5
Solution 6								
Tyrode solution with 1·35 mM-$[K]_e$ without NaCl	—	137	1·35	1·8	1·05	0·42	11·9	5
Solution 7†								
Na-free Tyrode solution with 1·35 mM-$[K]_e$	—	137	1·35	1·8	1·05	—	—	5

* In one series 68·5 mM-NaCl was replaced by 137 mM sucrose.

† Tris-buffer (5 mM tris(hydroxymethyl)-aminomethane + HCl, pH 7·4). Bubbled with 100 % O_2.

K^+ equilibrium potential. From the Nernst equation the K^+ equilibrium potential (E_K) at 35° C is derived by

$$E_K = 61 \times \log_{10} \frac{[K]_e}{[K]_i} \quad (mV) \tag{2}$$

and E_K at 5° C by

$$E_K = 55 \times \log_{10} \frac{[K]_e}{[K]_i} \quad (mV). \tag{3}$$

Statistical methods. Whenever possible, values are presented as means ± S.E. of means. The significance of difference of means was checked by Student's (t) test. n indicates the number of measurements.

RESULTS

Inulin space. For calculations of the intracellular cation concentrations during the experiments, appropriate values of the extracellular space are necessary. Therefore, the inulin space was measured in normal Tyrode solution at 35° C, in K-poor solutions at 4° C and in normal Tyrode solution at 35° C after hypothermia in a K-poor, Na-free incubation fluid. As indicated in Table 2 the value of the inulin space remained constant between the 30 and 60 min, both in normal Tyrode solution at 35° C and in K-poor solution at 4° C (series 1 and 2). These values were assumed to be representative for the extracellular space of the atria under the respective experimental conditions. During hypothermia in K-poor solution (series 2) the inulin space of the tissue was significantly diminished compared to the inulin space in normal Tyrode solution at 35° C (series 1) ($P < 0{\cdot}01$). Cooling the auricles in a Na-free, K-poor solution had no effect on the value of the inulin space during or after hypothermia (series 3 and 4). From these results the extracellular space of the atria was assumed to be about 300 ml./kg wet wt. during hypothermia and about 350 ml./kg wet wt. before and after cooling. These values are employed in further calculations.

$[K]_i$, $[Na]_i$, membrane potential and E_K after hypothermia. A. Hypothermia in K-poor Tyrode solution. Following equilibration, the auricles in this series were cooled for 4 hr in K-poor Tyrode solution (Table 1, solution 2) at 4° C. Afterwards they were rewarmed in normal Tyrode solution (Table 1, solution 1) for 60 min at 35° C. At determinate times during the course of the experiment some of the atria were removed from the bathing fluid and their cation and water content were measured. Under similar conditions the membrane potential of ten quiescent left auricles was recorded before and after hypothermia. At the end of the equilibration period $[Na]_i$ and $[K]_i$ amounted to $22{\cdot}9 \pm 2{\cdot}74$ m-mole/l. fibre water (f.w.) and $133{\cdot}0 \pm 9{\cdot}45$ m-mole/l. f.w. respectively. The membrane potential was about -70 mV (inside negative). These values are in accordance with measurements reported in the literature (cf. Glitsch, 1969). During the

TABLE 2. Inulin space [ml./kg wet wt.] of guinea-pig auricles under various experimental conditions

Series	Pre-treatment	Treatment	Time in inulin Tyrode solution: 10 min	30 min	40 min	60 min
1	30 min equilibration in Tyrode solution, $[K]_e$ 5·4 mM, at 35° C	Tyrode solution containing inulin, $[K]_e$ 5·4 mM, at 35° C	$n = 9$ $281 \pm 15{\cdot}1$	$n = 8$ $372 \pm 31{\cdot}8$	—	$n = 7$ $369 \pm 37{\cdot}3$
2	As in series 1	Tyrode solution containing inulin, $[K]_e$ 1·35 mM at 4° C	$n = 9$ $191 \pm 31{\cdot}2$	$n = 10$ $301 \pm 19{\cdot}8$	—	$n = 8$ $296 \pm 19{\cdot}9$
3	Additional 3·5 hr in Na-free solution, $[K]_e$ 1·35 mM at 4° C	Na-free solution containing inulin, $[K]_e$ 1·35 mM at 4° C	—	$n = 6$ $294 \pm 55{\cdot}8$	—	—
4	As in series 3. Additional 30 min in Na-free solution, $[K]_e$ 1·35 mM at 4° C	Tyrode solution containing inulin, $[K]_e$ 5·4 mM at 35° C	—	—	$n = 10$ $337 \pm 56{\cdot}7$	—

experiment there was no marked change in the water content of the preparations (about 800 ml./kg wet wt.). Fig. 1 shows the time course of the membrane potential during rewarming, together with the intracellular cation concentrations and the calculated K^+ equilibrium potential of the rewarmed atria. As expected, a gain of $[Na]_i$ and a loss of $[K]_i$ took place during cooling. Based on the low extracellular K concentration, the E_K value of the auricles increases during hypothermia. Rewarming causes a

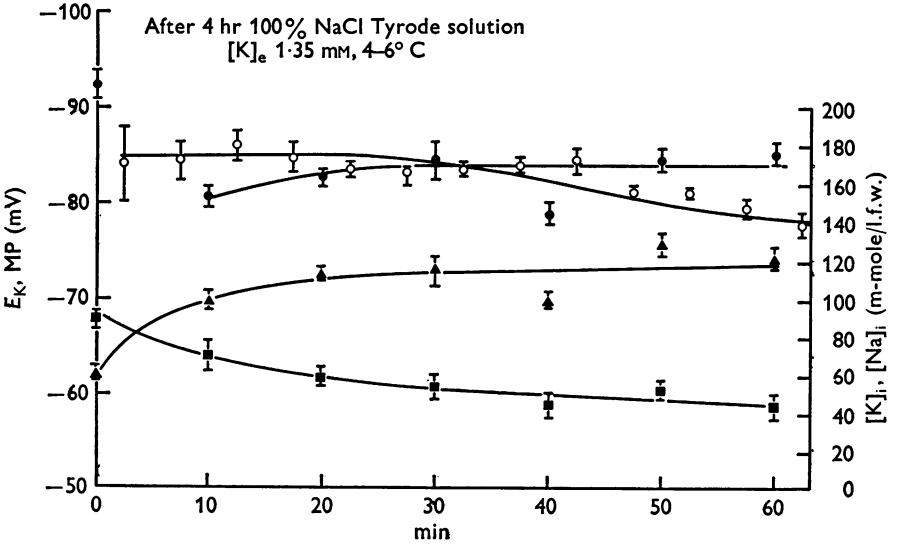

Fig. 1. K^+ equilibrium potential (E_K), membrane potential (MP) and intracellular K and Na concentrations ($[K]_i$ and $[Na]_i$) during rewarming of the auricles in series *A*. Left ordinate: E_K (●) and MP (○) (mV). Right ordinate: $[K]_i$ (▲) and $[Na]_i$ (■) (m-mole/l. fibre water). Abscissa: time (min). Following hypothermia in Tyrode solution, $[K]_e$ 1·35 mM.

net Na transport out of the cells, and therefore a decrease of $[Na]_i$, while $[K]_i$ increases. During the enhanced Na pumping the membrane potential hyperpolarizes beyond the resting potential. The hyperpolarization is maintained for up to 90 min, at which time the membrane potential reaches $-74{\cdot}2 \pm 1{\cdot}15$ mV (not shown in Fig. 1). Although the membrane potential seems to hyperpolarize beyond E_K during the beginning of rewarming, the significance of the difference could not be established at the 5% level ($0{\cdot}1 > P > 0{\cdot}05$).

B. Hypothermia in K-poor Tyrode solution containing 50% (68·5 *mM*) *NaCl.* Differing from series *A*, the atria of series *B* were cooled for 4 hr in a K-poor Tyrode solution, in which 68·5 mM-NaCl was replaced by a corresponding amount of choline-Cl (Table 1, solution 3). Rewarming was performed again in Tyrode solution (Table 1, solution 1) at 35° C. As before,

the water and cation content of the preparations were determined during the experiments. From these measurements and the known size of the extracellular space, $[Na]_i$, $[K]_i$, and the E_K values were calculated. Again, the water content of the auricles did not change appreciably throughout the experiments. Fig. 2 presents values of the membrane potential of 10 left atria after hypothermia.

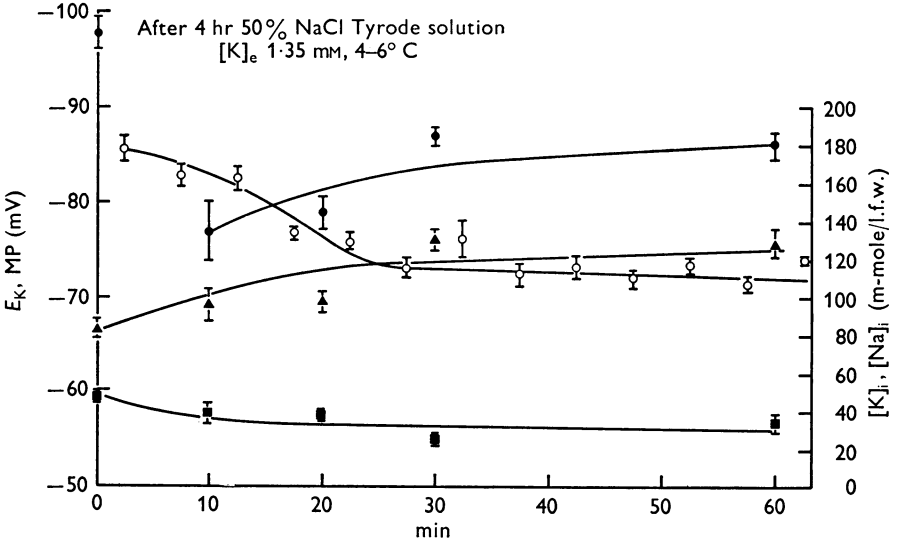

Fig. 2. K^+ equilibrium potential (E_K), membrane potential (MP) and intracellular K and Na concentrations ($[K]_i$ and $[Na]_i$) during rewarming of the auricles in series *B*. Left ordinate: E_K (●) and MP (○) (mV). Right ordinate: $[K]_i$ (▲) and $[Na]_i$ (■) (m-mole/l. fibre water). Abscissa: time (min). Following hypothermia in Tyrode solution containing 50% (68·5 mM) NaCl, $[K]_e$ 1·35 mM.

The corresponding intracellular Na and K concentrations and K^+ equilibrium potentials are also shown in the Figure. Due to the decrease in the Na concentration of the bathing fluid, the increase in $[Na]_i$ during hypothermia is less than in the previous series. As a consequence, the net Na transport out of the rewarmed cells is diminished. During the first 10 min of rewarming the membrane potential significantly exceeds the calculated E_K value ($P < 0{\cdot}025$). The hyperpolarization of the membrane potential decreases earlier than in the preceding experiments, and therefore the steady state of the membrane potential is reached earlier.

C. Hypothermia in K-poor Tyrode solution containing 25% (34·25 *mM*) *NaCl.* In this series hypothermia was performed in a K-poor Tyrode solution containing only 25% (34·25 mM) of the usual NaCl concentration of Tyrode solution, NaCl being replaced by choline-Cl (Table 1, solution 4).

Other experimental conditions remained unchanged. Mean values of the membrane potentials, of the intracellular Na and K concentrations and of E_K as determined in atria after hypothermia are presented in Fig. 3.

In the Na- and K-poor solution hypothermia causes only a small increase in $[Na]_i$. The intracellular Na concentration of the rewarmed auricles first diminishes, but later shows an increase. After 60 min of rewarming the Na concentration amounts to $60 \pm 9{\cdot}37$ m-mole/l. f.w.

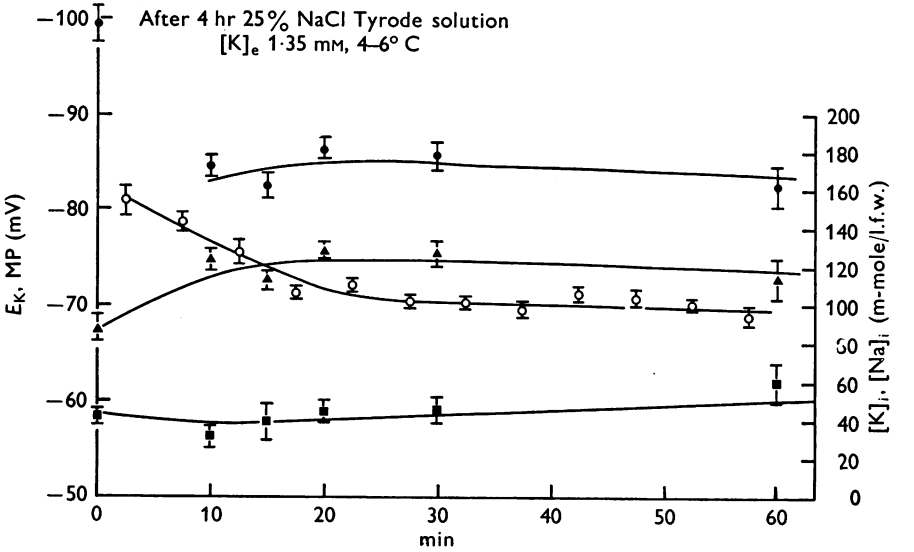

Fig. 3. K^+ equilibrium potential (E_K), membrane potential (MP) and intracellular K and Na concentrations ($[K]_i$ and $[Na]_i$) during rewarming of the auricles in series *C*. Left ordinate: E_K (●) and MP (○) (mV). Right ordinate: $[K]_i$ (▲) and $[Na]_i$ (■) (m-mole/l. fibre water). Abscissa: time (min). Following hypothermia in Tyrode solution containing 25% (34·25 mM) NaCl, $[K]_e$ 1·35 mM.

within the cells. Such a extraordinarily high value was measured only in this series. Therefore, it cannot be excluded that several damaged atria were responsible for this result. In contrast to the movement of Na ions, the intracellular K concentration rises at first during rewarming and then decreases. Compared to the Figs. 1 and 2, the intracellular Na concentration as well as the net Na transport from the cells at the beginning of the rewarming period are reduced. At the same time, the membrane potential does not hyperpolarize to the values recorded in series *A* and *B*, but only to −81 mV. As opposed to the foregoing series the membrane potential during rewarming does not exceed the calculated E_K values. Furthermore, the hyperpolarization diminishes earlier than in the experiments reported previously.

D. Hypothermia in K-poor Tyrode solution containing 10% (13·7 *mM*) *NaCl.* In series *D* the auricles were cooled in a K-poor Tyrode solution, in which 90% (123·3 mM) of the NaCl was replaced by an equivalent amount of choline-Cl (Table 1, solution 5). After hypothermia the intracellular Na concentration amounts to 39·2 ± 2·77 m-mole/l. f.w. (Fig. 4). A net Na transport from the cells cannot be definitively ascertained at the beginning of rewarming. Simultaneously the membrane potential hyperpolarizes only about 3–4 mV beyond the steady-state value of the rewarmed preparations. 10 min after the beginning of the rewarming period

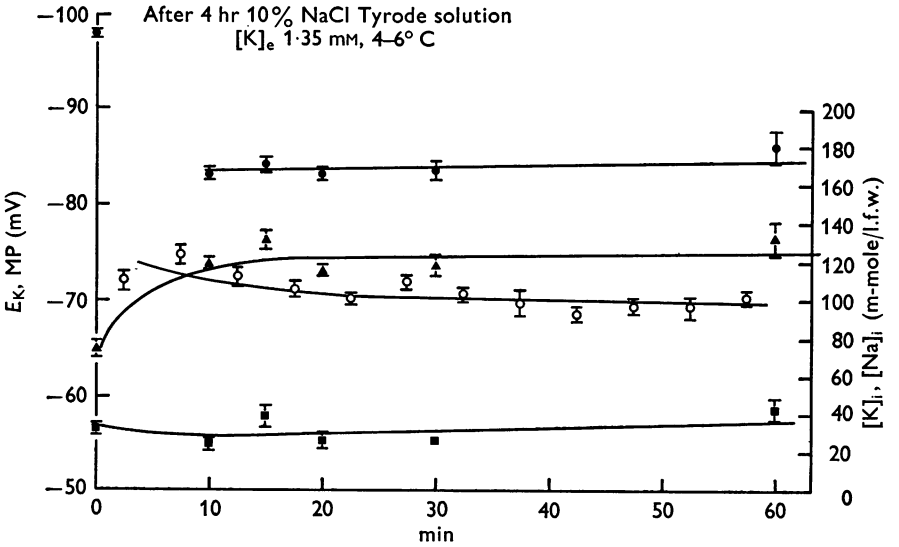

Fig. 4. K^+ equilibrium potential (E_K), membrane potential (MP) and intracellular K and Na concentrations ($[K]_i$ and $[Na]_i$) during rewarming of the auricles in series *D*. Left ordinate: E_K (●) and MP (○) (mV). Right ordinate: $[K]_i$ (▲) and $[Na]_i$ (■) (m-mole/l. fibre water). Abscissa: time (min). Following hypothermia in Tyrode solution containing 10% (13·7 mM) NaCl, $[K]_e$ 1·35 mM.

the membrane potential is 10 mV less negative than the calculated K^+ equilibrium potential.

E. Hypothermia in K-poor Tyrode solution without NaCl. With the other conditions as in series *A–D* hypothermia was induced in series *E* in a NaCl-free Tyrode solution. All NaCl in the bathing fluid was substituted by choline-Cl (Table 1, solution 6). In contrast to the previous series, hypothermia in NaCl-free solution caused a decrease in the intracellular Na concentration ($[Na]_i$ after equilibration: 29·2 ± 5·86 m-mole/l. f.w.; after hypothermia: 19·9 ± 2·26 m-mole/l. f.w.). During rewarming there was no net Na transport from the cardiac cells. On the contrary, as can be

seen from Fig. 5, a slow increase in the intracellular Na concentration takes place. At the same time the membrane potential does not hyperpolarize beyond the value measured at the end of the rewarming period. The membrane potential of the atria does not alter appreciably during rewarming and remains 16–20 mV less negative than the corresponding K^+ equilibrium potentials.

F. Hypothermia in K-poor, Na-free Tyrode solution. In series *F* the auricles were cooled in a Na-free, tris-buffered solution containing 1·35 mM-K (Table 1, solution 7). Hypothermia in this Na-free bathing fluid caused a

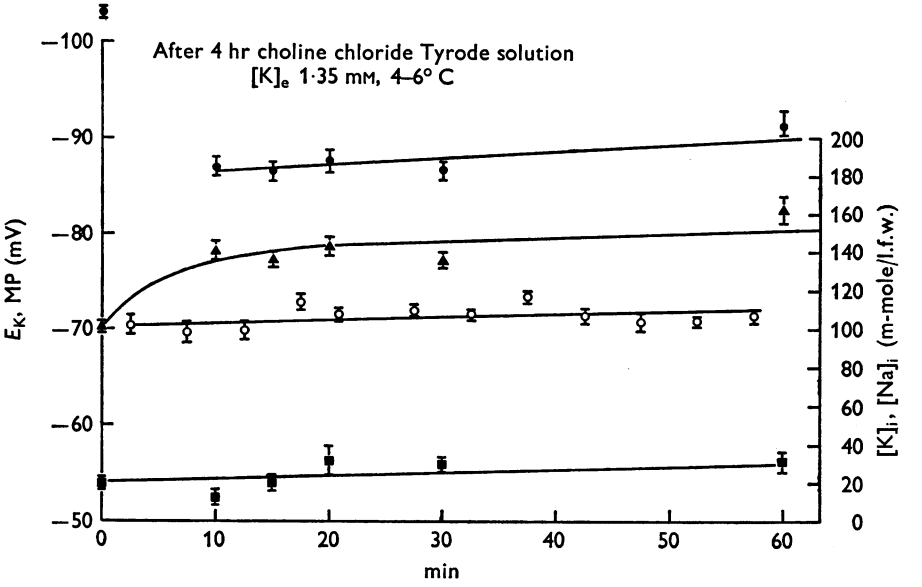

Fig. 5. K^+ equilibrium potential (E_K), membrane potential (MP) and intracellular K and Na concentrations ($[K]_i$ and $[Na]_i$) during rewarming of the auricles in series *E*. Left ordinate: E_K (●) and MP (○) (mV). Right ordinate: $[K]_i$ (▲) and $[Na]_i$ (■) (m-mole/l. fibre water). Abscissa: time (min). Following hypothermia in Tyrode solution without NaCl, $[K]_e$ 1·35 mM.

distinct decrease in the intracellular Na concentration ($[Na]_i$ after equilibration 26·3 ± 8·62 m-mole/l. f.w.; after hypothermia 9·75 ± 3·08 m-mole/l. f.w.). It is difficult to determine whether the remaining Na fraction was located in the extracellular or intracellular space of the preparations. As in series *E* a net Na transport *into* the cells increases the intracellular Na concentration during rewarming (Fig. 6), while the membrane potential does not hyperpolarize at all. Throughout the period of rewarming the membrane potential remains constant.

Relation between active Na efflux and hyperpolarization in rewarmed auricles. When the intracellular Na concentration had been increased during hypothermia, rewarming caused a net Na transport out of the cells. During this enhanced Na extrusion the membrane potential hyperpolarized beyond the steady-state value of rewarmed atria or even beyond the calculated K^+ equilibrium potential (series *A* and *B*). On the other hand, there was neither a net Na transport from the cells nor a hyperpolarization in rewarmed auricles in which the intracellular Na concentration was

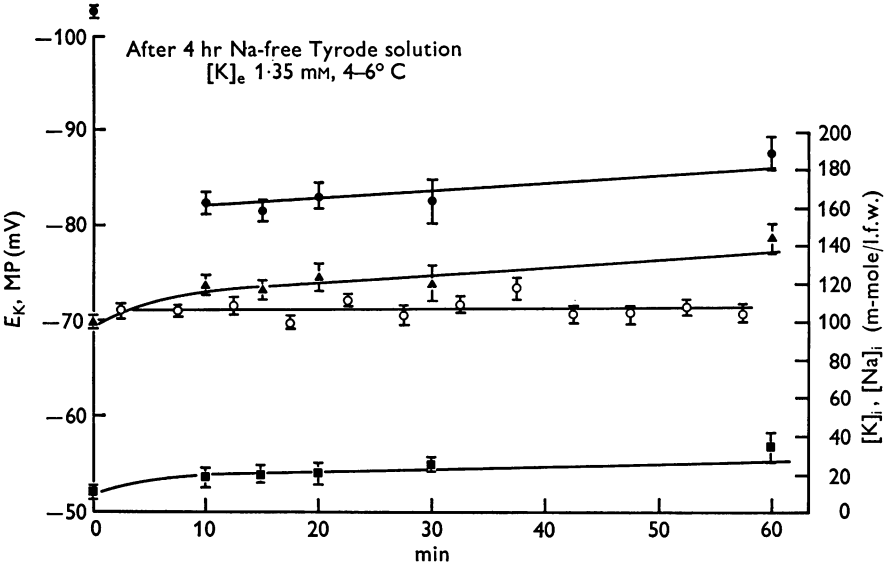

Fig. 6. K^+ equilibrium potential (*E*), membrane potential (MP) and intracellular K and Na concentrations ($[K]_i$ and $[Na]_i$) during rewarming of the auricles in series *F*. Left ordinate: E_K (●) and MP (○) (mV). Right ordinate: $[K]_i$ (▲) and $[Na]_i$ (■) (m-mole/l. fibre water). Abscissa: time (min). Following hypothermia in Na-free Tyrode solution, $[K]_e$ 1·35 mM.

decreased during hypothermia (series *E* and *F*). Therefore, the results of the experiments lend support to the hypothesis of an electrogenic Na pump contributing directly to the membrane potential of the atria during rewarming. If this is correct, a quantitative relation should exist between the active Na efflux from rewarmed auricles and the hyperpolarization of the membrane potential. From the experimental data the quantity of active Na efflux at the beginning of the rewarming period can be *estimated* in the following way:

The net movement of Na ions ($M_{Na\ net}$) across the cell membrane is related to the Na influx (M_{iNa}), and Na efflux (M_{eNa}) by

$$M_{Na\ net} = M_{eNa} - M_{iNa}. \qquad (4)$$

Application of the constant field equation (cf. Goldman, 1943; Hodgkin & Katz, 1949) to the Na influx yields

$$M_{1Na} = -P_{Na} \frac{FV}{RT} \frac{[Na]_e}{1 - e^{VF/RT}} \quad (5)$$

if one considers the direction of Na efflux as positive. P_{Na} is the permeability of the cell membrane for Na ions, V the membrane potential, and R, F and T have their usual meanings. From eqn. (4) the Na efflux can be derived easily if the net Na transport and the Na influx are known. From the present experiments in each series the net Na transport after 10 min rewarming was determined graphically from the size and the direction of the variation in the intracellular Na concentration. The simultaneous Na influx was estimated as follows: after hypothermia in Na-free

TABLE 3. Membrane potential, Na influx and net Na transport rates of the auricles in series *A–F* after 10 min of rewarming

Series	Membrane potential (mV)	Na influx (m-mole/l. f.w. min)	Net Na transport rate* (m-mole/l. f.w. min)
A	−85·3	1·50 × 1·16 = 1·74†	+1·5
B	−82·5	1·50 × 1·12 = 1·68	+0·8
C	−77·2	1·50 × 1·06 = 1·59	+0·2
D	−73·5	1·50 × 1·01 = 1·52	+0·1
E	−70·0	1·50 × 0·98 = 1·47	−0·1
F	−71·7	1·50 × 1·0 = 1·50	−0·4

* + indicates net Na-transport out of the cells.

† Na influx into rewarmed atria of series *F* times correction factor for differences in driving force relative to series *F*. For explanation see text.

solution, rewarming caused an increase in the intracellular Na concentration (series *F*, Fig. 6). The initial slope of this increase allows a graphical estimation of the Na influx into the atria of series *F*. According to eqn. (5) the Na influx is determined by the extracellular Na concentration, the Na permeability of the cell membrane and by a term, which represents the driving force of the membrane potential. This term varies, of course, from one series of experiments to the next, depending on the membrane potential of the rewarmed atria. Assuming P_{Na} and $[Na]_e$ to be constant during rewarming throughout the experiments, the Na influxes in series *A–E* differ from the Na influx in series *F* only in respect to the various driving forces of the membrane potential. Therefore, in order to obtain values for Na influx during rewarming in series *A–E* the Na influx in series *F* must be corrected by a factor which denotes the driving force of the membrane

potential in each series relative to the driving force in series F. This factor was derived from eqn. (5). Table 3 shows the mean values of the membrane potentials, the net Na transport rates and the Na influxes calculated from the first 10 min of rewarming in series A–F. As may be expected, the Na influx increases when the membrane potential of the rewarmed auricles increases. The active Na efflux of the atria was calculated from eqn. (4). The hyperpolarization of the membrane potential depending on the active Na efflux of the auricles after 10 min of rewarming is shown in Fig. 7. If

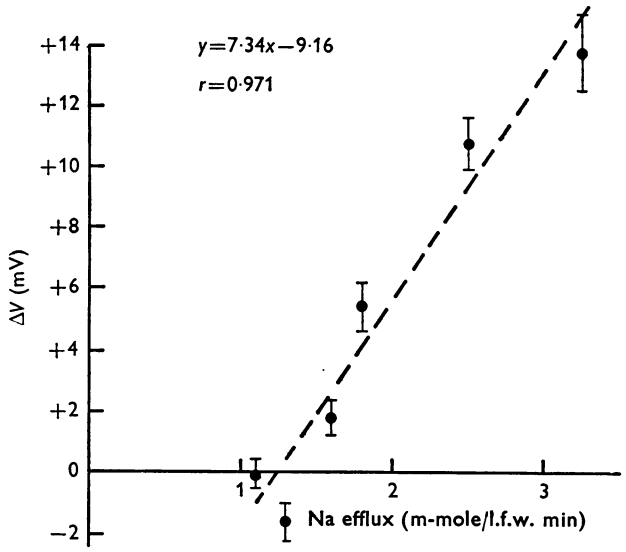

Fig. 7. Effect of active Na efflux on hyperpolarization of the membrane potential after 10 min of rewarming in series A–F. Ordinate: hyperpolarization of the membrane potential beyond the steady-state value at the end of rewarming (ΔV) (mV). Abscissa: active Na efflux (m-mole/l. fibre water min). Correlation coefficient: 0·971 ($P < 0{\cdot}01$). Equation of the regression line: $y = 7{\cdot}34x - 9{\cdot}16$.

the active Na extrusion is enhanced by an increase in the intracellular Na concentration, the hyperpolarization of the membrane potential increases as well. The correlation coefficient between both events is 0·97 suggesting a strong correlation between the hyperpolarization of the membrane potential and the active Na efflux of the atria after hypothermia ($P < 0{\cdot}01$).

Control experiments. It may be argued that at the beginning of rewarming the membrane potential of the auricles is mainly determined by the Cl^- equilibrium potential (E_{Cl}). Therefore, seven control experiments were performed, in which the effect of the E_{Cl} value on the membrane potential during rewarming was measured. For this purpose the atria were cooled in a K-poor Tyrode solution with 50 % (68·5 mM) of the usual NaCl concentration, the other 50 % being replaced by an

appropriate amount of sucrose (cf. Table 1, solution 3). Afterwards the membrane potential of the rewarmed auricles was recorded intracellularly in normal Tyrode solution. The results were compared with the membrane potentials of the rewarmed atria in series *B*. The intracellular Cl concentration of the auricles was assumed to be different in both series after hypothermia, because of the differing Cl contents of the solutions used during this period. Based on the different intracellular Cl concentrations at the beginning of rewarming, the E_{Cl} values of the atria should also be different in both groups. Fig. 8 shows the membrane potentials of the auricles in both series during rewarming. As illustrated by the Figure, the membrane potentials of the atria in both groups are nearly identical, although the E_{Cl} values probably differ. From this result it is concluded that the Cl^- equilibrium potential has no major effect on the membrane potential of the auricles during rewarming.

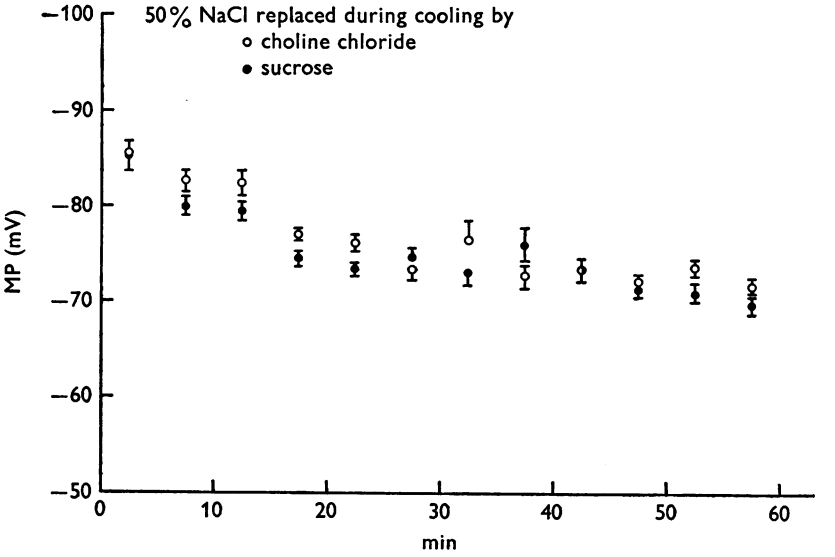

Fig. 8. Membrane potential of auricles during rewarming in normal Tyrode solution. Following hypothermia in Tyrode solution containing 50 % of the usual NaCl concentration, $[K]_e$ 1·35 mM. Replacement of NaCl by choline chloride (○) resp. sucrose (●). Ordinate: membrane potential (MP) (mV). Abscissa: time (min).

DISCUSSION

Before a definitive conclusion can be drawn from the experiments some of the results must be discussed in greater detail. As previously stated, the hyperpolarization of the membrane potential beyond the calculated E_K value during rewarming cannot be explained by an inaccuracy in the determination of the membrane and/or K^+ equilibrium potential (Glitsch, 1969). Especially, the hyperpolarization is not caused by a delayed equilibration of the extracellular space after switching from the K-poor to the K-rich bathing fluid at the beginning of the rewarming period. This

is clearly shown in series *E* and *F* (Figs. 5 and 6), where no hyperpolarization is observed under these conditions. Furthermore, the hyperpolarization is not affected by a variation of E_{Cl} as can be seen from Fig. 8. This is in line with reports of several authors who have shown that the hyperpolarization of the membrane potential in rewarmed fibres of frog skeletal muscles is not due to a shift of the Cl^- equilibrium potential to more negative values (Kernan, 1962; Kernan & Tangney, 1964; Cross *et al.* 1965). Measurements of the Cl concentration in papillary muscles of the cat have revealed an increase in the intracellular Cl content during hypothermia followed by a slow decrease during rewarming (Page & Storm, 1965). Obviously, the transitory hyperpolarization of the membrane potential in rewarmed auricles (series *A–D*) is not caused by a corresponding change of E_{Cl}. In addition, Fig. 8 presents evidence for the absence of any cholinergic effect due to intracellular accumulation of choline ions. A connexion between hyperpolarization and active Na transport is suggested primarily by the following results: First, the hyperpolarization of the membrane potential at the beginning of rewarming always occurs simultaneously with the enhanced Na extrusion from the cardiac cells. Secondly, the mechanism responsible for this hyperpolarization demonstrates some characteristics in common with the active Na pump of the atria. As can be seen from Figs. 1–6, a variation of the intracellular Na concentration affects both the hyperpolarization of the membrane potential and the net Na transport from the auricles at the beginning of the rewarming period. An increase in the Na concentration of the cells increases the hyperpolarization as well as the quantity of Na extruded from the intracellular space (Figs. 1 to 3). On the other hand, a decrease in the intracellular Na concentration during hypothermia in a Na-poor solution diminishes the Na extrusion from rewarmed atria and a hyperpolarization is not measured during rewarming (Figs. 5 and 6). Furthermore, the mechanism causing hyperpolarization is not activated by intracellular choline ions (Figs. 2 to 6) and neither is the active Na pump in cardiac muscle (Bosteels, Vleugels & Carmeliet, 1970).

Surprisingly, the intracellular K concentration of the auricles which had accumulated choline ions increased during rewarming as fast as that of atria with high intracellular Na concentration (compare Fig. 1 with Fig. 5). The reason for the K accumulation in Na-poor, choline-rich cardiac cells at the beginning of the rewarming period is not known. One could, however, speculate that an exchange of intracellular choline ions for extracellular K ions plays an important role in this K uptake (cf. Bosteels *et al.* 1970).

Thirdly, the calculation of the active Na efflux reveals a significant correlation between the amount of active sodium extrusion and the degree of hyperpolarization at the beginning of the rewarming period (Fig. 7).

In the calculation it is assumed that P_{Na} remains constant during rewarming in the series *A–F*, i.e. that it is independent of $[Na]_i$. This assumption is, of course, unproved, but seems reasonable for the sake of simplicity in the absence of experimental data referring to this problem in cardiac muscle.

In summary, it may be inferred from the results that the hyperpolarization of the membrane potential during rewarming is due to an enhanced active Na efflux. This conclusion is supported by the work of several authors. Page & Storm (1965) and later Tamai & Kagiyama (1968) have shown that the hyperpolarization in rewarmed heart tissues can be abolished by cardiac glycosides, which are known to be rather specific inhibitors of active Na transport. Furthermore, rewarming guinea-pig auricles in K-free solution causes neither a net Na transport from the cells nor a transitory hyperpolarization of the membrane potential (Glitsch, 1969).

At the beginning of the rewarming period the membrane potential of the atria with high intracellular Na concentration hyperpolarized beyond the steady-state value recorded at the end of this period. Afterwards the hyperpolarization decreased, while the K^+ equilibrium potential increased further. In series *B* the hyperpolarization even reached values significantly more negative than the calculated E_K value. If the hyperpolarization is caused by the active Na transport of the auricles, then these findings suggest a Na pump with electrogenic characteristics rather than a neutral Na–K exchange pump. They confirm, therefore, the results of the workers cited above, indicating an electrogenic active Na transport in cardiac muscle. In addition, the experiments reported here suggest an activation of the electrogenic Na pump in guinea-pig atria by intracellular Na ions.

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