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Activation of the Na^+/K^+ pump current by intra- and extracellular Li ions in single guinea-pig cardiac cells

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Abstract

Li^+ is the only ion that can replace the physiological intra- and extracellular activator cations of the Na^+/K^+ pump. In order to study this singular property of Li^+ in some detail, the activation of the Na^+/K^+ pump current (I_p) by intra- and extracellular Li^+ (Li_i^+; Li_o^+) was measured in isolated guinea-pig ventricular myocytes by means of whole cell recording at 34°C and a holding potential of −20 mV. I_p was identified as current blocked by dihydro-ouabain. Half-maximal I_p activation occurred at 23 mM Li_o^+ ($K_{0.5}$ value) in cells containing Na^+ (50 or 100 mM) and at 73 mM Li_o^+ in myocytes containing Li^+ (100 mM). The $K_{0.5}$ value of I_p activation by Li_o^+ increased with depolarisation, suggesting the transfer of 0.2 of an elementary charge across the electric field of the sacrolemma during Li_o^+-binding. An intracellular Li^+ concentration of 36 mM caused half-maximal I_p activation in cells superfused with Na^+- and Li^+-free media containing 1 mM K^+. In Na^+-free solutions, the I_p–V curve displayed a positive slope at negative membrane potentials. A negative slope at positive potentials was observed in Li^+-containing media. It is concluded that Li^+ is less efficacious and potent than the physiological pump activator cations. The shape of the I_p–V curves in Na^+-free solutions supports the view that the cardiac Na^+/K^+ pump contains a channel-like structure and suggests that there are voltage-sensitive steps in the pump cycle, apart from the binding of external cations. © 1997 Elsevier Science B.V.

Keywords: Na^+/K^+ pump; Pump current; Whole-cell recording; Cardiac myocyte; Li ion

1. Introduction

The Na^+/K^+ pump in the cell membrane of animal cells generates a pump current, I_p. Since the pump is activated by various species of extracellular monovalent cations, an effect of these ions on I_p would not be surprising. In fact, it is known for various cells (e.g. [1,2]), including cardiac cells [3,4], that Tl^+, Rb^+, Cs^+ or NH_4^+ mimic, to a certain degree, the activation of I_p by extracellular K^+. These ions are unable to replace intracellular Na^+ in the activation of the cardiac Na^+/K^+ pump [5]. However, a recent abstract [6] reports that Li ions can substitute for internal Na^+ in the mechanism of cardiac I_p activation. This is in contrast to earlier observations on cardiac preparations, which suggested rather that Li^+ cannot replace Na^+ at the intracellular cation binding sites of the Na^+/K^+ pump [5,7], but, is in line with findings in erythrocytes [8,9] and neurons [10], where Li^+ acts as a

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(poor) substitute for intracellular Na^+ in the mechanism of Na^+/K^+ pump activation. Interestingly, Li^+ is also known to be a weak extracellular activator cation of the Na^+/K^+ pump in red blood cells [8], nerve [11] and cardiac preparations [5]. In addition, Eisner and Lederer [12] found that extracellular Li^+ activates I_p in cardiac Purkinje fibres. Thus, Li^+ has the singular property among the monovalent activator cations of being able to activate the Na^+/K^+ pump at intra- and extracellular sites. The aim of the patch clamp experiments described below is to analyse quantitatively the effect of intra- and extracellular Li ions and membrane potential on the current generated by the Na^+/K^+ pump in cardiac cells. The voltage dependency of I_p has offered valuable information on ion translocation in the Na^+/K^+ pump cycle [2,13]. As Li^+ is widely used in the treatment of manic depressive disorders (for a recent review, see [14]), a detailed knowledge of the cellular actions of this ion species seems desirable.

2. Materials and methods

2.1. Preparation of single myocytes

Guinea-pigs ($\approx$ 400 g) were killed under deep ether anaesthesia by cervical dislocation. Ventricular myocytes were isolated at 35°C from the excised heart by means of a Langendorff perfusion with Ca^{2+}-free solutions containing collagenase and protease. The procedure has been described in detail previously [3,15]. The digested ventricles were cut into pieces and carefully stirred at room temperature in a small plastic beaker containing a low Ca^{2+}, enzyme-free medium. The Ca^{2+} concentration of the solution was stepwise increased to 1.8 mM within about 60 min. Isolated ventricular cells were then transferred using a Pasteur pipette to a culture dish (diameter, 3.6 cm) installed on the stage of an inverted microscope.

2.2. Solutions

Two basic solutions containing either 50 mM (A) or 100 mM Na^+ (B) were used in the patch pipettes for intracellular perfusion. Solution A contained (mM): 110 caesium aspartate, 40 NaOH, 10 EGTA, 40 HEPES, 5 $MgCl_2$ (free $Mg^{2+} \approx 2$ mM), 0.15 $CaCl_2$ (free $Ca^{2+} \approx 10^{-9}$ M), 5 glucose, 5 MgATP, 5 sodium creatine phosphate (adjusted to pH 7.3 at 34°C with HCl). Solution B contained (mM): 100 sodium aspartate, 10 EGTA, 40 HEPES, 6 $MgCl_2$ (free $Mg^{2+} \approx 3$ mM), 0.15 $CaCl_2$ (free $Ca^{2+} \approx 10^{-9}$ M), 5 glucose, 5 MgATP, 20 tetraethylammonium chloride (TEACl; adjusted to pH 7.3 at 34°C with NaOH). A medium containing 100 mM Li^+ instead of 100 mM Na^+ was obtained by replacing the sodium aspartate in solution B with lithium aspartate, the other constituents of solution B remained unchanged (pH adjusted with LiOH). Finally, a Na^+-free solution was made from solution B by substituting 100 mM TEACl for sodium aspartate. Thus, this Na^+-free medium contained 120 mM TEACl plus the residual constituents of solution B (pH adjusted to 7.3 with CsOH). High Na^+ concentrations of solutions A and B were selected in order to reduce the effect on the I_p amplitude of a possible Na^+ depletion beneath the cell membrane during strong activation of the Na^+/K^+ pump [16]. Solution A was used in the experiments if not stated otherwise. The standard extracellular superfusion medium contained (mM): 144 NaCl, 0–5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES and 10 glucose (adjusted to pH 7.3 at 34°C with NaOH). In order to decrease K^+ and Ca^{2+} conductances and the Na^+/Ca^{2+} exchange of the sarcolemma, 2 mM $BaCl_2$ and 5 mM $NiCl_2$ were added to the solution. In Na^+-free superfusion media, NaCl was replaced either by 150 mM choline chloride (plus $5 \cdot 10^{-6}$ M atropine sulphate), by 150 mM LiCl (pH of both solutions adjusted to 7.3 with LiOH) or by 150 mM *N*-methyl-D-glucamine (NMDG, pH adjusted to 7.3 with HCl). In Na^+-free superfusion media containing various Li^+ concentrations, [choline] plus $[Li^+]$ was always 150 mM.

2.3. Drugs

The cardiac glycoside, dihydro-ouabain (DHO; Sigma, Deisenhofen, Germany), was used to identify I_p in cells superfused with Na^+-free solutions. In these media, the affinity of the Na^+/K^+ pump for K_o^+ increased by a factor of ten and, thus, $[K^+]_o$ in the submillimolar range substantially activated I_p. Therefore, DHO was used (instead of a K^+-free solution) to check for exact zero I_p.

2.4. Experimental procedure and whole-cell recording

The experiments were carried out in culture dishes containing several hundred isolated, rod-shaped myocytes. A dish was fixed to the stage of an inverted microscope (IM; Zeiss, Oberkochen, Germany). The volume of the dishes was reduced to ≈ 0.3 ml by means of an annular plastic device, which was pressed down to the bottom of the dish. The device included an inlet and an outlet for the external media, which superfused the myocytes in the dish at ≈ 2 ml/min and 34°C. The cell under study was additionally superfused with test solutions that were successively applied close to the myocyte via a multibarrelled pipette (inner tip diameter, ≈ 150 μm). A command valve unit regulated solution release under gravitational force from reservoirs that were kept about 30 cm above the culture dish. The solution change at the surface of the cell was complete within 250 ms (cf. [3]). The temperature in the immediate vicinity of the cell dropped by 1–1.5°C during superfusion with one of the test media. The membrane current of the myocyte was measured at pre-set membrane potentials in the whole-cell recording mode of the patch clamp technique [17] by means of an Axoclamp 2A voltage clamp amplifier (Axon Instruments, Burlingame, CA, USA). The initial resistance of the patch pipettes filled with one of the intracellular solutions varied between 2 and 3.5 MΩ. Membrane current and voltage were recorded on a pen recorder (Multicorder; Watanabe, Tokyo, Japan). The holding potential was -20 mV throughout. I_p was identified as the outward current blocked by K^+-free and/or DHO-containing solutions. The cell surface area was estimated from the capacitive current flowing during small hyperpolarizing voltage pulses. The specific membrane capacitance was assumed to be 1 μF cm^{-2} (for more details, see [18]).

2.5. Statistics

Whenever possible, data are presented as means ± S.E.M., and S.E.M. is presented in the figures only if the size of the symbols is exceeded. The symbol, *n*, indicates the number of cells studied. Differences between means were tested by Student's one-tailed *t*-test. They were deemed significant if $P < 0.05$.

3. Results

3.1. I_p activation by K^+ or Li^+ in Na^+-free solution. Inhibition of I_p by DHO

Under normal physiological conditions, the Na^+/K^+ pump extrudes 3 Na^+ and takes up 2 K^+ into the cell during each cycle and, thus, I_p is an outward current. The bottom trace in Fig. 1 shows the membrane current (I) of a guinea-pig ventricular myocyte superfused with Na^+-free solution at the holding potential of -20 mV. The intracellular perfusion medium contained 50 mM Na^+ (solution A). The upper traces specify the solutions applied. First, the myocyte was superfused with a K^+-free medium in which NaCl was completely replaced by choline chloride. Active Na^+/K^+ transport was strongly inhibited by this solution and, thus, I_p was almost absent. The addition of 0.2 mM K^+_o evoked an outward current of 130 pA which was partially (82%) and reversibly inhibited by $5 \cdot 10^{-5}$ M DHO. The current was nearly blocked again following reapplication of the K^+-free, choline-containing solution. This current was considered to represent I_p. Switching to a K^+-free medium containing 150 mM LiCl instead of choline chloride shifted the current once more in the outward direction. The application of DHO ($5 \cdot 10^{-5}$ or 10^{-3} M) caused a concentration-dependent, reversible inward shift of the current and thus revealed the presence of I_p under these conditions. The I_p

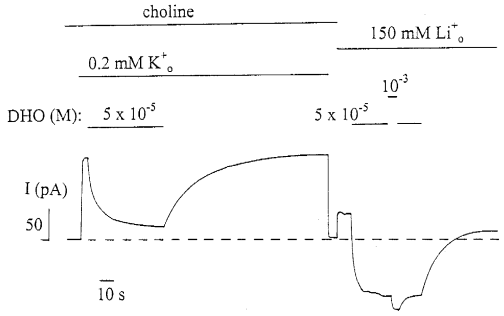

Fig. 1. Membrane current of a guinea-pig ventricular myocyte in various Na^+-free solutions. The current was measured at the holding potential (-20 mV) in media in which NaCl was replaced by choline chloride (left) or LiCl (right), as indicated by the lines above the current trace. The application of DHO is marked by horizontal bars. The lower end of the current calibration bar and the broken line indicate zero current level. Membrane capacitance, 158 pF.

inhibition evoked by $5 \cdot 10^{-5}$ M DHO amounted to 86% of the I_p blockade under 10^{-3} M DHO. In line with earlier observations [3,6,12], the experiment suggests that Li^+, like K^+, acts as an extracellular activator cation of the cardiac I_p.

The current shift upon switching from the choline-containing to the Li^+-containing solution is caused by two mechanisms. First, there is an activation of the outwardly directed I_p by Li_o^+. Secondly, there is, at the same time, an inward leak current because of a higher membrane permeability for Li^+ than for choline. The net result, compared to the current in K^+-free, choline-containing medium, can be an outward or an inward current shift, depending on the relative magnitude of I_p and the Li^+-leak current at -20 mV. In Fig. 1, a small net outward shift results when choline is replaced by Li^+. Upon applying DHO, the holding current shifted inwardly, since I_p is inhibited. The current level attained with $1 \cdot 10^{-3}$ M DHO indicates zero I_p.

3.2. Concentration–response curves of I_p inhibition by DHO in Na^+-free media

The application of various DHO concentrations to myocytes in experiments like that illustrated in Fig. 1 resulted in the concentration–response curves of I_p inhibition by DHO shown in Fig. 2. The figure displays the normalized inhibition of I_p versus the logarithm of the DHO concentration tested. The I_p inhibition caused by $1 \cdot 10^{-3}$ M DHO was arbitrarily set to 100%. I_p amounted to 0.64 ± 0.03 μA cm^{-2} ($n = 21$) in a choline solution containing 0.2 mM K^+ (●) and to 0.80 ± 0.02 μA cm^{-2} ($n = 16$) in myocytes superfused with the Na^+-, K^+-free medium containing 150 mM Li^+ (○). The sigmoid curves fitted to the data by least-squares non-linear regression obey the Hill equation:

$$\text{Percentage } I_p \text{ inhibition} = \frac{100 \times [\text{DHO}]^{n_H}}{(K'_D)^{n_H} + [\text{DHO}]^{n_H}}. \quad (1a)$$

The resulting Hill coefficient (n_H) of 1.08 for both curves reflects a one-to-one binding of DHO to the Na^+/K^+ pump (cf. [19]). The apparent K_D value K'_D ([DHO] for half-maximal I_p inhibition) amounted to $0.8 \cdot 10^{-5}$ M DHO in Li^+-containing medium and to $1.2 \cdot 10^{-5}$ M DHO in the choline-containing solution. Thus, the concentration–response curve of I_p inhibition by DHO in Li^+-containing medium was (significantly) shifted to the left, although I_p was slightly larger in the Li^+-containing solution. This is surprising because a stronger pump activation by a higher external activator cation concentration normally increases the K'_D value for DHO binding [19]. The present finding recalls an earlier observation of the authors that the inhibition of I_p by DHO in rat and guinea-pig ventricular cells occurs at somewhat lower [DHO] in Na^+-containing than in Na^+-free, choline-containing media, if the activation of I_p under both conditions is comparable [19]. Therefore, Li_o^+ seems to promote DHO binding to the Na^+/K^+ pump in a similar way as Na_o^+. This is also evident from the higher rate of I_p inhibition by $5 \cdot 10^{-5}$ M DHO when the current is activated by 150 mM Li_o^+ instead of 0.2 mM K_o^+ (Fig. 1). Table 1 summarizes the mean values of the apparent association and dissociation rate constants and the derived K'_D values of DHO binding from experiments like that shown in Fig. 1 (see [15] for details of the methodology used).

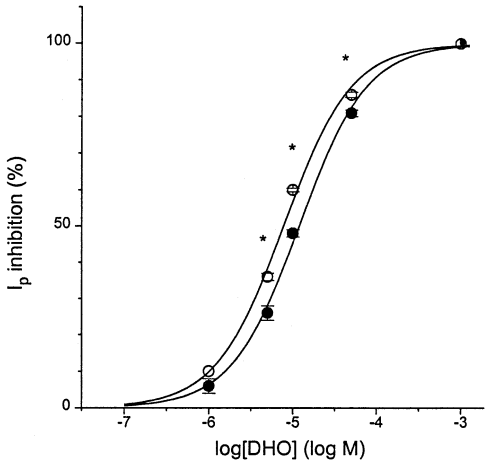

Fig. 2. Concentration–response curves of steady-state I_p inhibition by DHO in ventricular myocytes at the holding potential of -20 mV. I_p inhibition caused by $1 \cdot 10^{-3}$ M DHO was arbitrarily set to 100% (◑). Sigmoid curves fitted to the data points obey Eq. 1a. ●, 0.2 mM K_o^+, Na^+-free medium containing choline chloride; K'_D value ([DHO] for half-maximal I_p inhibition), $1.2 \cdot 10^{-5}$ M DHO ($n = 3$–8). ○, 150 mM Li_o^+; K'_D value, $0.8 \cdot 10^{-5}$ M DHO ($n = 2$–6). *, $P < 0.05$ for the difference between the data points. $r^2 = 0.99$.

Table 1
Kinetics of I_p inhibition by DHO in ventricular myocytes

I_p activated by:	k_1 10^3 M^{-1} s^{-1}	k_2 s^{-1}	K'_D 10^{-5} M	n
0.2 mM K_o^+, choline	1.9 ± 0.3 [a]	0.055 ± 0.003 [a]	3.4 ± 0.5 *	11
150 mM Li_o^+	7.2 ± 0.7 [a]	0.079 ± 0.005 [a]	1.2 ± 0.1 *	11

I_p was activated either by 0.2 mM K_o^+ (in choline medium) or by 150 mM Li_o^+.
k_1, apparent association rate constant.
k_2, apparent dissociation rate constant.
K'_D, mean of apparent K_D values determined for each cell (k_2/k_1).
[DHO] from $1 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$ M.
[a] Significant difference ($P < 0.05$).

To rule out a possible effect of choline on the dose–response curve, NMDG was also used as a Na^+ substitute, but no difference in the sensitivity of the pump towards DHO was found between myocytes in choline or NMDG-containing solution (data not shown).

3.3. I_p activation by various $[Li^+]_o$ in Na^+-containing cells

The concentration dependence of I_p activation by Li_o^+ was studied in some detail in Na^+-containing myocytes superfused with Na^+-free media at constant $[Li^+]_o + [\text{choline}]_o$ (150 mM). The measurements were carried out at the holding potential similar to the procedure depicted in the left part of Fig. 1. However, instead of 0.2 mM K_o^+, various $[Li^+]_o$ were applied to the myocytes. DHO-containing solutions ($1 \cdot 10^{-4}$ or $1 \cdot 10^{-3}$ M) were used to estimate the I_p amplitude at the various $[Li^+]_o$. In the presence of 150 mM Li^+, the amount of I_p blocked by $1 \cdot 10^{-4}$ M DHO amounted to 95% (see Fig. 2). No significant difference between I_p determined either with $1 \cdot 10^{-4}$ or $1 \cdot 10^{-3}$ M DHO was found when $[Li^+]_o$ was lower than 100 mM. Therefore, $1 \cdot 10^{-3}$ M DHO was applied in experiments where $[Li^+]_o$ exceeded 100 mM. The results are presented in Fig. 3 (●). Normalized I_p amplitudes were plotted versus $[Li^+]_o$. The pump current density amounted to 0.55 ± 0.04 $\mu A\ cm^{-2}$ ($n = 21$) at 150 mM Li_o^+. This I_p density was arbitrarily set to 100%. The curve fitted to the data represents the Hill equation:

$$I_p = \frac{I_{p,max} \times [Li^+]_o^{n_H}}{(K_{0.5})^{n_H} + [Li^+]_o^{n_H}}. \quad (1b)$$

The fitting procedure yielded a Hill coefficient, n_H, of 1.4 and an $I_{p,max}$ of 106%. The $K_{0.5}$ value, the $[Li^+]_o$ for half-maximal I_p activation, was 23 mM. A small I_p was still observed in the absence of Li_o^+ [0.021 ± 0.006 $\mu A\ cm^{-2}$ ($n = 8$); checked with $1 \cdot 10^{-3}$ M DHO]. It might be caused by a small amount of K^+ (≈ 10 μM; determined by atomic absorption spectrometry) present as an impurity in nominally K^+-free solutions and was subtracted from the I_p densities observed at the various $[Li^+]_o$. Experiments on four cells internally perfused with solution B (100

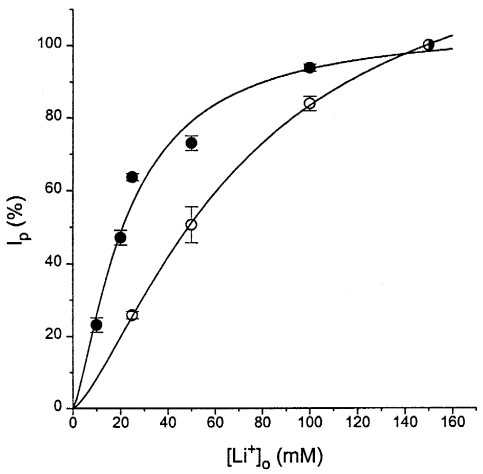

Fig. 3. I_p activation by Li_o^+ in ventricular myocytes containing Na^+ or Li^+. I_p amplitudes were estimated at -20 mV by the application of $1 \cdot 10^{-4}$–$1 \cdot 10^{-3}$ M DHO. I_p activated by 150 mM Li_o^+ was arbitrarily set to 100% (◑). At this $[Li^+]_o$, I_p amounted to 0.55 ± 0.04 $\mu A\ cm^{-2}$ in cells containing 50 mM Na^+ ($n = 21$) and to 0.33 ± 0.05 $\mu A\ cm^{-2}$ in myocytes that were internally perfused with 100 mM Li^+ (plus 20 mM TEACl; $n = 10$). $K_{0.5}$ value for Na^+-containing cells (●), 23 mM Li_o^+; $I_{p,max}$, 106%; n_H, 1.4 ($n = 8$–21); $r^2 = 0.98$. $K_{0.5}$ value for Li^+-containing myocytes (○), 73 mM Li_o^+; $I_{p,max}$, 137.5%; n_H, 1.4 ($n = 5$–10); $r^2 > 0.99$.

mM Na^+) resulted in the same I_p activation by Li_o^+. Compared to the activation of I_p by K_o^+ in Na^+-free, choline-containing media under otherwise similar conditions [19], Li_o^+ exhibits at least a 100-fold weaker potency in activating the cardiac Na^+/K^+ pump, and the maximal I_p activated by Li_o^+ tends to be smaller than the (maximal) I_p evoked by K_o^+ [1.02 ± 0.05 μA cm^{-2} ($n = 30$) in a Na^+-free (choline) medium containing 5.4 mM K^+].

3.4. I_p activation by various $[Li^+]_o$ in Li^+-containing myocytes

We tested to see if I_p activation by Li_o^+ differed in myocytes internally perfused with 100 mM Li^+ instead of 50 (or 100) mM Na^+. First, we checked whether Li ions actually activate the cardiac Na^+/K^+ pump at internal sites. The original records of different cells depicted in Fig. 4 illustrate the test. The pump current was activated in a Na^+-free, choline-containing solution by 1 mM K_o^+ (indicated by a fast upward deflection of the current trace). The upper record displays the change of I_p when a myocyte was internally perfused with a Na^+-free (TEA) pipette solution in order to reduce $[Na^+]_i$ to zero. I_p declined rapidly and, 90 s after establishing the whole-cell configuration, amounted to $< 10\%$ of the initial I_p amplitude, most probably because of the decline in $[Na^+]_i$. Intracellular perfusion with a Na^+-free medium containing 100 mM Li^+ caused a quite different time course of I_p decline (middle record). The I_p amplitude measured 90 s after the first I_p activation still reached $\approx 50\%$ of the initial I_p amplitude. About 2 min later, I_p amounted to 41% of the current measured at first and remained nearly constant thereafter for several minutes. For comparison, an original record of a cell internally perfused with 50 mM Na^+ (solution A) is also shown (bottom record). We conclude from the test that Li^+ activates the cardiac Na^+/K^+ pump at intracellular sites.

I_p activated by Li_o^+ in cells internally perfused with Li ions was estimated by applying superfusion media containing $1 \cdot 10^{-3}$ M DHO. The other experimental conditions remained the same as those in the experiments performed with Na^+ in the pipette and illustrated in Fig. 3 (●). The results are also shown in Fig. 3 (○). Normalized I_p densities were plotted versus $[Li^+]_o$. The I_p density measured at 150 mM

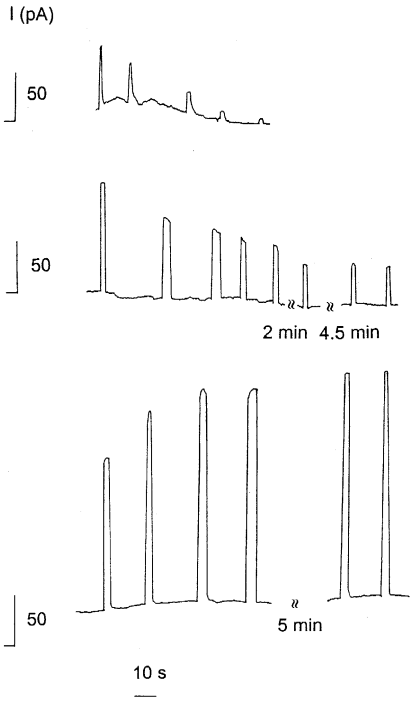

Fig. 4. Time course of I_p activation by 1 mM K_o^+ in three myocytes. Na^+-free (choline chloride) bathing medium was used. I_p activation causes a step-like current shift in the outward direction. Upper current trace: I_p in a cell internally perfused with Na^+-free solution containing 120 mM TEACl. Membrane capacitance, 174 pF. Middle trace: I_p in a myocyte that was internally perfused with 100 mM Li^+. Membrane capacitance, 120 pF. Interruption of the current trace indicates omission of records for the time period stated. Lower trace: I_p in a cell that was internally perfused with 50 mM Na^+. Membrane capacitance, 248 pF. Interruption of the current trace indicates omission of records for the time period stated. The lower end of the current calibration bars marks zero current level.

Li_o^+ amounted to 0.33 ± 0.05 μA cm^{-2} ($n = 10$). It was arbitrarily set to 100%. The curve fitted to the data obeys Eq. (1b), with $n_H = 1.4$, $I_{p,max} = 137.5\%$, and a $[Li^+]_o$ for half-maximal I_p activation of 73 mM ($K_{0.5}$ value; $n = 5$–10). Again, a small I_p was present in the nominal absence of K_o^+ and Li_o^+ (0.023 ± 0.004 μA cm^{-2} ($n = 10$); checked with $1 \cdot 10^{-3}$ M DHO). This value of I_p was subtracted from the I_p densities derived for the various $[Li^+]_o$. Compared to the data from Na^+-containing cells (●), the I_p density of myocytes internally perfused with Li^+ (○) is smaller at 150 mM Li_o^+ (0.33 versus 0.55 μA cm^{-2}) and at the other $[Li^+]_o$ tested. Furthermore, the $K_{0.5}$ value, the $[Li^+]_o$ required for half-

maximal I_p activation, was larger in myocytes containing 100 mM Li^+ than in cells intracellularly perfused with 50 (or 100) mM Na^+ (73 versus 23 mM Li_o^+).

3.5. Binding of Li_o^+ to the Na^+/K^+ pump is voltage-dependent

In single cardiac Purkinje cells, I_p activation by K_o^+ and its congeners Tl_o^+, NH_{4o}^+ and Cs_o^+ is voltage-dependent [4]. Depolarization increases and hyperpolarization decreases the extracellular concentration of activator cations required for half-maximal I_p activation ($K_{0.5}$ value). This means that hyperpolarization of the sarcolemma, at a constant concentration of an extracellular activator cation, or an increase in the ionic concentration at a constant membrane potential exerts an equivalent activation of I_p. We tested to see if the I_p activation by Li_o^+ in ventricular myocytes is also voltage-dependent. For this purpose, we carried out experiments as illustrated in Fig. 5A. The membrane potential of a myocyte was clamped to preset values (indicated by the upper trace) and the resulting current was measured in Na^+-free superfusion fluid containing 50 mM Li^+ with (right part of the figure) or without (left part) DHO ($1 \cdot 10^{-4}$ M). The cardiac glycoside was used to estimate I_p as the difference in current. As indicated by the dashed lines, the DHO-sensitive current (ΔI) is clearly smaller at $+20$ mV than at -40 mV. In this way, I_p was measured in media containing various [Li^+]. In all solutions, [choline chloride] plus

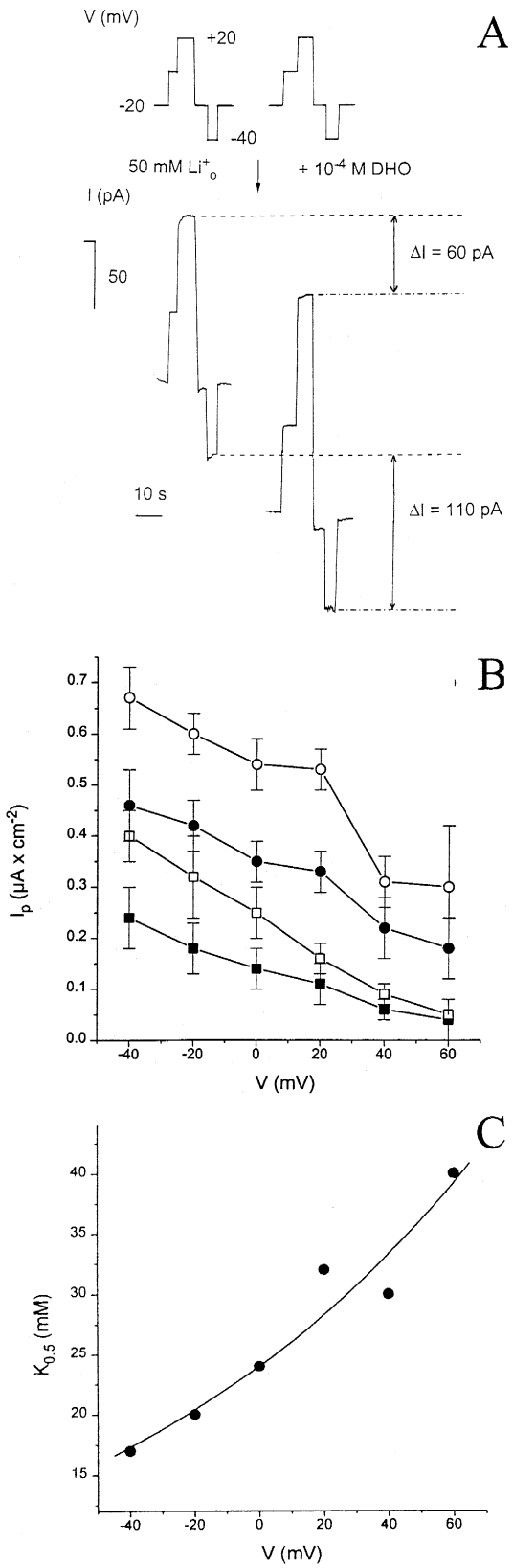

Fig. 5. I_p activation by Li_o^+ is voltage-dependent. I_p was estimated by application of $1 \cdot 10^{-4}$ M DHO. Pipette solution B (100 mM Na^+). (A) Original records of membrane current (bottom traces) at various membrane potentials (top traces) in Na^+-free (choline chloride) solution containing 50 mM Li^+ with (right part) or without DHO. The dashed lines indicate that the inward shift of membrane current (ΔI) caused by DHO is larger at -40 mV than at $+20$ mV. Membrane capacitance, 148 pF. The upper end of the current calibration bar marks zero current level. (B) I_p densities as a function of membrane potential at four $[Li^+]_o$. $[Li^+]_o$ plus [choline] was 150 mM throughout. ■, 10 mM Li_o^+ ($n = 2$–9); □, 20 mM Li_o^+ ($n = 2$–6); ●, 50 mM Li_o^+ ($n = 2$–9); ○, 150 mM Li_o^+ ($n = 2$–8). (C) $K_{0.5}$ value for I_p activation by Li_o^+ in myocytes as a function of membrane potential. The curve fitted to the data points obeys Eq. 2. $K_{0.5}$ value at zero potential [$K_{0.5(V=0\ mV)}$], 24 mM Li_o^+; δ, 0.22; $r^2 = 0.93$.

[LiCl] was 150 mM. Mechanisms different from the binding of external activator cations to the Na^+/K^+ pump govern the shape of the cardiac I_p–V curve at membrane potentials that are more negative than −40 mV [3,20]. Therefore, only I_p densities observed at more positive potentials were plotted versus voltage in Fig. 5B. The data were obtained at four $[Li^+]_o$. Surprisingly, the I_p density of the myocytes decreased with depolarization, even at 150 mM Li_o^+. This is in contrast to the cardiac I_p–V relationship at concentrations of K_o^+ or of the aforenamed congeners above their respective $K_{0.5}$ values. Under these conditions, I_p remained constant at potentials that were more positive than −40 mV [4]. As can be seen from Fig. 5B, I_p also declined at lower $[Li^+]_o$ upon depolarization. A closer inspection of the figure suggests that the percentage of I_p activated by the three lower $[Li^+]_o$ decreased with depolarization. This is more clearly seen in Fig. 5C where the Li_o^+ concentrations required for half-maximal I_p activation ($K_{0.5}$ values) were plotted as a function of the membrane potential (for details of the procedure, see [4]). The $K_{0.5}$ value increased by a factor of 2.5 over the potential range studied. The data points were fitted by a curve which obeys the exponential function:

$$K_{0.5(V)} = K_{0.5(V=0\ mV)}\exp(\delta FV/RT) \quad (2)$$

where $K_{0.5(V)}$ denotes the $K_{0.5}$ value at the membrane potential, V; $K_{0.5(V=0\ mV)}$ stands for the $K_{0.5}$ value at zero potential (24 mM Li_o^+), and R, T and F have their usual meanings. Delta (δ) influences the steepness of the function. It represents the fraction of the electrical field across the sarcolemma sensed by extracellular monovalent ions in the process of binding to the Na^+/K^+ pump [21] and was calculated to be 0.22. Thus, binding of Li_o^+ to the cardiac Na^+/K^+ pump is voltage-dependent and is facilitated by hyperpolarization.

3.6. Activation of I_p in myocytes at various intracellular $[Li^+]$

In order to study quantitatively the activation of I_p by intracellular Li^+, I_p was measured at four internal Li^+ concentrations. Each $[Li^+]_i$ was tested in a different group of myocytes ($n = 4$–16). The measurements were carried out at the holding potential of −20 mV. The media used for intracellular perfusion contained various $[Li^+]$, but [LiCl] plus [TEACl] was always 120 mM (Na^+-free solution B). The external Na^+-free (choline chloride) solution contained 1 mM K^+. At this $[K^+]_o$, the extracellular binding sites of the cardiac Na^+/K^+ pump for activator cations are nearly saturated [3,22]. In addition, I_p was estimated in cells intracellularly perfused with a medium containing no activator cation of the Na^+/K^+ pump. Under these conditions (1 mM K_o^+; 120 mM TEACl inside the myocytes), I_p amounted to 0.03 ± 0.01 μA cm^{-2} ($n = 8$; checked with $1 \cdot 10^{-3}$ M DHO). This I_p value was subtracted from the I_p densities observed at the various $[Li^+]_i$. Fig. 6 shows the results. The I_p density was plotted versus the Li concentration of the pipette solution ($[Li^+]_{pip}$). The current density increased with increasing $[Li^+]_{pip}$ towards a maximum value $I_{p,max}$ that amounted to 0.54 μA cm^{-2}, according to the Hill equation fitted to the data. Half-maximal I_p activation occurred at 36 mM Li_i^+. Thus, the maximal I_p density was lower (by a factor of two) and the $K_{0.5}$ value for the activation of I_p by intracellular Li^+ was larger (by a factor of four) than the corresponding values for I_p activation by Na_i^+ (see ref. [22]).

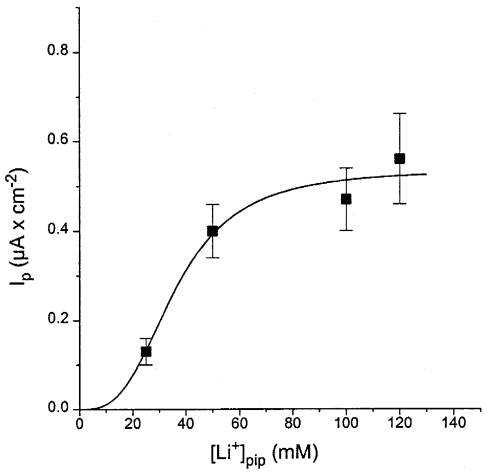

Fig. 6. I_p activation by internal Li^+ in myocytes superfused with Na^+-free (choline) medium containing 1 mM K_o^+. I_p densities are plotted versus $[Li^+]$ of the pipette solution for internal perfusion ($[Li^+]_{pip}$). Four different groups of four–sixteen cells were internally perfused at the holding potential with one of the four $[Li^+]_{pip}$. I_p was estimated by the application of $1 \cdot 10^{-3}$ M DHO. The curve fitted to the data points obeys Eq. 1b. $I_{p,max}$, 0.54 μA cm^{-2}; $K_{0.5}$ value, 36 mM Li_{pip}^+; $n_H = 3.1$; $r^2 = 0.97$.

3.7. I_p–V curves of cells containing Na_i^+ or Li_i^+ in Na^+-free solutions

We studied the I_p–V relationship of Na_i^+-containing ventricular myocytes superfused with Na^+-free media in the membrane potential range between −100 mV and +40 mV. Solution B (100 mM Na^+) was used for the internal perfusion of the cells in order to saturate the internal Na^+-binding sites of the Na^+/K^+ pump and to evoke maximal pump activation intracellularly. The external Na^+-free media contained either 150 mM Li^+ or 1 mM K^+ (NaCl replaced by choline chloride) to also strongly activate the pump at the external cation-binding sites of the pump. Mean I_p densities ± S.E.M. as a function of membrane potential are shown in Fig. 7. Open squares represent the I_p–V curve of cells in Na^+-, K^+-free solution containing 150 mM Li^+ ($n = 8$–9). Filled squares indicate the I_p–V relationship of myocytes superfused with Na^+-free (choline chloride) medium containing 1 mM K^+ ($n = 6$–8). Fig. 7 reveals a positive slope of the I_p–V curve at negative membrane potentials in myocytes superfused with the choline chloride solution containing 1 mM K^+ (■). The curve flattens at positive potentials. The I_p–V curve of cells in the medium containing 150 mM Li^+ (□) not only shows a positive slope at negative potentials up to −40 mV but also a markedly negative slope (significantly different from the slope observed in the choline chloride medium containing 1 mM K^+) at more positive voltages (as already noted in context with Fig. 5B).

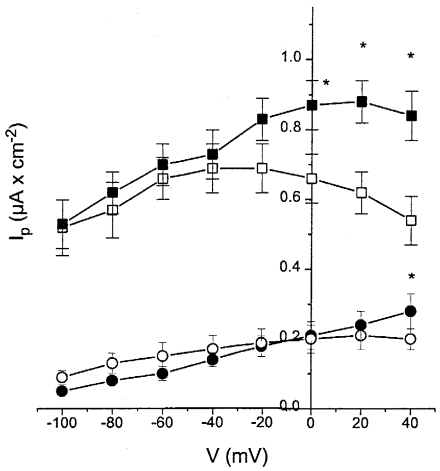

Fig. 7. I_p–V relationships of myocytes internally perfused with 100 mM Na^+ (squares) or 100 mM Li^+ (circles). I_p was estimated by the application of $5 \cdot 10^{-4}$–$1 \cdot 10^{-3}$ M DHO. ■, ●: 1 mM K_o^+ ($n = 6$–8); NaCl of the superfusion medium was replaced by choline chloride. □, ○: 150 mM Li_o^+ ($n = 6$–9). Both superfusion media were successively applied to the same cells containing 100 mM Li^+ (○, ●). *, $P < 0.05$ for the difference between corresponding data points. The positive slope of the curves at negative membrane potentials differs significantly from zero.

I_p–V relationships of myocytes internally perfused with a solution containing 100 mM Li^+ (circles) and superfused either with the K^+- and Na^+-free medium containing 150 mM Li^+ (○) or the choline chloride solution containing 1 mM K^+ (●) are also depicted in Fig. 7. Both media were successively applied to the same cells ($n = 6$–7). As in Na^+-containing myocytes, the I_p–V curve of the cells at 1 mM K_o^+ displays a positive slope at negative membrane potentials. The I_p–V relationship of the myocytes in the medium containing 150 mM Li^+ exhibits a more shallow slope at negative potentials. In these cells, I_p is nearly voltage-independent at positive membrane potentials. In this voltage range, the slopes of the I_p–V curves in Li^+-containing and K^+-containing solution differ significantly. The results suggest that internal perfusion of the cells with a solution containing 100 mM Li^+ instead of 100 mM Na^+ evokes a smaller I_p. The voltage dependence of I_p at negative potentials persists in Na^+-free solution, especially in the choline chloride medium containing 1 mM K^+.

4. Discussion

4.1. Activation of cardiac I_p by Li_o^+

The concentration-dependent inhibition by DHO of the Li_o^+-activated current (Fig. 1) clearly demonstrated that the activated current was indeed I_p. This is in line with earlier reports on Li_o^+ as an activator cation of the cardiac Na^+/K^+ pump current [3,6,12]. The data displayed in Fig. 5C indicate that 24 mM Li_o^+ is required at zero potential for half-maximal I_p activation in Na^+-containing ventricular myocytes superfused with Na^+-free solution. Under these conditions, K_o^+ exerts an ≈100-fold stronger I_p activation [19,22]. I_p tends to be smaller in Li^+-containing than in K^+-containing media (Figs. 4 and 7). This finding agrees well with earlier Na^+ efflux measurements on frog sartorius muscles in Na^+-free solution containing K^+ or Li^+ [23]. It also recalls biochemical

studies that demonstrated a smaller potency and efficacy of Li^+ compared to K^+ for the activation of the Na^+/K^+ ATPase [11,24]. The $[Li^+]_o$ required for half-maximal I_p activation is larger for cells internally perfused with Li^+ than in Na^+-perfused myocytes (73 versus 23 mM; Fig. 3). Interestingly, the $K_{0.5}$ value for the Na^+/K^+ pump activation by K_o^+ in Na^+- and Li^+-free media was very much the same in myocytes internally perfused either with Na^+ or Li^+ (data not shown). Thus, the increased $K_{0.5}$ value seems to be specific for Li_o^+. Li_o^+-induced alterations in the transition between the conformational E_2P-states in the Na^+ limb of the Na^+/K^+ pump cycle may be involved.

4.2. I_p activation by intracellular Li^+

According to a recent abstract [6], Li^+ can replace Na^+ at intracellular binding sites of the cardiac Na^+/K^+ pump in the mechanism of I_p activation. The results described above (Figs. 3, 4, 6 and 7) extend these observations. The I_p density is lower in myocytes internally perfused with media containing 100 mM Li^+ instead of 100 mM Na^+ (Fig. 7). The different current densities may be partly due to a lower affinity of the pump's intracellular binding sites for Li^+ (Fig. 6; $K_{0.5}$ value, 36 mM) than for Na^+ ($\approx$ 10 mM; see [22,25]). Since the difference between the I_p densities is large, even at high intracellular $[Na^+]$ and $[Li^+]$ (comp. Fig. 7), an additional factor seems to be involved. This factor may be a decreased $I_{p,max}$. Compared to Na^+, Li^+ is a less potent and less efficacious activator of ATP hydrolysis by the Na^+/K^+ ATPase in cells [8] and in K^+-free or K^+-containing media [11,26]. Since ATP splitting is a prerequisite for ion translocation by the Na^+/K^+ pump, it seems reasonable that $I_{p,max}$ is smaller in Li^+-containing than in Na^+-containing myocytes. Based on notoriously difficult net ion flux measurements, a change of the usual 3:2 coupling ratio for active Na_i^+–K_o^+ exchange to a ratio near or equal to 1:1 for the Li_i^+–K_o^+ exchange in erythrocytes has been suggested [9]. While the I_p data presented in Fig. 6 clearly show that the coupling ratio of active Li_i^+–K_o^+ exchange must be > 1, they do not exclude a ratio different from 3:2. Additional experimental evidence is required to prove a change in the coupling ratio.

4.3. I_p activation by Li_o^+ is voltage-dependent

The $K_{0.5}$ value for the activation of I_p by Li_o^+ is voltage-dependent (Fig. 5C). This and other observations [2] are interpreted to mean that binding of extracellular activator cations to the Na^+/K^+ pump might occur at the bottom of a 'high field narrow access channel' [27]. About 0.25 of an elementary charge traverses the electric field across the sarcolemma during the binding of extracellular cations. The data illustrated in Fig. 5C are in quantitative agreement with these considerations and lend further support to the hypothesis that the Na^+/K^+ pump molecule contains a channel-like structure.

4.4. I_p–V curves of ventricular myocytes in Na^+-free media containing K^+ or Li^+

The persistence of a positive slope in the I_p–V relationship at negative voltages in Na^+-free solutions is in line with earlier reports on isolated cardiac Purkinje cells [3,4] and *Xenopus* oocytes [28], but is at variance with other observations on *Xenopus* oocytes [29,30] and squid axons [21]. These inconsistent observations are most probably not due to the different Na^+/K^+ ATPase isoenzymes expressed in various cells. Guinea-pig ventricular myocytes express predominantly the α_1 isoenzyme [31]. However, in contrast to the present results, earlier studies on these cells revealed only a shallow (if any) voltage dependence of I_p at negative membrane potentials in Na^+-free media [20,32]. The latter findings constitute the experimental basis of the generally accepted hypothesis that voltage-dependent binding of Na^+ from the extracellular solution is an important mechanism for the decrease in I_p with hyperpolarization in cells superfused with Na^+-containing, 'physiological' media [2,13,30]. The I_p-V relationships shown in Fig. 7 suggest that this is probably not the only factor involved. As can be seen from the figure, the decrease in I_p with increasingly more negative potentials is most pronounced in Na^+-free solutions containing choline chloride, although choline has no effect on the cardiac Na^+/K^+ ATPase [5].

Another interesting feature of the I_p–V curve of Na^+-containing myocytes superfused with Li^+-containing medium is the negative slope that was observed at positive membrane potentials (Fig. 5B and Fig. 7). Such a negative slope is usually thought to be

caused by voltage-dependent binding of K^+ or its congeners to the Na^+/K^+ pump. It can be easily demonstrated in cells superfused with solutions containing low ($< K_{0.5}$ value) concentrations of external activator cations [1,4,29], but it is absent in cardiac cells at nearly saturating concentrations of these ions [4]. However, the figures display a negative slope of the I_p–V relationship at a high Li_o^+ concentration ($\approx$ six times the $K_{0.5}$ value at 0 mV) in myocytes internally perfused with a saturating $[Na^+]$. Thus, the negative slope of the I_p–V curves at positive potentials is probably not due solely to voltage-dependent Li_o^+-binding. An additional voltage-sensitive partial reaction of the pump cycle probably affects the slope of the I_p–V relationship under these conditions.

4.5. Li^+ transport by the Na^+/K^+ pump in Li^+-treated patients

The intracellular Li^+ concentration in Li^+-treated patients is normally less than 1.0 mM and is lower than expected from a passive distribution across the cell membrane [33]. Thus, Li^+-transport out of the cells occurs against an electrochemical gradient for Li^+. However, the present study suggests that a large active Li^+ efflux via the Na^+/K^+ pump is not performed if Li^+ acts as a partial agonist at the Na_i^+- and K_o^+-binding sites of the Na^+/K^+ pump. In vivo, the physiological activator cations Na_i^+ and K_o^+ are present at relatively high concentrations. Both cation species exhibit a higher apparent affinity than Li^+ for the respective binding sites. Thus, the main Li^+ efflux seems to be mediated by a mechanism that is different from that of active Na^+/K^+ transport.

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