The Existence of Electrogenic Na—Ca Exchange in Mammalian Heart Muscle

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Abstract. Membrane potentials were recorded from isolated rabbit papillary muscles at normal external sodium concentration and during perfusion with Na-free solution. During perfusion with Na-free solution (Na replaced by sucrose) hyperpolarization of about 20 mV was observed. This hyperpolarization reached its maximum after about 85 s and declined to a steady value within about 3 min. A mean maximal hyperpolarization of -108.9 ± 14.8 mV was observed. It exceeded the expected K equilibrium potential (about -80 mV, for external K^+ 4 mmol/l; Fozzard and Lee 1976). The addition of 3 mmol $LaCl_3$ irreversibly suppressed the Na-free hyperpolarization. These results indicate that Na-free hyperpolarization in adult mammalian heart muscle is related to electrogenic Na/Ca exchange.

Key words: Heart muscle — Na-free hyperpolarization — Electrogenic transport — Na–Ca exchange

Introduction

The existence of electrogenic Na–Ca exchange in mammalian heart muscle is still uncertain. The first experimental evidence for such a mechanism in intact preparations of mammalian heart was presented by Coraboeuf et al. (1980). These authors described hyperpolarization and a concurrent increase in mechanical tension resulting from perfusion with Na-free solution. However, all their experiments were performed in K-free solutions. The range of the potassium equilibrium potential remained therefore undefined. Crucial experimental evidence for the existence of electrogenic transport would be a hyperpolarization exceeding the K equilibrium potential. Experiments presented herein were directed to testing this hypothesis.

Material and Methods

Rabbits of either sex weighing 0.8—1.5 kg were sacrificed by cervical dislocation. The right ventricular papillary muscles were isolated and placed in a tissue bath where they were perfused with oxygenated Tyrode solution (30°C, flow rate 10 ml/min; bath volume 3 ml). The muscles were stimulated before changing the control solution to Na-free saline. The stimulation was stopped about 2 min before starting Na-free perfusion. Transmembrane potentials were recorded by means of conventional glass micro-electrodes filled with 3 mol KCl. The transmembrane potentials were recorded using an ENDIM 621.01 penrecorder (Schlotheim, GDR).

The normal Tyrode solution contained (in mmol/l): 150 NaCl, 4 KCl, 2.5 $CaCl_2$, 0.5 $MgCl_2$, 10 Tris HCl (pH 7.2—7.4), 11 glucose; it was saturated with 100% O_2. In Na-free solutions Na was replaced by sucrose (300 mmol/l). For better ionization $LaCl_3$ (3 mmol/l) experiments were performed at a pH of 7.0—7.2.

Results

Membrane potentials were shifted in a hyperpolarizing direction in Na-free solutions. These changes occurred with a latency of 24.5 ± 9.7 s, due mainly to the dead-volume of the perfusion system during the change of solution and the diffusion time in a multifibre preparation.

About 85 s after starting the test perfusion a mean maximum of nearly 30 mV was reached (Fig. 1). The hyperpolarization then slowly declined reaching a steady state value after about 3 min that was more negative than the control resting potential. After the Na-free perfusion was discontinued the membrane potential generally reached its control level.

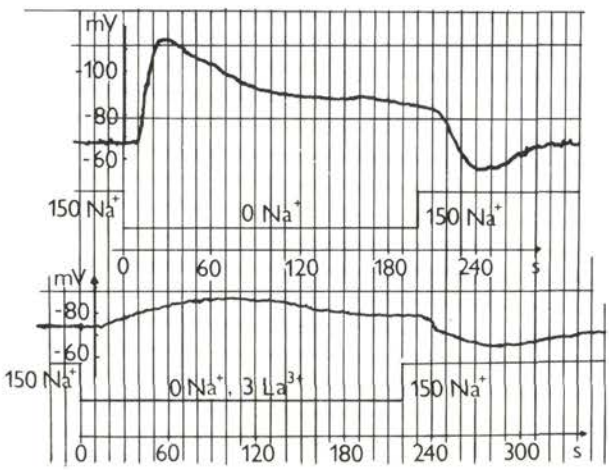

Fig. 1. The time course of changes in transmembrane potential in Na-free solution (top) and in Na-free solution containing 3 mmol/l La (bottom).

Table 1. Membrane potential in Na-free solutions (in mV)

	Mean	± SD	*n*
Control saline	−78.4	2.6	22
Na-free solution	−108.9*	14.8	11
Na-free + La (3 mmol/l)	−81.9	8.9	11

* statistically significant change ($p<0.01$; Student's *t* test)

If a high concentration (3 mmol/l) of La chloride was added to the Na-free solution the hyperpolarization was depressed. The time course of the remaining potential shift was not changed significantly compared to the change evoked by the Na-free solution alone.

Table 1 summarizes the experimental findings with the Na-free solution; the mean resting potential of about −78 mV was shifted to about −109 mV. In a Na-free solution containing 3 mmol/l La this potential change was not statistically significant. La induced changes in the resting potential were irreversible.

Discussion

The possible existence of electrogenic Na − Ca exchange mechanism in mammalian heart muscle remains the subject of controversy (Coraboeuf et al. 1980; Bridge et al. 1981; Hoerter et al. 1981; for review see Reuter 1981). Based on thermodynamic considerations the Ca flux due to coupled Na − Ca exchange must be outward if

$$n\mathrm{F}\,(E_{\mathrm{Na}} - E_{\mathrm{m}}) > 2\mathrm{F}\,(E_{\mathrm{Ca}} - E_{\mathrm{m}}),$$

where E_{Na}, and E_{Ca} are Na, Ca equilibrium potentials respectively; E_{m} is the transmembrane potential; F is the Faraday constant and n is the number of Na^+ ions that exchange for one Ca^{2+}. Therefore if the sodium equilibrium potential E_{Na} is reversed (in Na-free solution) Na efflux should occur (Mullins 1979). With $n>2$ the transport would be electrogenic. In the present analysis with Na-free solutions a net outward current to Na efflux would result in hyperpolarization. Such an effect was actually observed in the rabbit papillary muscle (Fig. 1). This hyperpolarization may result from: i) activation of the electrogenic Na − K pump; ii) the loss of external Na only; iii) changes in passive ionic permeabilities; iv) activation of electrogenic Na − Ca exchange. The possibility that hyperpolarization is a result of electrogenic Na − K antiport is very unlikely because Na-free solutions induce a decrease in internal Na activity (Ellis 1977) which tends to suppress pump activity. In Na^+-rich skeletal muscle fibres Na_i exerts a significant

inhibitory effect on the glycoside sensitive K^+ influx. In the absence of external sodium ions, potassium ions would be pumped back into the fibres due to reactivation of the Na − K pump (for review see Venosa 1979). This reactivation can produce a ouabain-sensitive hyperpolarization. In heart muscle cells, however, under control conditions (low Na_i) the existence of such a mechanism seems to be uncertain. Coraboeuf et al. (1980) described a hyperpolarization induced by external sodium removal in the presence of 10^{-6} mol/l ouabain. If the transmembrane potential is described sufficiently by

$$E_m = \frac{RT}{F} \ln \frac{K_e + \alpha Na_e}{K_i + \alpha Na_i},$$

where K_e, Na_e; K_i, Na_i are external and internal K and Na activities; $\alpha = P_{Na}/P_K$ is the Na : K permeability ratio (R, T and F are the usual constants); the changes in E_m at zero Na_e ($\alpha \sim 0.01$) can not exceed a maximum hyperpolarization of about 10 mV. Hyperpolarization due to changes in passive permeabilities (e.g. to an increase in K permeability) is also unlikely because the potential shift exceeds the expected E_K values. In the same preparations an intracellular K activity of 84.3 mmol/l has been measured which would correspond to K equilibrium potential of about −80 mV (Lee and Fozzard 1975; Fozzard and Lee 1976). The measured mean resting potential was found to be close to the expected E_K values (Table 1). It is therefore most likely that the Na-free hyperpolarization as much more negative than E_K. This experimental finding may be considered to be crucial evidence for the existence of an electrogenic transport mechanism. This is further supported by the blocking effect of La ions in the Na-free solution. La ions inhibit Na − Ca countertransport in the squid axon (0.1 mmol/l; Baker et al. 1969) and in the frog atrial trabeculae (3 mmol/l, Horačkova and Vassort 1979), but the effect is uncertain in mammalian heart muscle until 0.4 mmol/l La are added (Katzung et al. 1973; Coraboeuf et al. 1981). This is why a high (3 mmol/l) La concentration was used in the present study. At this concentration an irreversible inhibition of Na-free hyperpolarization was observed.

All of the present experimental findings support the proposed existence of an electrogenic Na − Ca exchange mechanism in mammalian heart muscle.

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