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Research Article

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5-(*N,N*-Dimethyl)Amiloride-sensitive Na-Li Exchange in Isolated Specimens of Human Atrium

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Abstract

To examine if a transmembrane Na-Li exchange similar to that reported to occur in human blood cells can be demonstrated in the heart, we incubated specimens of human atrium in cold (2–3°C) Li-Tyrode's solution. The Li-loaded, Na-depleted specimens were then transferred to warm (30°C) Na-Tyrode's solution. After transfer the membrane potential hyperpolarized to a level more negative than the equilibrium potential for K⁺. The hyperpolarization was inhibited by acetylstrophanthidin or K⁺-free solution indicating that it was due to current produced by the Na, K-pump responding to a Na load. This suggested that intracellular Li⁺ had been exchanged for Na⁺. The hyperpolarization was abolished by 10 μM 5-(*N,N*-dimethyl)amiloride while 10 μM bumetanide had no effect, findings that are consistent with the notion that the exchange of intracellular Li⁺ for extracellular Na⁺ occurs via an operational mode of the Na-H exchanger rather than being mediated through a mechanism involving the Na/K/2Cl cotransporter.

Introduction

It has been suggested that the increase in red blood cell Na-Li counter transport demonstrated in many studies to be associated with essential hypertension may reflect a pathogenetically important transport abnormality also present in cells of other tissues (1, 2). However, this hypothesis has been difficult to substantiate partly because a Na-Li counter transport resembling that in blood cells is yet to be demonstrated in other human organ systems and partly because the physiological role and mechanistic basis of this counter transporter is uncertain.

A study of transmembrane Li⁺ transport in cat papillary muscles has suggested that Li⁺ is passively distributed in the heart (3), implying that Na-Li counter transport does not occur in heart. However, because the rate and apparently even the presence or absence of Na-Li counter transport is species dependent (4), it should not be assumed that Li⁺ is also passively distributed in the human heart. We have addressed the question of whether Na-Li counter transport can be demonstrated in human cardiac tissue.

The counter transporter can function in either Na-Li, Li-Li, or Na-Na exchange modes (5), but none of these can be

of functional significance because normally Li⁺ is present in the human body only in very low concentrations (4) and Na-Na exchange can contribute nothing to maintaining homeostasis. The counter transporter could be an evolutionary remnant that has lost its physiological role (4) or, alternatively, it could be a mechanism that normally performs a more meaningful ionic exchange function. One such mechanism, the Na-H exchanger, can bind Li⁺ with high affinity and has many features in common with the Na-Li counter transport mechanism (2). It has therefore been suggested that Na-Li counter transport may be an operational mode of the Na-H exchanger. However, there also is evidence against this idea. An apparent lack of sensitivity of the Na-Li counter transport in red blood cells to amiloride (6, 7) suggests that the counter transport may not be mediated by the Na-H exchange mechanism, and inhibition by furosemide (5, 6) suggests an alternative hypothesis that Na-Li counter transport can be mediated by a Na/K/2Cl cotransporter, a mechanism in which Li⁺ can substitute for Na⁺ (8).

We have examined the effect of inhibition of both the Na-H exchanger and the Na/K/2Cl cotransporter on the development of an intracellular Na-load in exchange for a Li load in specimens of human atrial appendage. Li-loaded specimens were transferred to Li⁺-free Na-Tyrode's solution to allow oppositely directed concentration gradients to develop and thus facilitate Na-Li counter transport. This approach is similar to that used for its study in red blood cells (9). After transfer, membrane potential (E_m)¹ transiently hyperpolarized to levels more negative than the equilibrium potential for K⁺ (E_K), a phenomenon attributed to electrogenic pumping by the Na, K-pump of an intracellular Na load exchanged for the Li load. The hyperpolarization was abolished by 5-(*N,N*-dimethyl)amiloride, while bumetanide had no effect. These findings suggest that Na-Li exchange can occur in human cardiac tissue and that the exchange appears to be mediated by the Na-H exchanger rather than by the Na/K/2Cl cotransporter.

Methods

Specimens of atrial appendage, routinely excised to facilitate cannulation of the right atrium, were obtained from patients undergoing coronary artery bypass surgery. None of the patients had untreated hypertension and specimens from patients who had received cardiac glycosides preoperatively were excluded. The age of the patients was 64±9 yr (mean±SD). All institutional and U. S. Department of Health and Human Services guidelines for human subject research were followed. A second series of experiments using guinea pig atria (obtained as described in reference 10) was also performed.

Immediately after excision, unless otherwise indicated, specimens were placed in cold (2–3°C) Li-Tyrode's solution containing (in mM):

1. *Abbreviations used in this paper:* ACh, acetylcholine; E_K , equilibrium potential for K⁺; E_m , membrane potential; TTX, tetrodotoxin.

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LiCl 150, KCl 1, MgCl₂ 1.1, CaCl₂ 1.8, dextrose 11, Hepes buffer 5, and NaOH 2.25 (pH 7.5 at 30°C and 7.7 at 2°C). 70 min after excision, trabeculae dissected from the specimens were transferred to cold solutions of similar composition except for containing, unless otherwise indicated, 0.5 mM BaCl₂ (to increase membrane resistance by reducing K channel conductance, see reference 11), and 20 mM KCl. Changes in the K⁺ concentration were made by isosmolar substitution of KCl for LiCl. After an additional 20 min trabeculae were transferred to a 1.2-ml tissue chamber perfused (3–4 ml/min) with warm (30±0.2°C) Tyrode's solution containing 131 mM Na⁺ and 0.5 mM Ba²⁺, unless otherwise indicated. All warm solutions contained BaCl₂ and KCl in concentrations identical to those of the solution used during the last 20 min of the period of cooling. The compositions of the various solutions used are summarized in Table I. Either acetylcholinesterase inhibitor (0.5 μM) dissolved in ethanol (0.1 mg/ml), ouabain (0.1 mM), 5-(N,N-dimethyl)amiloride (10 μM) or tetrodotoxin (TTX, 10 μM) dissolved in water, or bumetanide (10 μM) from a 1.0 mM dimethylsulfoxide stock solution were added to the Na-Tyrode's solution in some experiments. All solutions were bubbled with 100% O₂.

The tissue while exposed to Li-Tyrode's solution was cooled to enable comparison of the time course of E_m subsequent to warming with the time course recorded subsequent to warming specimens loaded with Na⁺ by similar cooling in Na⁺-containing solutions. In addition, cooling was expected to minimize any toxic effects of incubation in Li-Tyrode's solution on cellular processes. Rewarming was required for activation of the Na, K-pump. E_m was recorded as soon as possible after the onset of rewarming using a recording system described previously (12). Glass microelectrodes filled with 3 M KCl, with resistances of 15–40 MΩ, and tip potentials of 5 mV or less were used. Impalements were regarded as reliable if the potential registered by the electrode changed virtually instantaneously when the electrode entered or was withdrawn from the cell. Independence of the recorded potential from minor vertical movements of the microelectrode was used as additional evidence for the reliability of impalements. Satisfactory impalements were usually achieved within 2 min after transfer of the tissue to warm Na-containing solutions. If a reliable impalement was not achieved within 4 min, the results are not reported. Any voltage offset recorded upon withdrawal of the electrode had to be <±4 mV and was accounted for when reporting E_m . Impalements

Table I. Composition of Solutions*

Solution	Li ⁺	Na ⁺	TEA ⁺	K ⁺	Ba ²⁺
1	0	150	0	1	0
2	0	131	0	20	.5
3	0	151	0	0	.5
4	148	2.25	0	1	0
5	129	2.25	0	20	.5
6	0	2.25	150	1	0
7	0	2.25	129	20	.5
8	149	2.25	0	0	.5
9	0	20	130	1	0
10	0	20	111	20	.5
11	0	25	106	20	.5
12	25	106	0	20	.5
13	25	25	81	20	.5

* Concentrations are in mM. In addition the solutions contained: Ca²⁺ 1.8, Mg²⁺ 1.1, dextrose 11 and Hepes buffer 5. The 2.25 mM Na⁺ in solutions 4–8 originated from NaOH used to adjust the solutions' pH. All solutions contained the same concentration of Cl⁻ (157 mM).

were usually made at closely adjacent sites every 2–4 min to check the reference potential and account for any minor voltage drift.

The Li⁺ content of atrial tissue was determined in a second series of experiments. Because extracellular ionic equilibration should be faster in thin-walled than in thick-walled atria, tissue from guinea pigs rather than humans was used for these experiments. After isolation from excised hearts the atria were treated according to a protocol identical to that used for human specimens except that E_m was not recorded. For determination of Li⁺ content the atria were blotted between sheets of filter paper, weighed and then oven-dried at ~ 80°C for 48 h to constant weight. The volume of tissue-water was determined by subtracting the dry weight from the wet weight. The dried atria were then dissolved in 1 ml of concentrated nitric acid, the solution was diluted to a final volume of 25 ml, and the Li⁺ concentration was measured using an (Varian Techtron model 1200; Varian Associates, Palo Alto, CA) atomic absorption spectrophotometer equipped with a Li-natural cathode. The Li⁺ content was then calculated. Intracellular Li⁺ concentrations were determined from the total Li⁺ content of the tissue and the volume of tissue-water according to the formula given in reference 3. The extracellular space was assumed to be 0.3 liter/kg wet tissue (10).

Results are expressed as mean±SE. Analysis of variance (13) was used in the statistical analysis. In total, 69 experiments on human atrial specimens and 7 experiments on guinea pig atria are reported.

Results

Time course of E_m during extrusion of an Na load. Since electrogenic extrusion of intracellular Na⁺ accumulated during exchange of Na⁺ for a Li-load was used to demonstrate Na-Li counter transport, the time course of E_m during extrusion of an Na load by the Na, K-pump was characterized. To induce Na-loading, five specimens were incubated in Na-Tyrode's solutions cooled to 2–3°C for 90 min (14). The solutions contained 1 mM K⁺ during the first 70 min (solution 1, Table I) and 20 mM K⁺ and 0.5 mM Ba²⁺ (solution 2) during the last 20 min of cooling. The 20 mM K⁺ solution used during the last 20 min of cooling was also used to rewarm the specimens and to obtain the reported data.

The time course of mean E_m after warming to 30°C is summarized in Fig. 1. E_m became increasingly negative, reaching a maximal level (E_{max}) at 14.0±2.3 min after the onset of warming of -79.2±1.4 mV, before decaying towards less negative levels. Mean E_{max} was 28 mV more negative than the level expected for E_K in human atrial tissue in 20 mM K⁺ under steady state conditions (i.e., ~ -51 mV). We have demonstrated previously that such a hyperpolarization of E_m upon

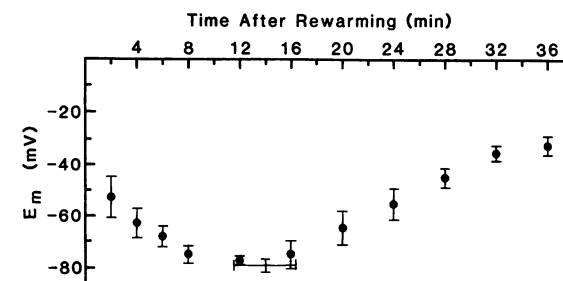


Figure 1. Hyperpolarization of Na-loaded specimens. The time course of mean E_m (±SE) recorded during rewarming is plotted for five specimens cooled for 90 min in Na-Tyrode's solutions. Mean E_{max} and the mean duration from the onset of rewarming until E_{max} was reached is indicated by the crossed error bars.

rewarming of cooled human atrial specimens results from electrogenic extrusion of an intracellular Na load by the Na, K-pump (12).

Time course of E_m of Li-loaded specimens. Nine specimens incubated for 90 min in cold Li-Tyrode's solutions (solutions 4 and 5) to induce an intracellular Li-load were transferred to warm Na-Tyrode's solution containing 20 mM K^+ (solution 2). The time course of mean E_m after the transfer is depicted in Fig. 2 A. Initially, during the first minute or two after rewarming, E_m rapidly fell to ~ -40 mV and then, over the next 8–10 min, E_m transiently hyperpolarized reaching a maximal level of -59.4 ± 0.6 mV after 11.3 ± 0.9 min before again decaying towards more positive levels. The mean E_{max} was more negative ($P < 0.01$) than the E_K expected under steady state conditions in 20 mM K^+ (~ -51 mV). Since the intracellular K^+ concentration of cardiac tissue has been shown to decrease during exposure to Li-substituted solutions (3), the difference between E_{max} and the actual E_K at the time E_{max} was obtained is probably even greater than that calculated from the presumed steady state value for E_K . Therefore it appears that E_m of specimens incubated in Li-Tyrode's solution also can transiently hyperpolarize to levels that are negative to E_K after they are transferred to Na-Tyrode's solution.

In some experiments, when E_m was near E_{max} , small boluses (60 μ l) of Tyrode's solution containing 200 mM acetylcholine (ACh) were added to the tissue bath at the inflow orifice to substantiate that E_m actually hyperpolarized to levels negative to E_K . The effect of ACh in one such experiment is shown in Fig. 2 B. A small transient depolarization was recorded following the administration of ACh-containing solution. Because ACh increases atrial membrane K conductance (14), it is expected to enhance inward, depolarizing K current at potentials negative to E_K and enhance outward, hyperpo-

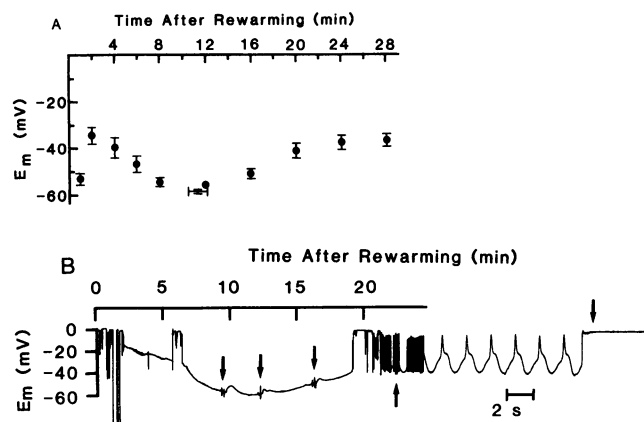


Figure 2. (A) Hyperpolarization of Li-loaded specimens. The time course of mean E_m recorded after transferring nine specimens from cold Li-Tyrode's solution to warm Na-Tyrode's solution is plotted. (B) Typical time course of E_m of Li-loaded specimen. E_m was recorded after transferring the specimen from cold Li-Tyrode's solution to warm Na-Tyrode's solution. Transient depolarizations were induced when 60 μ l boluses of solution containing ACh were added at the inflow orifice of the tissue bath 9, 12, and 16 min after the transfer (administered at the times indicated by arrows). The electrode was placed extracellularly at ~ 19 min to verify the zero mV level. Reimpalement was achieved at 22 min and the specimen was found to be spontaneously active. After reimpalement ACh caused a small hyperpolarization and a brief arrest of spontaneous activity.

larizing K current at potentials positive to E_K (15). The depolarization induced by ACh (Fig. 2 B) therefore confirms that E_m was negative to E_K . The response to ACh also indicates that under these experimental conditions, 0.5 mM Ba^{2+} does not completely block ACh-sensitive K channels in human atrial tissue, a result consistent with findings that a concentration of 1 mM Ba^{2+} is required to block ACh-induced K currents in Purkinje strands completely (15). Even higher concentrations of Ba^{2+} might be required for atrial tissue that presumably has a greater density of ACh receptors than ventricular tissue.

Requirement of the hyperpolarization for Li- or Na-loading. Experiments were performed to examine the requirement for intracellular Li^+ or Na^+ in the mechanism generating the hyperpolarization depicted in Figs. 1 and 2. Six specimens were incubated for 90 min in cold tetraethylammonium chloride (TEA-Cl)-substituted Tyrode's solution (solutions 6 and 7) and then transferred to warm Na-Tyrode's solution containing 20 mM K^+ (solution 2).

The time course of mean E_m recorded after the transfer is depicted in Fig. 3. After impalement E_m initially rapidly depolarized. A similar brief early depolarization occurred when Li-loaded specimens were transferred to Na-Tyrode's solution. After the brief early depolarization, whereas Li-loaded specimens subsequently hyperpolarized transiently to levels more negative than E_K (see Fig. 2 A), no such hyperpolarization occurred after transferring specimens cooled in TEA-Tyrode's solution. Thus, intracellular loading with either Li^+ or Na^+ was required for the development of the hyperpolarization depicted in Figs. 1 and 2.

Effect of reducing the Na and K channel conductances. Hyperpolarization of E_m to levels more negative than E_K is usually attributed to enhanced electrogenic Na, K-pump activity (16, 17). However, to explain the hyperpolarization depicted in Figs. 2, A and B, additional putative mechanisms must be considered. An outward transmembrane concentration gradient for Li^+ should develop after the transfer of the Li-loaded specimens to Li^+ -free Na-Tyrode's solution, and since the Na channels in sarcolemmal membranes are permeable to Li^+ (3, 18), the hyperpolarization could be due to an outward diffusion of Li^+ via Na channels. In principle, the hyperpolarization might also arise from an outward diffusion of Li^+ through any K channels not blocked by 0.5 mM Ba^{2+} . Experiments were performed to examine these possibilities.

Specimens incubated for 90 min in cold Li-Tyrode's solu-

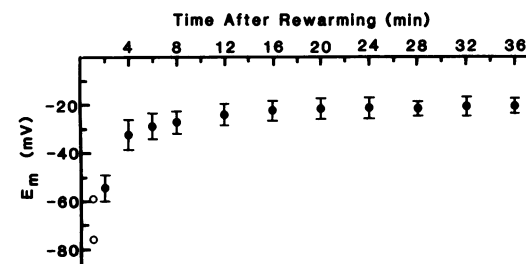


Figure 3. Time course of mean E_m upon transfer of 6 specimens incubated in cold TEA-Tyrode's solution to warm Na-Tyrode's solution. Note that, as for Li-loaded specimens (Fig. 2 A), E_m rapidly depolarized after the initial impalement. However, in contrast to the Li-loaded specimens, no subsequent hyperpolarization developed.

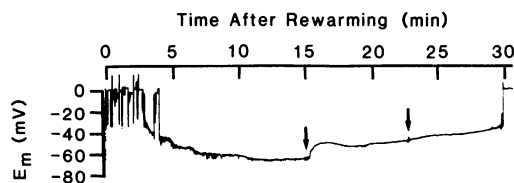


Figure 4. Effect of TTX on time course of E_m . E_m was recorded after transferring a specimen from cold Li-Tyrode's solution to warm Na-Tyrode's solution containing TTX. ACh (administered at the time indicated by arrows) caused a transient depolarization when E_m was near E_{max} but had no effect subsequently when E_m had depolarized to ~ -46 mV suggesting that E_K at that time was ~ -46 mV.

tions (solutions 4 and 5) were transferred to warm Na-Tyrode's solution containing 20 mM K^+ , 0.5 mM Ba^{2+} (solution 2) and, to decrease Na channel conductance (19), 10 μ M TTX. An example of the time course of E_m recorded after the transfer is depicted in Fig. 4. A transient hyperpolarization to -65 mV developed, and a bolus of solution containing ACh caused a brief depolarization when E_m was near E_{max} , indicating that E_m could hyperpolarize to levels negative to E_K despite exposure to TTX. Mean E_{max} in three experiments (-68.3 ± 2.1 mV) was more negative than mean E_{max} in analogous experiments performed without TTX in Na-Tyrode's solution (-59.4 ± 0.6 mV, Fig. 2 A) while the time from the transfer until E_{max} was reached was similar (12.7 ± 1.6 min vs. 11.3 ± 0.9 min). Since the amplitude of the hyperpolarization was increased rather than decreased by TTX, it is unlikely that an outward Li current flowing through Na channels underlies the hyperpolarization.

Experiments were also performed to examine the effect of K channel conductance on the transient hyperpolarization. Four specimens incubated for 90 min in cold Li-Tyrode's solutions were transferred to warm Na-Tyrode's solution containing 20 mM K^+ . In contrast to the experiments described above, none of the solutions contained $BaCl_2$. Mean E_{max} , reached 12.0 \pm 2.0 min after transfer, was less negative than mean E_{max} in analogous experiments performed with Ba^{2+} in the solutions (-51.5 ± 2.1 mV vs. -59.4 ± 0.6 mV, Fig. 2 A). This indicates that an outward Li current through Ba^{2+} -sensitive K channels cannot account for the transient hyperpolarization. Conversely, the fact that an increased membrane resistance induced by either Ba^{2+} or TTX caused an increase in the amplitude of the hyperpolarization, is consistent with the notion that the hyperpolarization was due to a mechanism involving electrogenic exchange or active transport.

Effect of Na, K-pump inhibition. The effect of Na, K-pump inhibition on E_m was examined to determine if the hyperpolarization were due to enhanced electrogenic Na, K-pump activity. The Na, K-pump was inhibited by exposure of specimens to acetylcholinesterase or K^+ -free, Na-Tyrode's solution. Six specimens incubated in cold Li-Tyrode's solutions (solution 4 and then 5) were transferred to warm Na-Tyrode's solution (solution 2) containing 20 mM K^+ and 0.5 μ M acetylcholinesterase. The time course of mean E_m recorded after the transfer is depicted in Fig. 5. During the first minute E_m was similar to that observed in analogous experiments for which acetylcholinesterase had not been included in the Na-Tyrode's solution (see Fig. 2 A). However, within 2 min E_m depolarized to about -40 mV and remained at that level throughout the next 26 min, a finding consistent with a fast

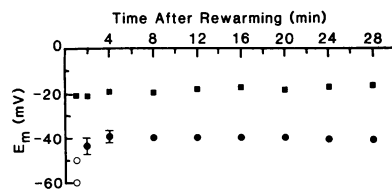


Figure 5. Effect of Na, K-pump inhibition on time course of mean E_m . E_m was recorded after transferring specimens from cold Li-Tyrode's solution to warm Na-Tyrode's solution

containing 20 mM K^+ and 0.5 μ M acetylcholinesterase (circles, $n = 6$) or to K^+ -free solution (squares, $n = 6$). Error bars are not shown when contained within the symbols. Note that Na, K-pump inhibition abolishes the hyperpolarization recorded in analogous experiments performed without pump inhibition (Fig. 2).

onset of the inhibition of Na, K-pump-induced hyperpolarization by acetylcholinesterase.

In another series of experiments six cooled Li-loaded specimens were transferred to warm K^+ -free, Na-Tyrode's solution (solution 3). To wash K^+ out of the interstitial space, the tissue was placed in cool K^+ -free, Li-Tyrode's solution (solution 8) during the last 20 min before transferring to warm Na-containing solution. The time course of mean E_m is depicted in Fig. 5. E_m was at an unchanging level from the time of the first impalements and was less negative than the E_m of those specimens exposed to acetylcholinesterase (0.5 μ M) in solution 2 containing 20 mM K^+ . Abolition of the hyperpolarization by inhibiting the Na, K-pump (Fig. 5) indicates that electrogenic Na^+ pumping underlies the ability of Li-loaded specimens to transiently hyperpolarize after transfer to warm Na-Tyrode's solution. The results depicted in Fig. 5 also suggest that, if Na-Li counter transport occurs after the transfer, the counter transport must be electroneutral.

Effect of Li^+ on Na, K-pump activity. The hyperpolarization of Li-loaded specimens could be due to electrogenic pumping of Li^+ by the K^+ -dependent, acetylcholinesterase-sensitive, Na K-pump if Li^+ can substitute for Na^+ at ligand-binding pump sites located on the cytosolic side of the sarcolemma. Alternatively, if Na-Li counter transport occurs, electrogenic pumping of Na^+ which is exchanged for Li^+ after the transfer to Na-Tyrode's solution could underlie the hyperpolarization. Extracellular Li^+ competes with Na^+ during cellular uptake of Na^+ via Na-Li counter transport in red blood cells (20). This implies that if the hyperpolarizing mechanism involves counter transport, addition of Li^+ to the Na-containing solution should reduce uptake of Na^+ via Na-Li counter transport, and hence the hyperpolarization caused by electrogenic pumping of Na^+ exchanged for Li^+ should decrease. Since the inhibitory effect of Li^+ on Na^+ uptake via the counter transport system is competitive, the inhibition should be accentuated by reducing the concentration of Na^+ in the Tyrode's solution. Therefore, the effect on E_m of adding Li^+ to solutions containing "normal" or "low" concentrations of Na^+ was examined.

Control experiments in which Li-loaded specimens were transferred to solutions with a reduced concentration of Na^+ and no Li^+ were performed first. For these controls, six specimens were incubated in cold Li-Tyrode's solutions (solutions 4 and 5) for 90 min and then transferred to a warm solution containing 25 mM Na^+ , 20 mM K^+ and, to render it isosmolar with the other solutions, 106 mM TEA-Cl (i.e., "low" Na-Tyrode's solution, solution 11). The time course of mean E_m recorded after the transfer is summarized in Fig. 6, A. Mean

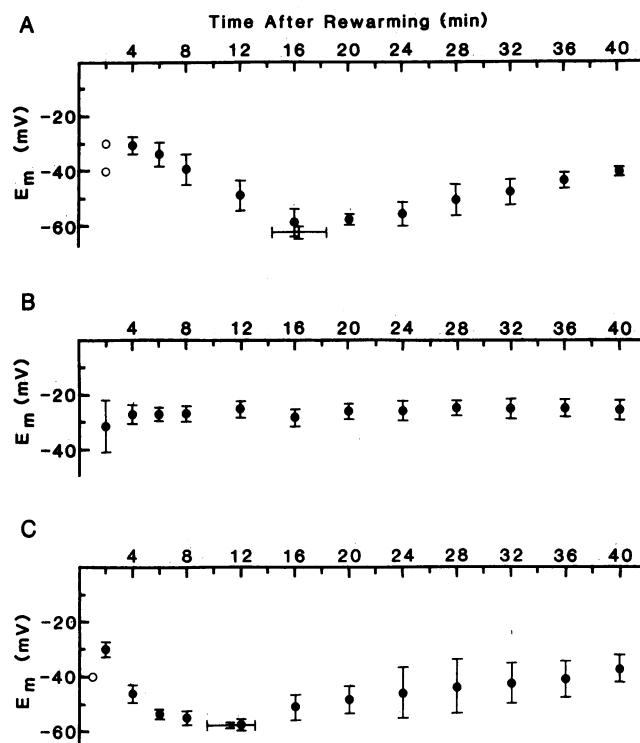


Figure 6. Effects of extracellular Li^+ on the time course of mean E_m at "low" and "normal" concentrations of Na. (A) E_m recorded after transferring six specimens from cold Li-Tyrode's solution to warm low Na-Tyrode's solution. (B) E_m recorded after transferring six specimens from cold Li-Tyrode's solution to warm low Li^+ , low Na-Tyrode's solution. The transient hyperpolarization recorded in the control experiments (A) was abolished by extracellular Li^+ . (C) E_m recorded after transferring five specimens from cold Li-Tyrode's solution to warm low Li^+ normal Na-Tyrode's solution. The inhibitory effect of extracellular Li^+ on the transient hyperpolarization (B) was overcome by the increase in the Na^+ concentration.

E_{max} (-62.2 ± 1.7 mV) was similar to mean E_{max} of Li-loaded specimens transferred to solution containing 131 mM Na^+ (-59.4 ± 0.6 mV, Fig. 2 A). However, the hyperpolarization developed more slowly in the 25 mM Na^+ solution (time from transfer until E_{max} was recorded was 16.3 ± 2.1 min in the 25-mM solution and 11.3 ± 0.9 min in the 131-mM solution, $P < 0.05$). As in other cases (see Figs. 2 B and 4) ACh administered when E_m was near E_{max} , induced a very brief depolarization indicating that E_m was more negative than E_K (data not shown).

After incubation in Li-Tyrode's solutions (solutions 4 and 5) the effect on the time course of E_m of 25 mM Li^+ in a warm solution closely resembling that used for the series of control experiments described above (solution 11) was examined. The solution contained 25 mM Na^+ , 20 mM K^+ , 81 mM TEA-Cl, and 25 mM Li^+ (i.e., low Li^+ , low Na-Tyrode's solution, solution 13). The time course of mean E_m recorded after the transfer of six specimens is depicted in Fig. 6 B. No transient hyperpolarization was observed. E_m remained stable at ~ -30 mV throughout the period of recording in contrast to the hyperpolarization observed in similar solutions not containing Li^+ (see Fig. 6 A). This suggests that extracellular Li^+ inhibited the exchange of an intracellular ion for extracellular Na^+ .

If the absence of a transient hyperpolarization (Fig. 6 B)

were due to competitive inhibition of cellular Na^+ -uptake by Li^+ , it should be possible to overcome the inhibition by increasing the Na^+ concentration in the rewarming solution containing 25 mM Li^+ . This was examined by transferring five cooled Li-loaded specimens to warm solution containing 106 (rather than 25) mM Na^+ , 20 mM K^+ and 25 mM Li^+ (solution 12). The time course of mean E_m is summarized in Fig. 6 C. A mean E_{max} of -57.6 ± 1.1 mV was recorded 11.2 ± 1.8 min after the transfer, and ACh, administered when E_m was near E_{max} , induced a depolarization (data not shown), again indicating that E_m had been negative to E_K . Thus the inhibitory effect of 25 mM Li^+ on the transient pump-induced hyperpolarization (Fig. 6 B) was overcome by increasing the Na^+ concentration from 25 to 106 mM. This indicates the involvement of extracellular Na^+ in the process underlying the transient hyperpolarization and supports the conclusion that an intracellular ion-load had been replaced by a Na-load. In addition, these findings also indicate that the inhibition by Li^+ of the hyperpolarization observed in the solution containing 25 mM Na^+ (Fig. 6 B) was not due to a functionally significant direct inhibitory effect of extracellular Li^+ on the electrogenic Na, K-pump.

Effect of Na, K-pump inhibition on tissue content of Li^+ .

The effect of Na, K-pump inhibition on the transient hyperpolarization of Li-loaded specimens (Fig. 5) transferred to Na-Tyrode's solution indicates that the hyperpolarization was due to electrogenic Na, K-pump activity. The results presented in Fig. 6 indicate that the hyperpolarization was not due to electrogenic pumping of Li^+ by the Na, K-pump. To further substantiate this conclusion we examined the effect of pump inhibition on the tissue content of Li^+ . In these experiments guinea pig atria were incubated for 90 min in cold Li-Tyrode's solution before being transferred to warm Na-Tyrode's solution containing 20 mM K^+ (identical to solution 2 except for not containing Ba^{2+}). The atria were exposed to 0.1 mM ouabain before and after the transfer to Na-Tyrode's solution in one series of experiments. No ouabain was used in a second series.

At the end of the cooling period the atria contained 2.8–2.9 μg Li^+ /mg dry weight ($n = 2$). These values reflect Li^+ contained in the extracellular and the intracellular compartments. The Li^+ concentration calculated from the data was 110–118 mmol/liter intracellular water, values in good agreement with similar data for cat papillary muscles (3). The tissue content of Li^+ in the atria was also determined 15 min after transfer to warm Na-Tyrode's solution. Li^+ in tissue exposed or not exposed to ouabain was similar (0.58 ± 0.07 , $n = 3$ vs. 0.65 ± 0.06 μg /mg dry weight, $n = 3$, or 27.1 ± 5.1 vs. 27.8 ± 2.8 mmol/liter intracellular water) suggesting that Na, K-pump inhibition had no effect on the extrusion of intracellular Li^+ . This finding supports the conclusion of the previous section that the hyperpolarization of Li-loaded human atrial specimens does not result from electrogenic pumping of Li^+ , and is consistent with the hypothesis that Li^+ efflux is mediated by a mechanism involving exchange with extracellular Na^+ .

Effect of inhibition of Na/K/2Cl co-transport and Na-H exchange. Because exchange of an intracellular Li-load for extracellular Na^+ might involve either an operational mode of the Na/K/2Cl cotransporter or of the Na-H exchanger, we examined the effect on E_m of inhibiting each of these systems.

Six specimens, Li-loaded by incubation in cold Li-Tyrode's solutions (4 and 5), were transferred to warm Na-Tyrode's solution (2). To inhibit the Na/K/2Cl cotransporter solutions

2 and 5 contained 10 μM bumetanide (8). The time course of mean E_m recorded during rewarming is shown in Fig. 7. Mean E_{max} (-59.7 ± 1.5 mV), reached 11.0 ± 1.4 min after the onset of rewarming, was not significantly different from the mean E_{max} in analogous experiments performed without bumetanide in these solutions (-59.4 ± 0.6 mV, Fig. 2 A). This indicates that the Na/K/2Cl cotransporter is unlikely to be involved in the sequence of events resulting in a Na, K-pump-induced hyperpolarization after transfer of Li-loaded specimens to Na-Tyrode's solution.

Six specimens, cooled for 90 min in Li-Tyrode's solutions (4 and 5) were transferred to warm Na-Tyrode's solution (2). Solutions 2 and 5 contained 10 μM 5-(*N,N*-dimethyl)amiloride, a Na-H exchange inhibitor. The time course of mean E_m during rewarming is shown in Fig. 8. After the initial impalement E_m rapidly depolarized. In analogous experiments performed without 5-(*N,N*-dimethyl)-amiloride in the solutions a similar early depolarization occurred (see Fig. 2 A and Fig. 7, 1- and 2-min mark). However, in these latter experiments E_m subsequently hyperpolarized transiently to levels more negative than E_K (see Fig. 2 A and Fig. 7, 10- and 12-min mark). A comparison of the results of the two experimental paradigms indicates that the hyperpolarization can be abolished by 5-(*N,N*-dimethyl)amiloride. Control experiments on Na-loaded rather than Li-loaded atrial tissues indicated that the drug, in the concentration used, had no substantial effect on electrogenic Na⁺ pumping in human atrial tissue (data not shown). This finding indicates that the pump-induced hyperpolarization of the Li-loaded tissue was not abolished because the Na, K-pump had been inhibited and it suggests that the Na-H exchanger is involved in the sequence of events that result in electrogenic pumping of an intracellular Na-load developed after transferring Li-loaded tissue to Na⁺-containing Tyrode's solution.

Determinants for the time course of E_m . In Li-loaded specimens transferred to Na-Tyrode's solution, the duration of the pump-induced hyperpolarization to voltages negative to E_K was long (> 17 min in a typical experiment, see Fig. 2 B) in comparison with time constants of < 2 min reported for the decay of Na, K-pump currents (21). This could be interpreted to mean that Na-Li counter transport rather than extrusion of Na⁺ by the pump is the rate limiting determinant for the time course of E_m . However, other factors also are expected to contribute to the prolonged pump-induced hyperpolarization. If, for example, intracellular pump-sites for Na-binding were saturated, the time required for extrusion of a large Na load

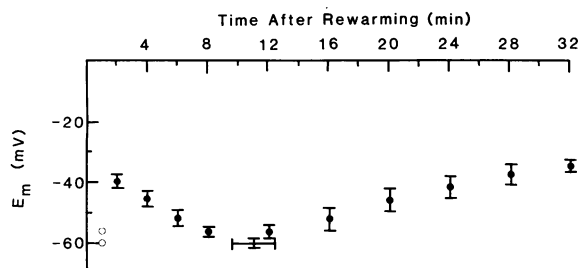


Figure 7. Effect of bumetanide (10 μM) on the time course of E_m of Li-loaded tissue. Six specimens cooled for 90 min in Li-Tyrode's solutions were transferred to warm Na-Tyrode's solution. The open circles indicate data from individual experiments.

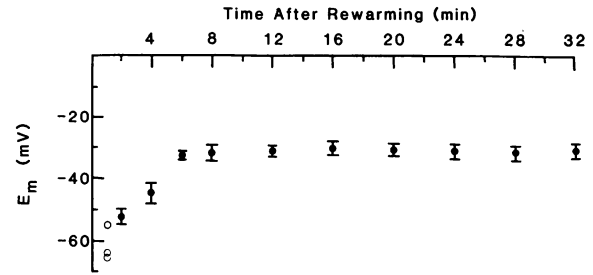


Figure 8. Effect of 5-(*N,N*-dimethyl)amiloride (10 μM) on the time course of E_m of Li-loaded tissue. Six specimens cooled for 90 min in Li-Tyrode's solutions were transferred to warm Na-Tyrode's solution.

should be longer than that expected from the time constant for the decay of pump current for when the elevation of intracellular Na⁺ activity is modest (12).

To evaluate the effect of the size of the Na-load on the time course of E_m , a specimen was cooled for 90 min in solutions containing 20 mM Na⁺. These solutions also contained 130 mM TEA-Cl and 1 mM K⁺ (during the first 70 min, solution 9) or 111 mM TEA-Cl, 20 mM K⁺ and 0.5 mM Ba²⁺ (during the subsequent 20 min, solution 10). Cooling in these solutions should result in a smaller Na-load than cooling in solutions with normal Na⁺ concentration and, because the sarcolemmal membrane has a very low permeability for TEA⁺ (18), less intracellular K⁺ should be lost during cooling. Since the combination of increasing K⁺ gradients and decaying electrogenic pump current should be the principal determinants for the time course of E_m during rewarming (see reference 12 for discussion), E_m should reach its most negative level earlier than the E_m of specimens rewarmed after cooling in normal Na-Tyrode's solution and, if saturation of intracellular pump-sites contributes to a prolongation of the hyperpolarization, the subsequent rate of decay should be faster.

The time course of E_m during rewarming of the cooled specimen in normal Na-Tyrode's solution containing 20 mM K⁺ and 0.5 mM Ba²⁺ (solution 2) is depicted in Fig. 9. E_{max} (-81 mV) was similar to E_{max} of specimens rewarmed after cooling in normal Na-Tyrode's solutions (-79.2 ± 2.8 mV, Fig. 1), but it was reached earlier and the subsequent decay of E_m was faster (half-time for decay 3 vs. 10 min).

The similarity between E_{max} after cooling with low or with normal Na⁺ concentrations should not be taken to indicate that pump-sites were saturated at an intracellular Na⁺ concentration of 20 mM because the intracellular Na⁺ concentration

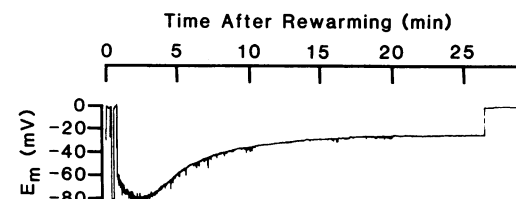


Figure 9. Effect of cooling in low Na-Tyrode's solution on time course of E_m . E_m was recorded after transferring a specimen from cold low (20 mM) Na-Tyrode's solution to warm normal Na-Tyrode's solution. Comparison with Fig. 1 suggests that the amplitude but not the duration of the hyperpolarization is independent of the size of the Na load.

after cooling may be higher than the extracellular concentration due to an inwardly directed electrical gradient during cooling. However, the longer duration of the hyperpolarization with the presumed increase in the Na-load is consistent with the notion that pump electrogenesis saturates at some experimentally, readily attainable level of intracellular Na⁺ activity. Quantitative estimates of this level cannot be derived from the time course of E_m because E_m is determined by a complex interaction between the effects of intracellular Na⁺ on Na, K-pump activity and the transmembrane gradient for K⁺ acting in combination with the voltage dependencies of both pump current (22) and the Ba²⁺-induced decrease in K channel conductance (15). However, in principle it is not unreasonable to suggest that the prolonged hyperpolarization observed when Li-loaded specimens were transferred to Na-containing solutions (Fig. 2) can be explained by saturability of intracellular Na, K-pump sites rather than being indicative of a relatively slow rate of Na-Li exchange.

Discussion

Specimens of human atrial tissue were incubated in Li-Tyrode's solution to replace partially intracellular K⁺ and Na⁺ with Li⁺. The specimens were then transferred to Na-containing solutions to allow outward Li⁺ and inward Na⁺ gradients to develop. After transfer, E_m transiently hyperpolarized to levels more negative than those recorded during the subsequent steady state. The hyperpolarization could have been caused by a change in transmembrane ionic conductances or by an electrogenic mechanism involving ionic exchange or active transport.

Because Na channels are permeable to Li⁺ (18), it is possible that an outward flow of Li⁺ ions through Na channels could have generated a hyperpolarizing current upon transfer of the specimens to Li⁺-free solution. However, were this the case, the amplitude of the hyperpolarization should have been reduced by TTX rather than augmented, as was found experimentally. Therefore, it is reasonable to conclude that an ion flow through Na channels does not account for the hyperpolarization. Li-loading may have increased K channel conductance (3). In principle, the increase might result in an outward flow of Li⁺ through K channels and thus hyperpolarize E_m . However, the depolarizing response to ACh (Figs. 2 B and 4) indicates that the hyperpolarization was not due to outward current through ACh-sensitive K channels, and the increase in amplitude of the hyperpolarization induced by Ba²⁺, relative to that obtained in experiments performed without Ba²⁺, indicates that the hyperpolarization was not due to outward current through Ba²⁺-sensitive K channels.

An outward flow of Li⁺ through channels insensitive to TTX, ACh and Ba²⁺ must, a priori, be considered a very unlikely mechanism for the hyperpolarization. Strong evidence against this possibility is provided by the experiments summarized in Fig. 6 C. When Li-loaded specimens were transferred to Li⁺-containing (25 mM) Na-Tyrode's solution, E_{max} was -57.6 ± 1.1 mV. At the time E_{max} was recorded intracellular Li⁺ should have been considerably less than the Li⁺ concentration in the cooling solution (129 mM). It follows that the equilibrium potential for Li⁺ should be less negative than -43 mV (calculated from the Nernst equation), i.e., at least 14 mV less negative than the E_{max} recorded experimentally. This indicates that the hyperpolarization cannot be accounted for by an

outward flow of Li⁺ through any species of ionic channel. Since hyperpolarization of Li-loaded specimens cannot be accounted for by change in an ionic conductance, an electrogenic mechanism should be considered. The inhibitory effect of acetylthiocholine (Fig. 5) indicates that electrogenic Na, K-pump activity must be the mechanism responsible for the hyperpolarization.

Since Li⁺ largely should have replaced intracellular Na⁺ and K⁺ during incubation in Li⁺-substituted solutions (3), enhanced pump activity could be caused by pumping intracellular Li⁺ or, alternatively, by pumping an intracellular Na-load that developed after transferring the tissue to Na-containing solutions. The biological and physical properties of Li⁺ and Na⁺ are in several respects quite similar (23). Li⁺ can, for example, replace Na⁺ in the mechanism generating the upstroke of cardiac action potentials (3), and the similarity between the physicochemical properties of the two ions can interfere seriously with interpretation of ion-selective-microelectrode recordings (24). For this study, the ionic selectivity of intracellular Na, K-pump sites is crucial for interpretation of the experimental findings. Previous studies (3, 17) have provided evidence against substantial outward transport of Li⁺ by the sarcolemmal Na, K-pump. However, glycoside-induced pump inhibition was not utilized in those studies. In the present study the effect of such inhibition on the Li⁺-content of guinea pig atrial tissue was examined. In agreement with the previous studies (3, 17) Na, K-pump activity or lack thereof had no apparent effect on Li⁺ efflux from myocytes.

In contrast, outward transport of Li⁺ by the Na, K-pump has been demonstrated in red blood cells, although under extreme experimental conditions (20, 25). However, the selectivity of Na⁺ over Li⁺ is so great that ouabain-sensitive Li⁺ efflux is undetectable unless the intracellular Na⁺ concentration is less than 1 mM (23). Such a low concentration could not possibly be achieved during incubation in the solutions used in this study (Table I) indicating that electrogenic pumping of Li⁺ by the Na, K-pump is unlikely to have caused the hyperpolarization of the Li-loaded specimens. In addition, if the hyperpolarization were due to pumping of Li⁺, extracellular Li⁺ would not be expected to be inhibitory at low extracellular Na⁺ concentration (Fig. 6 B) yet have no effect at normal extracellular Na⁺ concentration (Fig. 6 C). Therefore, it is reasonable to conclude that the hyperpolarization of E_m to levels more negative than E_K is not due to electrogenic pumping of intracellular Li⁺ by the Na, K-pump. Since the Na, K-pump-induced hyperpolarization observed upon transfer of Li-loaded tissue to Na-Tyrode's solution cannot be attributed to electrogenic pumping of Li⁺, it must have been due to electrogenic pumping of an intracellular Na-load that developed after the transfer.

The cellular content of Ca²⁺ probably increased during incubation in the cold Li-Tyrode's solution, and, upon transfer to warm Na-Tyrode's solution, the accumulated Ca²⁺ should be exchanged for Na⁺ via the Na-Ca exchange mechanism. This may have generated inward current (26), and the transient depolarization recorded during the first few min after the transfer (Fig. 2 A) could in principle be caused by such a current. However, Ca²⁺ should also accumulate intracellularly during incubation of specimens in cold Na-Tyrode's solution. A transient depolarization induced by Na-Ca exchange current should therefore also have occurred upon transfer of these specimens to warm Na-Tyrode's solution. This was not ob-

served (Fig. 1) suggesting that the transient depolarization of Li-loaded specimens (Fig. 2 A) was not directly related to a transient Ca-overload and an associated Na-Ca exchange current.

A Ca load accumulated during cooling in Li-Tyrode's solution may, nevertheless, have contributed indirectly to the time course of E_m shown in Fig. 2 A. Exchange of some intracellular Ca^{2+} for Na^+ may have provoked a small Na-load immediately after transfer and before when E_m first could be recorded. It is possible that the early depolarization of Li-loaded specimens was due to a rapidly decaying outward Na, K-pump current activated by the putative short-lived, early transient Na-load² occurring prior to when Na-Li exchange causes the Na load of interest. Alternatively, the initial changes in E_m could indicate that exchange of Li^+ for Na^+ is electrogenic. This alternative hypothesis, however, is inconsistent with the finding that the initial, relatively negative but rapidly depolarizing E_m seen in Li-loaded specimens (Fig. 2 A) was abolished when the Na, K-pump was inhibited by K^+ -free solutions (Fig. 5). A similar rapidly occurring initial depolarization was also observed after specimens cooled in Li^+ -free TEA-Tyrode's were transferred to Na-Tyrode's solution (Fig. 3), indicating that this particular depolarization was not dependent upon an intracellular Li load. It too may have involved a rapidly decaying hyperpolarizing Na, K-pump current associated with extrusion of a Na load developed because of an exchange of Na^+ for an initial Ca load in a manner analogous to that discussed above for Li-loaded specimens.

While exchange of intracellular Ca^{2+} for extracellular Na^+ may be involved indirectly in the mechanism underlying the initial, relatively negative, rapidly depolarizing E_m of Li-loaded specimens (Fig. 2 A), such an exchange cannot sponsor the subsequent hyperpolarization because the latter was found to be dependent upon an intracellular Li-load (compare Fig. 2 with Fig. 3). In addition, were Na-Ca exchange involved, the hyperpolarization of Li-loaded specimens should not be abolished by extracellular Li^+ (Fig. 6 B) because the Na-Ca exchanger does not appear to have affinity for Li^+ (27). Therefore, the evidence is against the notion that Na-Ca exchange underlies the hyperpolarization of E_m negative to E_K .

The persistence of the hyperpolarization in the presence of 10 μ M bumetanide (Fig. 7) indicates that the Na/K/2Cl co-transporter also is not involved in the mechanism underlying the hyperpolarization. This could be taken to indicate that the mechanism for Na-Li exchange in heart is different from that mediating Na-Li exchange in red blood cells. Inhibition of Na/K/2Cl co-transport with furosemide is said to inhibit Na-Li exchange in blood cells (5, 6). It should, however, be noted that millimolar concentrations of furosemide were needed to inhibit cotransport. Loop diuretics at such concentrations have been reported to exhibit nonspecific inhibitory effects on membrane transport systems other than the Na/K/2Cl cotransporter (8).

The Na, K-pump-induced hyperpolarization of the Li-loaded specimens was abolished by 10 μ M 5-(*N,N*-dimethyl)

amiloride (compare Fig. 8 with Fig. 2), suggesting that the Na-H exchanger could be involved in the mechanism causing the hyperpolarization. In principle, if an intracellular acid-load developed as a consequence of the low extracellular Na^+ concentration during exposure to cold Li-Tyrode's solution, a 5-(*N,N*-dimethyl)amiloride-sensitive exchange of Na^+ for intracellular protons should occur after transfer to Na-Tyrode's solution. The subsequent electrogenic Na-pumping of the Na load would then cause the hyperpolarization. However, development of a significant acid-load is unlikely. Cooling of the Li-Tyrode's solution should have inhibited any exchange-carrier-mediated changes in intracellular ionic composition (28). Consequently, intracellular pH should be determined by extracellular pH and E_m . E_m in cold Li-Tyrode's solution was ~ -15 mV (data not shown) and extracellular pH was ~ 7.7 . From these values intracellular pH, derived from the Nernst equation, should have been ~ 7.4 , i.e., considerably more alkaline than expected under physiological conditions. In addition, the time course of E_m during rewarming of Li-loaded specimens in the presence of 5-(*N,N*-dimethyl)amiloride was similar to the time course recorded when specimens cooled in low-Na, TEA-substituted Tyrode's solution were rewarmed in 5-(*N,N*-dimethyl)amiloride-free Na-Tyrode's solution (compare Fig. 3 with Fig. 8). This indicates that an intracellular Li load is required for a Na, K-pump-induced hyperpolarization to develop via a 5-(*N,N*-dimethyl)amiloride-sensitive mechanism.

In principle, intracellular Li^+ could cause a Na-load to develop by stimulating exchange of intracellular protons for extracellular Na^+ . Various agents can stimulate the Na-H exchanger by shifting the "set-point" for an intracellular "modifier" site in an alkaline direction (see reference 29 for review). However, when the loss of intracellular protons and Na^+ which must have occurred during cooling in Li-Tyrode's solution, and the relatively large buffer capacity of cells is considered, an unreasonably large shift in the "set-point" would seem to be required for such a mechanism to induce the Na load. In addition, experimental evidence suggests that Li^+ actually inhibits rather than stimulates the exchange of intracellular protons for extracellular Na^+ (30, 31). Therefore, it appears that a mechanism involving an actual exchange of intracellular Li^+ for extracellular Na^+ and the subsequent electrogenic Na-pumping of the consequent Na load offers the most likely explanation for the hyperpolarization occurring when Li-loaded tissue is transferred to Na-Tyrode's solution.

The rate of the Na-Li exchange cannot be determined from the present study. However, it can be concluded from the experiment depicted in Fig. 9 that the exchange rate is not necessarily the rate limiting determinant for the prolonged pump-induced hyperpolarization. This conclusion is consistent with findings from experiments on guinea pig atria. The decrease in the intracellular Li^+ concentration 15 min after transfer from Li-Tyrode's solution in this study was severalfold larger than the Na, K-pump-mediated decrease reported for the intracellular Na^+ concentration in Na-loaded guinea pig atria after 15 min under conditions expected to induce nearly maximal pump stimulation (see Fig. 1, reference 10).

It is interesting that the intracellular Li^+ concentration of guinea pig atria in this study 15 min after transfer to Na-Tyrode's solution (~ 27 mmol/liter intracellular water) was lower than the Li^+ concentration 30 min after transfer of cat papillary muscles in similar experiments (~ 66 mmol/liter in-

2. Any Na load developed in exchange for Ca^{2+} in specimens cooled in Na-Tyrode's solution may not be important quantitatively because cooling can induce very large Na-loads (10). This may explain why an early transient depolarization did not develop in the experiments depicted in Fig. 1.

tracellular water) (3). This might be taken to indicate that Na-Li counter transport occurs faster in guinea pig than in cat heart. Alternatively, the differences could be due to differences between delays in extracellular diffusion of Li^+ , a delay that for cat papillary muscle may be quite substantial during the first 30 min after the transfer (3). However, it does not seem plausible that delays in extracellular diffusion also accounts for 25 mmol Li^+ /liter intracellular water measured even 2 h after the transfer of cat papillary muscles (3). Such findings suggests that not all intracellular Li^+ is freely exchangeable. The possibility that a fraction of intracellular Li^+ is not freely exchangeable, in combination with quantitative uncertainties regarding transmembrane ionic gradients (arising from delays in extracellular ionic equilibration), makes an accurate kinetic analysis of Na-Li counter-transport difficult to perform in intact cardiac tissue. Such an analysis might require use of sarcolemmal membrane vesicles to ensure well-defined transmembrane ionic gradients.

Inhibition of the hyperpolarization in Li-loaded specimens by 5-(*N,N*-dimethyl)amiloride (Fig. 8) also suggests that the mechanism for the hyperpolarization recorded in the absence of drug (Fig. 2) involves an exchange of intracellular Li^+ for extracellular Na^+ . This is consistent with a recent report of inhibition of Na-Li exchange in canine smooth muscle sarcolemmal membrane vesicles (28) by another derivative of amiloride, ethylisopropylamiloride. However, considerably higher concentrations (0.1–1.0 mM) than used for the present study were needed to effectively inhibit the exchange, despite ethylisopropylamiloride being an order of magnitude more potent than 5-(*N,N*-dimethyl)amiloride (32). This could be due to species or tissue-dependent differences in drug-sensitivity or to differences in the experimental designs of the studies.

Both 5-(*N,N*-dimethyl)amiloride and ethylisopropylamiloride are potent inhibitors of the Na-H exchanger. Inhibition of Na-Li exchange might therefore be due to inhibition of the mechanism which usually mediates Na-H exchange. However, amiloride and its derivatives also can block Na, K-pumping (33), Na-Ca exchange (34) and ion permeation of Na and K channels (35, 36), at least in high concentrations. This suggests that inhibition of Na-Li exchange could have been due to effects on any of these transport systems. The Na-Ca exchanger does not have affinity for Li^+ (27), however. Therefore it is unlikely that Na-Li exchange could be mediated by an operational mode of the Na-Ca exchanger. In the concentrations used in this study, 5-(*N,N*-dimethyl)amiloride was found to have essentially no effect on electrogenic Na pumping, indicating that, although the Na, K-pump dealt with the resulting Na load, it did not mediate the Na-Li exchange. Finally, blockade of Na and K channels with TTX and Ba^{2+} did not inhibit hyperpolarization in Li-loaded specimens (Fig. 4), indicating that exchange of Li^+ for Na^+ involved neither Na nor K channels in the mechanism for the hyperpolarization. Although it cannot be excluded entirely that another currently unknown transport system, sensitive to 5-(*N,N*-dimethyl)amiloride, is able to transport Li^+ , it would seem that the effect of this drug on the counter transport system that usually mediates Na-H exchange offers the most reasonable explanation for inhibition of Na-Li exchange. This, in turn, suggests that Na-H exchange and Na-Li exchange are mediated by the same transport system. As first pointed out by Aronson (1), such common identity could have important implications for under-

standing the association between abnormalities in Na-Li counter transport and essential hypertension.

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