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Lithium and KB-R7943 effects on mechanics and energetics of rat heart muscle

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ABSTRACT

The role of calcium influx on energy expenditure during cardiac contraction was studied. For this purpose, the described ability of lithium and KB-R 7943 (KBR) to diminish Ca entry through Na–Ca exchanger (Ponce-Hornos & Langer, *J Mol Cell Cardiol* 1980, **12,** 1367, Satoh *et al., Circulation* 2000, **101,** 1441) were used. In isolated contractions (contractions elicited after at least 5 min of rest) LiCl 45 mmol L^{-1} decreased pressure developed and pressure–time integral from 42.3 ± 2.7 and 14.5 ± 1.2 to 32.1 ± 3.4 mN mm^{-2} and 8.3 ± 0.9 mN mm^{-2} s, respectively. A similar effect was observed in regular contractions (at 0.16 Hz stimulation). The presence of KBR (5 μmol L^{-1}) in the perfusate induced a slight but not significant decrease in pressure developed and pressure–time integral in steady-state contractions. As it was previously described, the heat involved in a heart muscle contraction can be decomposed into several components (H_1, H_2, H_3 and H_4), but only one (H_3) was associated with force generation. While H_3 decreased with lithium in both types of contractions, H_3/PtI ratio remained unaltered, indicating that the economy for pressure maintenance was unaffected. To further investigate the role of Ca entry on force development, a condition in which the contraction is mainly dependent on extracellular calcium was studied. An 'extra' stimulus applied 200 ms after the regular one in a muscle stimulated at 0.16 Hz induces a contraction with this characteristic (Marengo *et al., Am J Physiol* 1999, **276,** H309). Lithium induced a strong decrease in pressure–time integral and H_3 associated with this contraction (43 and 45%, respectively) with no change in H_3/PtI ratio. Lithium also reduced (53%) an energy component (H_2) associated with Ca cycling. The use of KBR showed qualitatively similar results [i.e. a 33% reduction in pressure–time integral associated with the extrasystole (ES) with no changes in H_3/PtI ratio and a 30% reduction in the H_2 component]. Li and KBR effects appear to be additive and in the presence of 45 mmol L^{-1} Li and 5 μmol L^{-1} KBR the extrasystole was abolished in 77%. Lithium and KBR effects particularly for the extrasystole can be explained through the inhibition of Ca entry via Na–Ca exchange giving support to the participation of the Na–Ca exchanger in the Ca influx from the extracellular space. In addition, the results also suggest the possibility of an effect of Li on an additional Ca sensitive locus (different than the Na–Ca exchanger). In this connection, in isolated contractions lithium decreased the energy release fraction related to mitochondrial processes (H_4) increasing the economy of the overall cardiac contraction.

Keywords calorimetry, cardiac energetics, EC coupling, heart, heat, KB-R 7943, lithium, Na–Ca exchange.

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The cardiac muscle contraction is triggered by a complex series of events. Upon excitation, Ca enters the cell from the extracellular space through voltage operated Ca channels and possibly the Na–Ca exchanger (Morad & Cleeman 1987, Levi *et al.* 1994, Vornanen *et al.* 1994, Su *et al.* 2001). Once inside the cell, this Ca induces the release of more Ca from the sarcoplasmic reticulum (SR) (Fabiato 1983, 1985) and as a consequence

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cytosolic Ca increases and activates the myofilaments. The return to the resting mechanical state requires the removal of Ca until resting cytosolic Ca levels are achieved. This can be carried out by SR-Ca pump and sarcolemmal (SL) systems such as SL-Ca pump and SL-Na–Ca exchanger (Reuter & Seitz 1968, Bers & Ellis 1982, Philipson & Ward 1986, Philipson 1992). Bridge *et al.* (1990) showed that all the Ca entering via voltage dependent Ca channels were removed via Na–Ca exchange. On the other hand, Leblanc & Hume (1990) based on experiments in which they induced a transient net influx of Ca via the Na–Ca exchanger suggested a role for the Na–Ca exchanger in the activation of contraction. Recent evidence (Su *et al.* 2001) suggested the existence of a Na fuzzy space where Na can modulate Ca influx via the Na–Ca exchanger. In addition, under conditions of intracellular Na overload, there is an intracellular Na-dependent Ca influx fraction, that can be inhibited by intracellular Li (Ponce-Hornos & Langer 1980). The route used by Ca during either the contraction or the relaxation process is of energetic relevance. For instance, while the SR-Ca pump uses one adenosine triphosphate (ATP) for the removal of two Ca, the sarcolemmal Ca pump has a stoichiometry of 1Ca : ATP (Philipson 1997). Similarly, Ca removal via the Na–Ca exchanger involves the entrance of 3Na/Ca removed and the removal of those 3Na via the Na–K pump will have to be carried out with the hydrolysis of one ATP. Furthermore, the increase of cytosolic Ca will have a different energetic effect depending on the mechanism used. If Ca enters the cell via an SL-Ca channel the energy accumulated in the electrochemical gradient during the resting period will be totally used, while if it enters via the Na–Ca exchanger part of that energy will be recovered in the form of a Na gradient.

It has been reported that the heat released by a cardiac muscle contraction induced after at least 5 min of rest (isolated contraction) can be decomposed into four components of heat released (Ponce-Hornos *et al.* 1995). Three of them (H_1, H_2 and H_4) were independent of pressure development (P) and pressure–time integral (PtI), and were proposed to be related to calcium binding to intracellular sites (H_1), ionic exchange (H_2) and mitochondrial activity (H_4) (Ponce-Hornos *et al.* 1995, Consolini *et al.* 1997). The remaining component, H_3, was correlated with PtI and it was mainly attributed to the actomyosin adenosine triphosphatase (ATPase) activity (Ponce-Hornos *et al.* 1995). For contractions obtained during sustained stimulation at 0.16 Hz (steady-state contraction) only three fractions of heat released were determined (Ponce-Hornos *et al.* 1995). They correspond to H_1, H_2 and H_3 of the isolated contractions. A relevant aspect of these measurements was that it allowed us to evaluate the tension independent heat (TIH) fraction simultaneously with the tension dependent heat (TDH) component in the presence of pressure development. This energetic approach was used together with Li and KB-R 7943 (KBR) to further investigate the role of Na–Ca exchanger during the contractile event at mechanical and energetic levels. The results support the idea that, particularly during the extrasystole, in addition to its role as Ca removal mechanism during relaxation Na–Ca exchanger could also be participating as a mechanism of Ca influx, even in the absence of intracellular Na overload. It was also found in isolated beat that Li modifies an energy release fraction related to mitochondrial processes increasing the economy of the overall cardiac contraction.

METHODS

Biological preparation

Female Wistar rats, weighing 260–320 g at the time of sacrifice, were heparinized (2000 U) and anaesthetized with a pentobarbital sodium overdose. The beating hearts were removed from the thorax and retrograde perfusion by the Langendorff method, with a Krebs-bicarbonate solution, was initiated at room temperature (20–24 °C). The right atria was carefully dissected from the heart and a small cut in the septal wall, close to the aorta, was performed in order to prevent spontaneous contractions. A latex balloon was placed into the left ventricle and the muscle was then mounted in a Kel-F frame between two stainless steel hooks (Ponce-Hornos *et al.* 1990, 1995). After cannulation and mounting, the ventricle was placed in the inner chamber of a calorimeter (Ponce-Hornos *et al.* 1982, 1995) and the latex balloon connected to a Statham P23Db pressure transducer so that pressure developed during isovolumic contractions could be measured. At the end of each experiment the tissue was removed from the calorimeter, weighed in a preweighed vial, and dried at 110 °C to constant weight so that water content could be calculated. The average water content in the present experiments was 81.6 ± 0.4% ($n = 20$).

Solutions

The heart was perfused at 25 °C and at a constant rate (4.8 mL min^{-1}) with a Krebs-bicarbonate solution containing (in mmol L^{-1}): NaCl 120, KCl 6, $MgCl_2$ 1.0, NaH_2PO_4 0.5, $CaCl_2$ 0.5, $NaHCO_3$ 25, dextrose 6.0 and LiCl 0, 15, 30 or 45, and made isoosmotic to 45 mmol L^{-1} LiCl by adding 90, 60, 30 and 0 mmol L^{-1} sucrose, respectively. Osmolarity of the

solutions were measured with a vapour pressure osmometer (Wescor Model 5100 C, Wescor Inc, Logan, UT, USA) and the differences in osmolarity between solutions were less than 6%. Solutions containing KB-R 7943 (Tocris Cookson Inc., Ellisville, MO, USA) 5 μmol L^{-1} were prepared by adding it from a stock solution 12 mmol L^{-1} [in dimethyl sulphoxide (DMSO)] to both, 90 mmol L^{-1} sucrose and 45 mmol L^{-1} LiCl solutions. The solutions were bubbled with 95% O_2–5% CO_2 to achieve a pH of 7.3–7.4. Under these experimental conditions, mechanical and energetic parameters remain reproducible for more than 6 h.

Mechanical and heat measurements

The technique for on line measurement of heat production and mechanical activity of isolated heart muscle has been described previously in detail (Ponce-Hornos *et al.* 1982, 1990, 1995). Briefly, the calorimeter was submerged in a constant temperature bath. The temperature of the calorimeter bath (25 °C) was controlled with a cooling–heating bath (±0.003 °C) in which the perfusate was also equilibrated. Calorimeter calibration was accomplished by passing 2.1 kHz sine wave through the muscle by means of the stimulating electrodes (Ponce-Hornos *et al.* 1982). The calorimetric response was significantly improved by increasing the number of thermosensitive junctions (from 62 in the original calorimeter to 254 in the present calorimeter). This led to an improvement in sensitivity, and at the perfusion rate used for the biological experiments the rate constant of the calorimeter was about 0.03 s^{-1}. The minimum output of the thermosensitive units recorded in the present experiments was higher than 40 μV whereas the electrical noise was 1 μV at a maximum gain (1 μV mm^{-1}). With this method it was possible to continuously and simultaneously record: rest pressure, pressure development (P), pressure–time integral (PtI), perfusion pressure, total heat production (H_t) and resting heat production (H_r). The heat and the left ventricular pressure data were digitized from either 25 or 50 mm s^{-1} records or with the use of an A/D converter (TL-1 DMA Axon Instruments Inc, Foster City, CA, USA). The sampling frequency ranged between 1 and 50 samples per second. The 50 samples per second rate was used for capturing the first few seconds in an isolated contraction (Fig. 1b), for the remaining part of the energetic event lower sampling frequencies were used. For the steady stimulation protocols all contractions were sampled at a rate of 50 samples per second. From the record at high speed, PtI was calculated. Once the muscle was placed in the inner chamber of the calorimeter, at least 60 min equilibration period with control solution (0 LiCl – 90 mmol L^{-1} sucrose) was allowed to elapse before any experimental intervention. During this equilibration period, optimal pressure development was functionally established under stimulation (0.16 Hz) by gradually inflating (every 3–4 min) the latex balloon. Under these conditions resting pressure development was increased in steps of about 1–2 mN mm^{-2} until stable pressure development showed no detectable increase at regular gain [0.5 (mN mm^{-2}) mm^{-1}]. A muscle was accepted for study if (1) during the equilibration period a minimum of 9 mN mm^{-2} steady pressure was developed at 0.16 Hz stimulus frequency at a resting pressure lower than 5.0 mN mm^{-2} and (2) it remained quiescent in the absence of stimulation.

Heat signal analyses

The analysis of the energy components released by a contraction was performed as described elsewhere (Ponce-Hornos *et al.* 1995). Briefly, when power applied is interrupted before the integration time, the calorimetric output from zero time to its peak value, can be fitted by the following equation (Ponce-Hornos *et al.* 1995):

$$\dot{H}_t = \dot{H}_0 \left[1 - A_0 e^{-\beta t} - 8\pi^{-2} \sum_{i=0}^{\infty} A_i e^{-\alpha_i t} \right] e^{-\tau t} \qquad (1)$$

where $A_0 = (\mu 4\pi^{-2}\beta^{-1})^{0.5} \tan[(\mu 4\pi^{-2}\beta^{-1})^{0.5}]$; $\mu =$ cooling rate constant of the calorimeter; $\beta =$ diffusion delay constant; $A_i = 1/\{(2i+1)^2 [1 - (2i+1)^2 \mu\beta^{-1}]\}$; $\alpha_i = (2i+1)^2\mu$; τ is the rate constant of the declining fraction; t is time, and H_0 represents the fitted value of the power. The area under the whole calorimetric output curve represents the total energy released during the event. Therefore, from the fitted parameters it was possible to calculate the energy involved in each component of heat released. The various fractions of energy released during a contraction were fitted as a linear combination of components each described by Equation (1) as shown elsewhere (Ponce-Hornos *et al.* 1995). Figure 1a shows the original record of both heat and pressure signals from a typical isolated contraction. Figure 1b represents the digitized heat signal output associated with the contraction shown in Figure 1a fitted to a linear combination of four heat release components described by Equation (1). As already pointed out elsewhere (Ponce-Hornos *et al.* 1995) due to the delay associated with the calorimetric response, the time to peak of each component should not be considered as the time to peak of the process associated with it (for detailed explanation, see Ponce-Hornos *et al.* 1995). Therefore, from this analysis, the amount of energy released by each component can be calculated, but no information is obtained on the time course of that release.

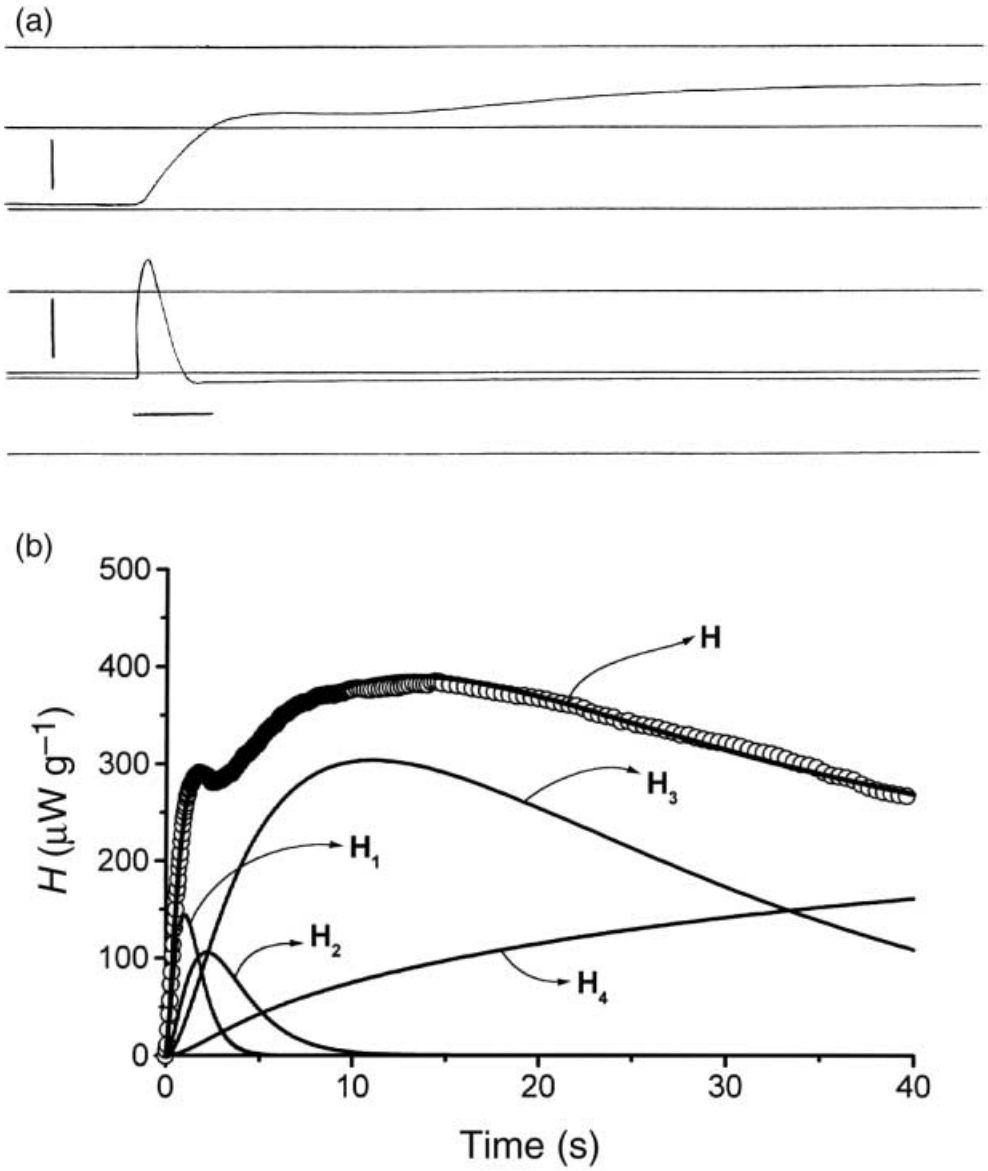

Figure 1 (a) Original record of an isolated contraction showing the heat production (upper trace, vertical bar at left in the heat record corresponds to 100 μW) and pressure development (lower trace, vertical bar corresponds to 20 mN mm^{-2}). The time scale represented by a horizontal bar corresponds to 1 s. (b) Digitalized data of heat production of the beat showed in (a). The fitted curve is shown as the continuous line on circles (H) and the four heat components (H_1, H_2, H_3 and H_4) that describe the data [according to Equation (1) in the text] are also labelled correspondingly.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM) and statistical significance was settled at $P < 0.05$ level. When more than two groups were compared a two-way analysis of variance was used. For paired comparisons a paired t-test was used. Regression analysis of myothermic records was performed with the use of a non-linear regression technique that uses the Marquardt algorithm running on an AT 486 compatible desk computer. Regression coefficients higher than 0.97 for $n > 240$ data points were obtained. Unless otherwise indicated, myothermic measurement results included in the present work are quoted per gram wet weight.

RESULTS

Effects of lithium on the isolated contraction

After the equilibration period with control sucrose perfusate the ventricles were kept at rest for at least 5 min and then a single stimulus was applied (3–5 V, 5–8 ms square wave pulse). Simultaneous mechanical

and myothermic responses were recorded. Afterwards, and during quiescent conditions, the muscles were exposed to solutions that contained LiCl 15, 30 and 45 mmol L^{-1}. Single contractions were recorded under all three LiCl levels.

As shown in Figure 2 the presence of Li in the perfusion media induced changes in the mechanical pattern in a dose dependent manner. Developed pressure (P) significantly decreased in the presence of Li (from 42.3 ± 2.7 to 32.1 ± 3.4 mN mm^{-2} for control and 45 mmol L^{-1} LiCl, respectively). The decrease in P was accompanied with a parallel decrease in the maximal rate of contraction ($+dP/dt$) (from 372 ± 36 to 270 ± 32 mN mm^{-2} s^{-1} for control and 45 mmol L^{-1} LiCl, respectively), so that the $(+dP/dt)/P$ ratio remained unchanged and all data were pooled and averaged. The averaged value of $(+dP/dt)/P$ for all 28 data points was 8.8 ± 0.3 s^{-1}. In contrast, maximal rate of relaxation ($-dP/dt$) was not affected by the presence of Li in the perfusion media at any of the Li levels tested, being the averaged value for all 28 data points 148 ± 7 mN mm^{-2} s^{-1}. Consequently, as shown in Figure 2c, the ratio between $-dP/dt$ and P increased with Li (from 3.2 ± 0.2 to 4.6 ± 0.2 s^{-1} for control and 45 mmol L^{-1} LiCl, respectively). This indicates that mechanical relaxation would be facilitated in the presence of Li. Total contraction time (TCt) significantly decreased with Li (from 0.66 ± 0.02 to 0.53 ± 0.02 s, for control and 45 mmol L^{-1} LiCl, respectively) without changes in time to peak tension (0.19 ± 0.01 s, $n = 28$). This decrease in TCt together with the decrease in P rendered a change in PtI from 14.5 ± 1.2 to 8.3 ± 0.9 mN mm^{-2} s for control and 45 mmol L^{-1} LiCl, respectively (see Fig. 2a).

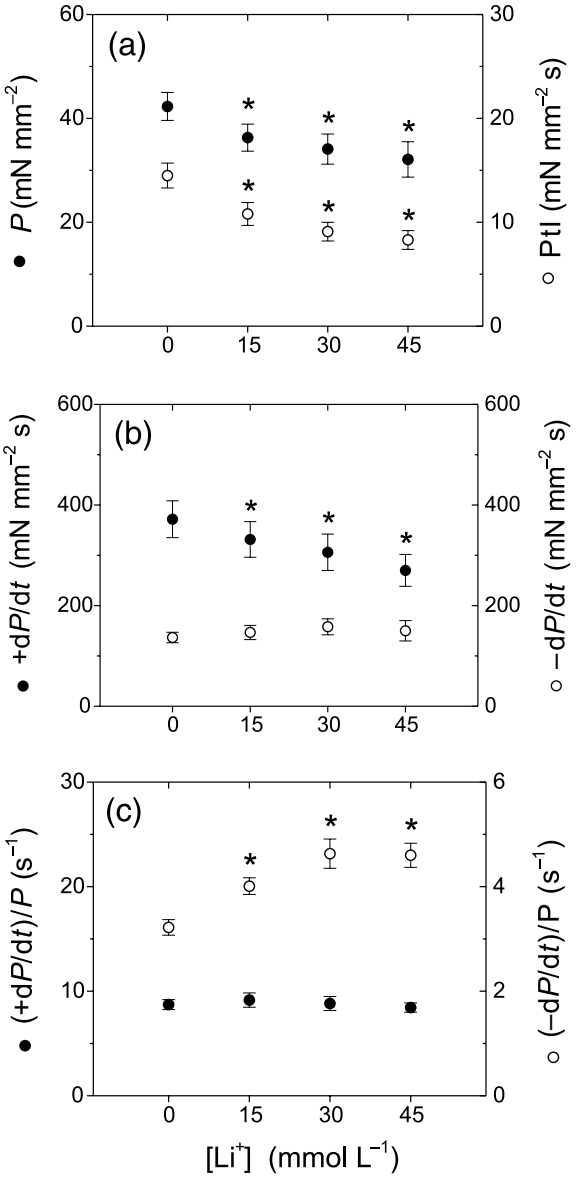

Figure 2 Mechanical performance of isolated heart beats at various Li levels. (a) (●) developed pressure (P) and (○) pressure–time integral (PtI). (b) (●) maximal rate of contraction ($+dP/dt$) and (○) maximal rate of relaxation ($-dP/dt$). (c) (●) ratio between maximal rate of contraction and pressure development ($(+dP/dt)/P$) and (○) ratio between maximal rate of relaxation and developed pressure ($(-dP/dt)/P$). Data are mean values and vertical bars represent one SEM for seven experiments. *$P < 0.05$ (paired t-test).

As previously reported (Ponce-Hornos *et al.* 1995) the heat released during an isolated beat could be decomposed into four fractions of heat, three of them independent of pressure development (H_1, H_2 and H_4) and one dependent on the development of pressure (H_3). The presence of Li in the perfusion media induced a significantly dose dependent decrease ($P < 0.05$) in the total heat released by a single beat (H_a) from 31.3 ± 4.6 to 12.3 ± 1.2 mJ g^{-1} (in the absence and in the presence of 45 mmol L^{-1} LiCl, respectively). The average decrease in H_a was −13.8 ± 3.3, −18.4 ± 3.9 and −19.0 ± 3.6 mJ g^{-1} for 15, 30 and 45 mmol L^{-1} LiCl, respectively. As shown in Figure 3, H_1 (energy fraction related to Ca binding) was unchanged in the presence of LiCl. The above mentioned fall in H_a was related to the decrease in H_2, H_3 and H_4 fractions of heat released by a single beat (Fig. 3). Lithium induced a significant decrease in H_2 (the energy fraction related to transport processes) from 3.1 ± 0.4 mJ g^{-1} under control conditions to 2.3 ± 0.3 mJ g^{-1} in the presence of 45 mmol L^{-1} LiCl. The fall in the pressure dependent heat fraction (H_3) induced by Li (from 8.5 ± 0.7 to 5.4 ± 0.7 mJ g^{-1} for control and 45 mmol L^{-1} LiCl, respectively) was accompanied by a parallel decrease in PtI (see above, Fig. 2) so that the H_3/PtI ratio remained unaltered. The averaged mean value for all 28 data points was 0.62 ± 0.02 mJ g^{-1} mN^{-1} mm^2 s^{-1}. This

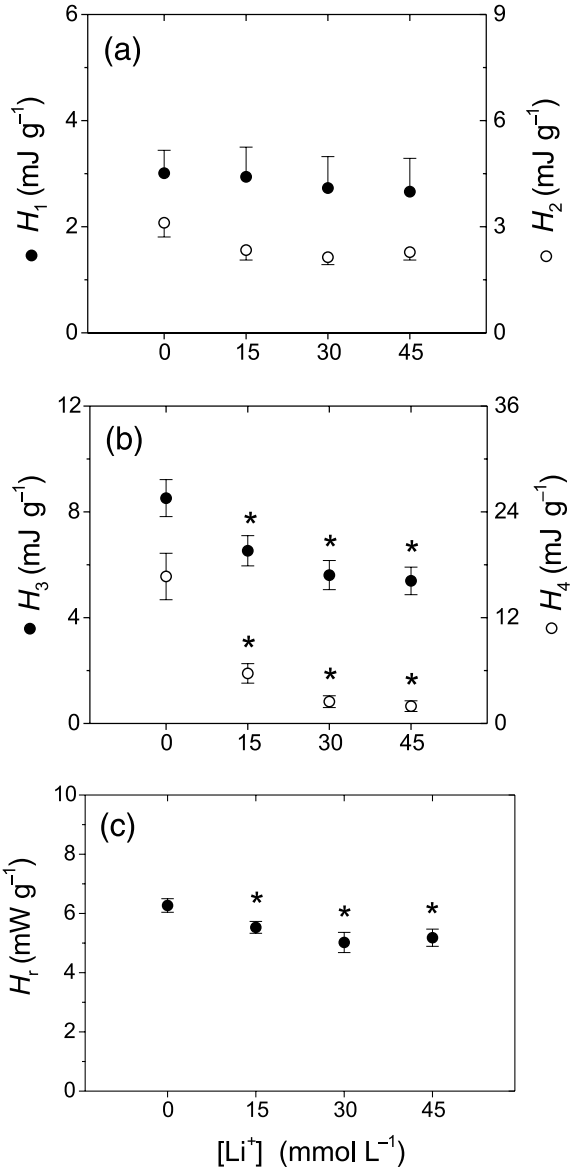

Figure 3 Myothermal performance of isolated heart beats at various Li levels. (a) (●) first fraction of heat released (H_1) and (○) second fraction of heat released (H_2). (b) (●) third fraction of heat released (H_3) and (○) fourth fraction of heat released (H_4). (c) Resting heat rate (H_r). Data are mean values and vertical bars represent one SEM for seven experiments. *$P < 0.05$ (paired t-test).

indicates that the economy for pressure maintenance remained unchanged in the presence of Li. Lithium induced a significant decrease in H_4 (Fig. 3) from 16.7 ± 2.6 mJ g^{-1} under control conditions to 2.0 ± 0.6 mJ g^{-1} in 45 mmol L^{-1} LiCl. In addition, resting heat rate also decreased significantly ($P < 0.05$) in the presence of LiCl in a dose dependent manner from 6.27 ± 0.23 to 5.13 ± 0.49 mW g^{-1} for control and 45 mmol L^{-1} LiCl, respectively (Fig. 3c).

Effects of lithium on steady-state contraction

The observed mechanical and energetic effects of Li on isolated contractions suggested a decreased Ca availability for contraction. A decreased Ca availability in the presence of Li could be related either, to the observed increase in Ca removal velocity relative to P (Fig. 2) and/or to the fall in Ca entry to the cytosol from SR and/or the extracellular space. To further study the last possibility, eight experiments were performed in which during stimulation at constant frequency (0.16 Hz) an extrasystolic stimulus was applied 200 ms after the regular one in the presence and in the absence of 45 mmol L^{-1} LiCl. It has been shown that the entry of Ca associated with such a stimulus is mainly extracellular in origin (Morad & Cleeman 1987, Marengo *et al.* 1999). Therefore, the mechanical and energetic evaluation of the associated contraction or extrasystole (ES) is appropriate to study the above mentioned hypothesis. The energetic and mechanical responses associated with ES were calculated as the difference in both parameters between the paired contraction (PC) and the regular (RC).

Lithium induced a significant decrease ($P < 0.05$, $n = 8$) in P (from 19.5 ± 2.6 to 16.4 ± 2.5 mN mm^{-2}) and PtI associated with steady-state beats (see Fig. 4a). As shown in Figure 4a, the portion of PtI associated with ES ($PtI_{PC} - PtI_{RC}$) was also diminished by Li (from 1.7 ± 0.3 to 1.0 ± 0.2 mN mm^{-2} s). This seems to indicate that in the presence of Li the amount of calcium that enters the cytosol during the ES is less than that of control condition.

As previously described (Ponce-Hornos *et al.* 1995) only three heat fractions (H_1, H_2 and H_3) were observed in the heat signal associated with steady-state contractions, instead of the four fractions found in isolated contractions. As it happened with isolated contractions, H_3 fell in the presence of Li (Fig. 4b). The fall in H_3 was accompanied by a parallel decrease in PtI, so that H_3/PtI ratio remained unaltered (Fig. 4c), being the averaged value for 16 data points 0.63 ± 0.03 mJ g^{-1} mN^{-1} mm^2 s^{-1}. As it was observed in single beats, while H_2 fraction of heat released decreased with Li (from 2.2 ± 0.4 to 1.7 ± 0.3 mJ g^{-1}, $n = 8$, $P < 0.05$), no change in H_1 was observed in steady-state beats (−0.23 ± 0.19 mJ g^{-1}).

The heat released by the ES was calculated by subtraction of the heat signal of the RC contraction from that of the PC one. This extra heat released was fitted to three or two heat released fractions. The number of fractions of heat released associated with the ES was dependent on whether the extrasystolic stimulus induced an increment in pressure development over the regular beat or it simply prolonged pressure maintenance (Marengo *et al.* 1999). The pressure dependent

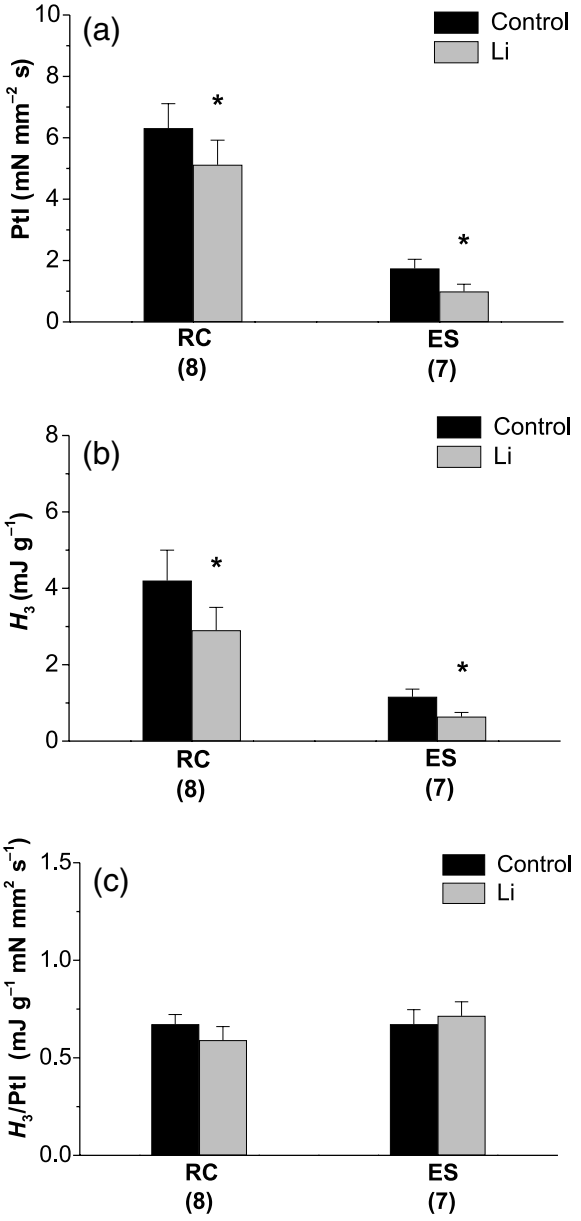

Figure 4 Effects of Li (45 mmol L^{-1}) on regular contraction (RC) and extrasystole (ES). (a) Pressure–time integral (PtI) under control condition and in the presence of Li. (b) Force related component (H_3) in the absence and in the presence of Li. (c) Economy of pressure maintenance (H_3/PtI) under control condition and in the presence of Li. Bars represent mean ± SEM. Numbers in parenthesis indicate number of experiments. *$P < 0.05$ (paired *t*-test).

component (H_3) associated with ES decreased in the presence of Li (Fig. 4b) but the H_3/PtI ratio remained unaltered. The mean of the pooled H_3/PtI values was 0.69 ± 0.06 mJ g^{-1} mN^{-1} mm^2 s^{-1} ($n = 14$), indicating that the energy cost for pressure maintenance of the extrasystole is not modified by Li (Fig. 4c). While the first heat fraction (H_1) associated with the ES showed no change in the presence of LiCl (−0.01 ± 0.02 mJ g^{-1}, $n = 7$, NS), the magnitude of the second heat fraction released during the ES (H_2) fell ($P < 0.05$) when Li was present in the perfusion media (−0.24 ± 0.07 mJ g^{-1}).

As it happened in isolated beat experiments, resting heat rate for these experiments significantly fell ($P < 0.05$) in the presence of 45 mmol L^{-1} LiCl from 6.35 ± 0.31 to 5.41 ± 0.25 mJ g^{-1} ($n = 8$).

Effects of KBR and KBR-lithium on steady-state contraction

In order to further investigate the role of the Na–Ca exchanger the effects of a specific inhibitor for the exchanger at a concentration that affects the Ca entry mode was studied (Satoh *et al.* 2000). Five experiments where performed in which the same protocol used for the lithium intervention during steady stimulation was applied. That is, during stimulation at constant frequency (0.16 Hz) an extrasystolic stimulus was applied 200 ms after the regular one in the presence and in the absence of 5 μmol L^{-1} KBR. The energetic and mechanical responses associated with ES were calculated as the difference in both parameters between the PC and RC.

KBR induced a slight but not significant decrease in P (from 21.1 ± 2.2 to 20.0 ± 2.1 mN mm^{-2}) and PtI associated with steady-state beats (Fig. 5a). The PtI associated with the ES ($PtI_{PC} - PtI_{RC}$) was diminished by KBR (Fig. 5a) from 1.8 ± 0.2 to 1.2 ± 0.1 mN mm^{-2} s ($P < 0.05$, $n = 5$). This seems to indicate that as it happened with Li, in the presence of KBR the amount of calcium that enters the cell during the ES is less than that of control condition. Once the measurements in the presence of KBR were performed, Li (45 mmol L^{-1}) was also added to the perfusate. The addition of Li in the presence of KBR induced a decrease in P (to 17.2 ± 2.2 mN mm^{-2}) and PtI (to 5.7 ± 0.8 mN mm^{-2} s) of the steady-state contraction (Fig. 5a). A similar behaviour was observed regarding PtI of the ES, decreasing in the presence of Li and KBR to 0.4 ± 0.1 mN mm^{-2} s (Fig. 5a). While the fall in PtI associated with ES induced by KBR alone was over 33% of the control value, when Li was added in the presence of KBR the fall reached about 78%. As it was shown above, the decrease in PtI associated with the ES observed when Li was present in the perfusion media was about 43% of the control value. Therefore, the effects of Li and KBR appear to be additive. Although to a lesser extent, H_3 slightly fell in the presence of KBR and it was further reduced when Li was added (Fig. 5b). The changes in H_3 were accompanied by parallel changes in PtI, so that H_3/PtI ratio remained unaltered (Fig. 5c). The averaged value for 15 data points was 0.67 ± 0.03 mJ g^{-1} mN^{-1} mm^2 s^{-1}. KBR induced a slight but not significant decrease in H_2 fraction of heat release (from 2.2 ± 0.3 to

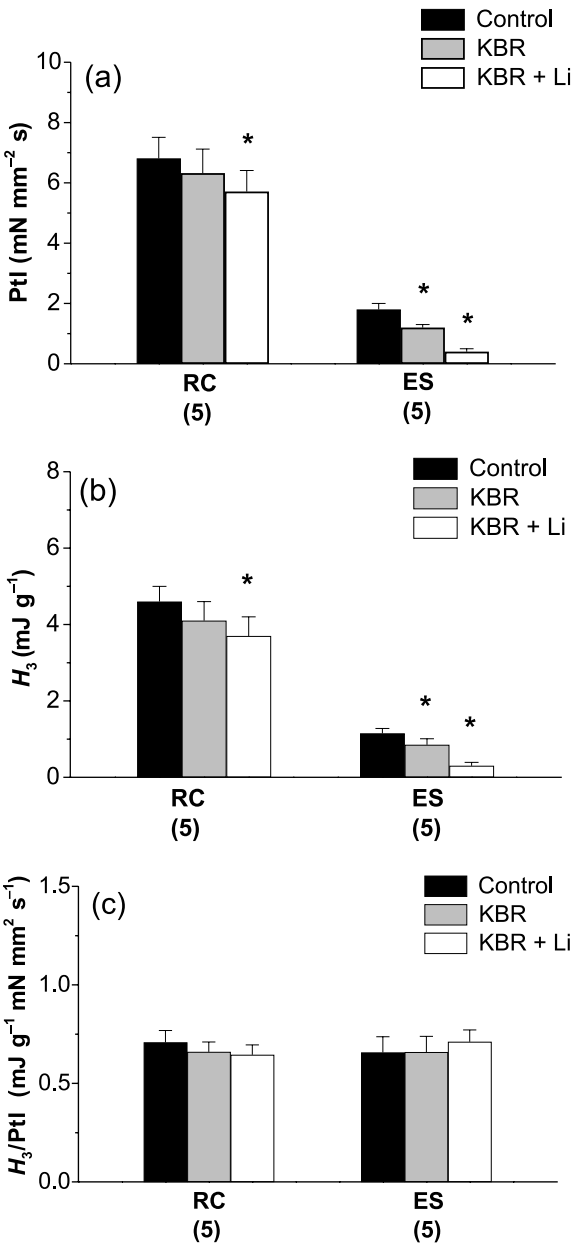

Figure 5 Effects of KBR (5 μmol L^{-1}) and KBR (5 μmol L^{-1}) plus Li (45 mmol L^{-1}) on regular contraction (RC) and extrasystole (ES). (a) Pressure–time integral (PtI) under control condition and in the presence of Li. (b) Force related component (H_3) in the absence and in the presence of Li. (c) Economy of pressure maintenance (H_3/PtI) under control condition and in the presence of Li. Bars represent mean ± SEM. Numbers in parenthesis indicate number of experiments. *$P < 0.05$ (paired t-test).

1.8 ± 0.3 mJ g^{-1}) and it was further reduced (to 1.2 ± 0.2 mJ g^{-1}) when Li was added. No significant change in H_1 was observed neither in the presence of KBR nor in the presence of Li and KBR.

The pressure dependent component (H_3) associated with ES decreased in the presence of KBR and it was further reduced when Li was added (Fig. 5b). As shown in Figure 5c, the H_3/PtI ratio remained unaltered. The mean value of the pooled H_3/PtI values was 0.68 ± 0.05 mJ g^{-1} mN^{-1} mm^2 s^{-1} ($n = 15$), indicating that the energy cost for pressure maintenance of the ES is not modified by KBR. While the first heat fraction (H_1) associated with the ES showed no change in the presence of KBR (−0.03 ± 0.03 mJ g^{-1}), the magnitude of the second heat fraction released during the ES (H_2) fell ($P < 0.05$) when KBR was present in the perfusion media (from 0.42 ± 0.06 to 0.29 ± 0.08 mJ g^{-1}). When Li was added in the presence of KBR, no changes were detected in H_1 but a further decrease in H_2 was observed reaching a value of 0.12 ± 0.03 mJ g^{-1}.

DISCUSSION

Resting heat production measured under control conditions for the present experiments (6.31 mW g^{-1}), was higher than previously reported by us and other investigators (4.09–4.78 mW g^{-1}) in rat heart (Gibbs & Loiselle 1978, Loiselle 1987, Bonazzola *et al.* 1992, Ponce-Hornos *et al.* 1995, Márquez *et al.* 1997). The difference can be attributed to the increase in osmolarity of the perfusate used for the present work. In five experiments (data not shown) we found that resting heat rate increases with osmolarity by about 40%. In this connection, an osmolarity-dependent oxygen consumption and heat production increases were reported in quiescent rat cardiac muscle (Daut *et al.* 1989, Hanley *et al.* 1994).

The presence of LiCl decreases resting heat rate. It was reported that Li enters the cell through pathways that are also used by Na (Gow & Ellis 1996). Also, it has been shown that Li can displace equimolar amounts of Na and K out of the cell (Carmeliet 1964, Gow & Ellis 1991) but it is not actively eliminated via Na/K pump (Smith 1974). Therefore, a decrease in intracellular Na would lead to a decreased activity of the Na/K pump with a consequent decrease in resting heat rate.

The release of heat during an isolated contraction could be decomposed into four fractions of heat: H_1 (that can be related to Ca binding processes), H_2 (that was tentatively related to Ca removal processes), H_3 associated with actomyosin interaction and H_4 associated with Ca-sensitive mitochondrial processes (Ponce-Hornos *et al.* 1995, Consolini *et al.* 1997). The decrease in the heat released by an isolated contraction induced by Li can be ascribed to the fall of three (H_2, H_3 and H_4) of the four fractions of heat release. Note that it has been shown that all three fractions (H_2, H_3 and H_4) are dependent on the extracellular calcium concentration (Ponce-Hornos *et al.* 1995, Consolini *et al.* 1997). Also the observed changes in the myothermal response to Li were accompanied by a decrease

in PtI with no changes in H_3/PtI ratio, indicating that the economy of pressure maintenance was not modified. Therefore, the effect of Li on P and PtI seems not to be a consequence of an effect on the myofilaments–Ca interaction, but most probably related to a decrease in the availability of calcium for the contractile event. A decrease in Ca availability could be related to either a diminished Ca influx from extracellular space (which would induce less Ca release from the SR), or to an increase in Ca removal [suggested by the increase in relaxation velocity relative to P ($(-dP/dt)/P$) induced by Li]. In this regard, while maximal rate of relaxation remains unchanged in the presence of Li the ratio $(-dP/dt)/P$ increases indicating that the final result is an improved relaxation per unit of developed pressure. This can be explained if Li inhibition on the Na–Ca exchanger is proportionally higher for the Ca entry mode than for the Ca removal mode of the exchanger. It should be noted that Li accumulates in the cell so that it will compete with intracellular Na (which is at a relatively low concentration) and subsequently will inhibit the Ca inward mode of the exchanger. On the contrary, as extracellular Na is high compared with extracellular Li (even for the highest concentration used for Li, the ratio Na/Li for the extracellular media is higher than three) an effect of extracellular Li should become less noticeable. In addition, intracellular Na concentration is reduced in the presence of Li and consequently it should increase the Na electrochemical gradient for the Na–Ca exchanger in its Ca removal mode, facilitating relaxation. Such an increase in Na gradient [in line with the increase in $(-dP/dt)/P$ ratio induced by Li] would lead to a higher participation of Na–Ca exchanger as a mechanism of Ca removal. The decrease observed in all three fractions of heat released (H_2, H_3 and H_4) and in particular the decrease of H_2 fraction (the fraction related to Ca removal processes) with LiCl suggests a diminished Ca availability to the contractile event associated with a decrease in cytosolic Ca influx.

To test the hypothesis of a Ca influx through Na–Ca exchanger, the mechanical and energetic responses associated with an ES were evaluated. Lithium decreased both, the PtI and the H_2 energy component associated with an ES. Because of the fact that the Ca entering during ES is mainly extracellular in origin (Marengo *et al.* 1999), a decrease in PtI and H_2 further supports the idea of a diminished Ca influx from the extracellular space. Work performed on rat ventricular myocytes strongly suggests that the majority of the Ca that activates contraction is released from the SR, this Ca release being induced by the Ca entering the cell via L-type Ca channels (Wier 1991, Wier *et al.* 1995, Cheng *et al.* 1996, Wang *et al.* 2001). In this matter, it has been proposed that Na–Ca exchange is unable to trigger Ca release from the SR or activate contraction under physiological conditions (Sham *et al.* 1995, Morad & Suzuki 1997, Satoh *et al.* 2000). The results presented here agree with it and they suggest that, under physiological conditions and steady stimulation the participation of the Na–Ca exchanger (evaluated via Li and KBR effects on mechanical and energetic parameters), is relatively small (6–16%). On the other hand, under a pathological condition such as an ES the role of the Na–Ca exchanger in providing Ca for the contraction becomes more relevant.

From the present data it cannot be established if Na–Ca exchange mechanism participates in bringing Ca into the cells for the activation of the contraction directly, or as a Ca triggering mechanism for SR Ca release (Levi *et al.* 1993, Morad & Suzuki 1997). Inspite of that uncertainty, during the ES, in both cases (Li and KBR) an inhibition of the exchanger in the Ca entry mode results in a depressed PtI. In fact, it has been shown (Ponce-Hornos & Langer 1980) that in Na-overloaded muscles Li decreases Ca uptake. It could be speculated that an increased cellular Na (particularly in the fuzzy space proposed by Su *et al.* 2001) could be the major increase in driving force for the Ca influx via the exchanger during the ES. Under such a condition the Na–Ca exchanger activity would be enhanced and therefore more exposed to the inhibitory effect of KBR and Li.

The effect of Li and KBR could either be additive on the Na–Ca exchanger or, in the case in which the Na–Ca exchange is inhibited in a high degree by KBR (as suggested by Satoh *et al.* 2000 and Shigekawa & Iwamoto 2001 for the KBR concentration used in the present work), the possibility of an additional effect on force development (not mediated by a reverse Na/Ca exchange) by Li arises. In addition to the improvement on relaxation induced by Li [indicated by the increase in the $(-dP/dt)/P$ ratio, which is not affected by KBR], the present work shows that the H_4 energy component (a Ca dependent energy component associated with the mitochondria) in the isolated contraction is affected by Li. The fact that the fourth fraction of heat release (H_4) decreases in the presence of Li also agrees with the capacity of Li to stimulate the mitochondrial Ca efflux. A stimulation of mitochondrial Ca efflux combined with a diminished Ca influx could affect the level of Ca necessary for enzyme activation in the mitochondrial matrix and consequently decreasing the mitochondrial futile cycle (Carafoli *et al.* 1980, McCormack *et al.* 1990, Garlid *et al.* 1991, Gunter *et al.* 1994). It is of interest to note that because of this decrease in the fourth energy component the overall energetic effect of Li for the isolated contractions is to improve muscle economy.

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