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The role of calcium in stimulation of sugar transport in muscle by lithium

Luisa Bigornia and Ivan Bihler

Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, R3E 0W3 (Canada)

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We have investigated the relation between the stimulation of sugar transport by Li^+ and Li^+-induced changes in cellular Ca^{2+} distribution. The fluxes of 3-*O*-[^{14}C]methyl-D-glucose and ^{45}Ca were measured in hemidiaphragm, soleus, and cardiac muscles of the rat, and cellular levels of Ca^{2+}, Na^+ and K^+ were determined. Li^+ increased in parallel the fluxes of 3-*O*-[^{14}C]methyl-D-glucose and ^{45}Ca in rat hemidiaphragm and soleus muscles. Sugar transport and Ca^{2+} efflux were also stimulated by Li^+ in Ca^{2+}-free medium, suggesting that in addition to increasing sarcolemmal Ca^{2+} influx, Li^+ may also cause the release of Ca^{2+} from intracellular storage sites, presumably the mitochondria. Mitochondria were isolated from preparations of rat ventricular muscle exposed to Li^+, and their Ca^{2+} content was determined. In rat cardiac muscle, Li^+ stimulation of sugar transport was associated with decreased mitochondrial Ca^{2+} levels (indicating mitochondrial Ca^{2+} release) only under conditions of deteriorating mitochondrial function. Thus, Li^+-induced changes in cellular Ca^{2+} distribution, which would increase cytosolic Ca^{2+} levels, were associated with stimulation of sugar transport. These observations support the hypothesis that the increased availability of cytosolic Ca^{2+} regulates the activity of the sugar transport system in muscle.

Introduction

Bhattacharya [1,2] first observed that lithium (Li^+) increased the basal uptake of glucose in rat diaphragm and epididymal fat pads. Further studies [3,4] showed that the action of Li^+ differed quantitatively and qualitatively from that produced by insulin in the same tissue. The mechanism whereby lithium (Li^+) stimulates sugar transport in muscle is not known. Li^+ did not significantly alter the concentration of glycolytic intermediates, adenine nucleotides (ATP, cAMP) [5] and creatine phosphate concentrations [6] under conditions where the rate of glucose metabolism was markedly enhanced. These results suggest that such factors are probably not involved in Li^+ activation of sugar transport [7]. However, Li^+ does affect the cellular distribution of calcium. Studies have shown that Li^+ increased sarcolemmal Ca^{2+} influx in heart muscle [8], induced the efflux of Ca^{2+} from heart mitochondria [9], and inhibited Ca^{2+} uptake into sarcoplasmic reticulum [10–12]. Changes in Ca^{2+} distribution which increase cytosolic Ca^{2+} have been associated with the activation of the sugar transport system, and it was thus hypothesized that an increased availability of Ca^{2+} for binding to a specific site may activate sugar transport [13].

The object of this study was to examine the relation between the stimulation of sugar transport by Li^+ and effects of Li^+ on cellular Ca^{2+} fluxes. Li^+ increased in parallel the fluxes of 3-*O*-[^{14}C]methyl-D-glucose and ^{45}Ca in rat skeletal muscles. In rat cardiac muscle, a relation between Li^+ stimulation of sugar transport and Li^+-induced release of mitochondrial Ca^{2+} was apparent

when mitochondrial function was depressed. The results of this study are consistent with a role of Ca^{2+} in the regulation of sugar transport. A preliminary report of some of this work has appeared earlier [14].

Methods

3-O-[^{14}C]Methyl-D-glucose and ^{45}Ca influx experiments

3-*O*-[^{14}C]Methyl-D-glucose influx was measured in isolated intact rat hemidiaphragm and in different preparations of rat cardiac muscle. ^{45}Ca influx was measured only in rat hemidiaphragms. Intact rat hemidiaphragms were isolated from young male Sprague-Dawley rats, weighing 50–70 g, and incubated as described by Kono and Colowick [15]. For experiments using ventricular muscle slices, hearts from Sprague-Dawley rats weighing 400–600 g were perfused 20 min without recirculation to remove blood, and the ventricular muscle was cut into 0.3 mm thick slices using a Stadie-Riggs tissue slicer. Hemidiaphragms or cardiac slices were then preincubated for 20–30 min, followed by 30 min (hemidiaphragms) or 10–20 min (cardiac slices) incubation under the same conditions, but with the addition of ^{14}C-labelled and unlabelled nonmetabolized sugar analogue, 3-*O*-[^{14}C]methyl-D-glucose (total concentration, 5.0 mM), and [G-^{3}H]inulin, a marker for the extracellular space. Intact resting left atria were isolated from Sprague-Dawley rats weighing 300–350 g, and prepared for perifusion as described by Sawh and Bihler [16]. Isolated left atria were preperifused in an open circuit for 15 min at 30°C followed by a 15 min recirculating perifusion with medium containing the sugar and inulin. Krebs-Henseleit bicarbonate solution (pH 7.4) containing 1.25 mM Ca^{2+} and 4 mM pyruvate but no glucose, saturated with 95% CO_2/5% O_2 and maintained at 37°C, was used for perifusion and incubation of tissues.

After incubation or perifusion, the muscles were treated and analysed for radioactivity and ion content as described before [17]. The same procedure was followed for measurements of Ca^{2+} uptake, except that tracer amounts of $^{45}CaCl_2$ were added instead of ^{14}C-labelled 3-*O*-methyl-D-glucose. Sugar transport and Ca^{2+} uptake were calculated as percent equilibration, i.e., the concentration in the intracellular water space was expressed as a percentage of the final concentration in the incubation medium. Data were presented as rates (μmol/ml cell water per min) of sugar or Ca^{2+} transport:

$$\{[\text{percent equilibration} \times \text{substrate concentration in medium (mM)} \times 1000]\} \times \{\text{incubation time (min)}\}^{-1}$$

3-O-[^{14}C]Methyl-D-glucose and ^{45}Ca efflux experiments

Efflux experiments were performed with soleus muscles isolated from Sprague-Dawley rats weighing 50–75 g. The procedures for isolation and incubation of the soleus muscles were as described by Kohn and Clausen [18]. The muscles were loaded for 60 min with 2 μCi/ml $^{45}CaCl_2$ or 6 μCi/ml 3-*O*-[^{14}C]methyl-D-glucose (and 1 mM unlabelled 3-*O*-methyl-D-glucose). This was followed by a washout of radioactivity into a series of tubes containing 4 ml unlabelled medium for a total of 200 min. This washout period consisted of an initial 70 min wash, followed by successive 10-min washes. One muscle in each pair was switched at 90 min to wash medium containing the test factor (e.g. Li^{+}). After the last efflux period, the muscles were lightly blotted on filter paper, weighed, and then treated and analysed for radioactivity as described before [17]. An aliquot of medium from each washout period was measured for its radioactive content. The amount of radioisotope retained in the tissue at each time interval of the efflux period and the percentage of radioisotope released from the tissue per min was calculated as described by Clausen [19]. The radioisotope content (A_t) of the tissue at various times during the washout was calculated by adding successively in reverse order the amount of radioisotope released into each vial (a_t) during the 10 min periods:

$$A_{t=10} = A_t + a_t$$

The percent fractional loss was calculated as fol-

lows:

$$[\text{amount in medium } (a_t)/\text{amount in tissue } (A_t) \times \text{min}] \times 100$$

Data were expressed as fractional efflux rates of control and test muscles, during the 100–110 min period, i.e., 10–20 min after change in the composition of solution bathing the test muscle.

Experiments with mitochondria

Hearts from Sprague-Dawley rats weighing 400–600 g were perfused 5–10 min without recirculation to remove blood. In experiments using intact ventricular muscle, open circuit perfusion was followed by a 20 min recirculating perfusion with a Li^+-containing buffer in the presence or absence of extracellular Ca^{2+}. The perfusion buffer was supplemented with 4 mM pyruvate and 5 mM glucose to delay the deterioration of the heart [20]. In experiments using muscle slices, ventricular muscle was prepared as described above, and the slices were incubated for 40 min in a Li^+-containing buffer in the presence or absence of extracellular Ca^{2+}. Ventricular muscle mitochondria were isolated from whole hearts or cardiac slices according to the method of Nayler et al. [21]. To prevent changes in the mitochondrial Ca^{2+} levels during isolation, the homogenization and differential centrifugation steps were carried out in a medium lacking Na^+ and EDTA, and containing Ruthenium red (5 μM). The following solutions were used: (1) homogenizing medium (0.21 M mannitol, 0.07 M sucrose, 0.01 M Tris-phosphate (pH 7.6), 0.005 g/ml Nagarse, 5 μM Ruthenium red); and (2) suspending medium (0.21 M mannitol, 0.07 M sucrose. 0.01 M Tris-HCl (pH 7.4), 5 μM Ruthenium red). Bovine serum albumin (1%) was included in the homogenizing medium to prolong mitochondrial survival, but was omitted from the suspending medium to avoid interference with protein determinations. Mitochondrial function was monitored by measuring the parameters of oxidative phosphorylation, which include the ADP:O ratio, respiratory control index (RCI), and oxygen consumption rate (Q_{O_2}) [22]. Protein was determined by the method of Lowry et al. [23], and cytochrome oxidase activity by the method of Schnaitman et al. [24].

Na^+ and K^+ were determined by emission flame photometry using the lithium internal standard procedure. Results were expressed as millimolar concentrations in the intracellular water. Total tissue or mitochondrial Ca^{2+} content was determined by atomic absorption spectrophotometry.

Results were analysed using the two-tailed Student's *t*-test [25].

Results

Rat hemidiaphragm

Intact isolated rat hemidiaphragms were initially used in sugar and Ca^{2+} transport studies, because the earlier observations of Li^+ stimulation of sugar transport were made in rat hemidiaphragm muscle [1,4]. The right and left hemidiaphragm were randomly assigned to treatment and control preincubation flasks in a paired experimental design. Table I shows the effects of Li^+ on 3-*O*-[^{14}C]methyl-D-glucose uptake by isolated intact rat hemidiaphragm. Significant and dose-dependent increases in 3-*O*-methyl-D-glucose uptake were seen with the addition of 2, 10 and 20 mM Li^+ to Ca^{2+}-containing Krebs-Henseleit bicarbonate buffer and with complete replacement of $[Na^+]_o$ (145 mM) with Li^+, both in the presence and absence of extracellular Ca^{2+}.

To prevent complications from Li^+-induced hyperosmolarity, iso-osmolarity was maintained by removing extracellular Na^+ in proportion to the amount of Li^+ added. In the low-Na^+ control, an equivalent amount of mannitol was added. Table I shows the effects of low Na^+, Li^+ and Ca^{2+}-free medium on 3-*O*-methyl-D-glucose transport and ^{45}Ca uptake by isolated intact rat hemidiaphragm. Reduction of $[Na^+]_o$ from 145 mM to 95 mM significantly increased 3-*O*-methyl-D-glucose transport ($P < 0.001$) and ^{45}Ca uptake ($P < 0.001$). The addition of 20 or 50 mM Li^+ to low-Na^+ media caused further significant increases in 3-*O*-methyl-D-glucose transport and corresponding increases in ^{45}Ca uptake. The results show that in the presence of extracellular Ca^{2+}, Li^+ stimulation of sugar transport was associated with an increase in Ca^{2+} influx. In Ca^{2+}-free low Na^+ medium, 50 mM Li^+ also significantly increased sugar transport ($P < 0.001$). This suggests that in addition to increased sarcolemmal Ca^{2+} influx, the stimulation

TABLE I

EFFECT OF Li^+ ON 3-*O*-[^{14}C]METHYL-D-GLUCOSE AND $^{45}CaCl_2$ UPTAKE IN INTACT ISOLATED RAT HEMIDIAPHRAGM

The buffer designations indicate the concentrations of relevant ions in millimoles per liter (mM). D-Mannitol (mann) was added for osmotic balance in low Na^+ controls (see text). The Na^+-free, $Li(HCO_3)$ buffer consisted of 120 mM LiCl and 25 mM $Li(HCO_3)$. Data represent mean ± S.E., and numbers in brackets denote the number of experiments. The last column shows the ratio of uptake in treated versus control muscles.

Control (C)	μmol/ml per min	Treatment (T)	μmol/ml per min	T/C
(A) 3-*O*-[^{14}C]Methyl-D-glucose				
(1) Ca^{2+} present				
145 Na^+	18 ± 2(20)	2 LiCl	23 ± 2(20) [b]	1.28
145 Na^+	20 ± 2 (6)	10 LiCl	30 ± 4 (6) [b]	1.50
145 Na^+	19 ± 2 (6)	20 LiCl	37 ± 5 (6) [b]	1.95
145 Na^+	18 ± 1 (8)	$-Na^+$, $Li(HCO_3)$	63 ± 7 (8) [c]	3.50
145 Na^+	28 ± 1 (6)	125 Na^+, 40 mann	26 ± 1 (6) [a]	0.93
125 Na^+, 40 mann	16 ± 2 (6)	125 Na^+, 20 Li^+	26 ± 2 (6) [c]	1.63
145 Na^+	27 ± 1 (5)	95 Na^+, 100 mann	56 ± 3 (5) [c]	2.07
95 Na^+, 100 mann	25 ± 5(11)	95 Na^+, 50 Li^+	43 ± 6(11) [c]	1.72
(2) Ca^{2+}-free				
145 Na^+	20 ± 3(11)	$-Na^+$, $Li(HCO_3)$	127 ± 8(11) [c]	6.35
95 Na^+, 100 mann	37 ± 2(10)	95 Na^+, 50 Li^+	60 ± 4(10) [c]	1.62
(B) ^{45}Ca				
125 Na^+, 40 mann	27 ± 1 (5)	125 Na^+, 20 Li^+	32 ± 1 (5) [c]	1.2
124 Na^+	25 ± 1 (8)	95 Na^+, 100 mann	34 ± 3 (8) [c]	1.36
95 Na^+, 100 mann	30 ± 1(18)	95 Na^+, 50 Li^+	43 ± 2(18) [b]	1.13

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

of transport by Li^+ may be associated with the release of intracellular stores of Ca^{2+}.

Table II shows the effects of other sugar transport regulating factors in the presence and absence of 50 mM Li^+. In 145 mM Na^+ medium, insulin (0.25 mU/ml), K^+-free medium, and hyperosmolarity (10 mM mannitol) significantly increased 3-*O*-methyl-D-glucose transport and ^{45}Ca uptake. In 95 mM Na^+ medium with 50 mM Li^+, these factors maintained their stimulatory effect on sugar transport and ^{45}Ca uptake. These results indicate that Li^+ did not inhibit the mechanisms whereby insulin, K^+-free medium, and hyperosmolarity increase sugar transport. In addition, these regulators, like Li^+, consistently increased ^{45}Ca uptake corresponding to increased sugar transport.

Table III presents the effects of Li^+ on the intracellular concentrations of Na^+ and K^+. In standard Krebs-Henseleit bicarbonate medium, Na^+ and K^+ were approx. 22 and 137 mM, respectively. Na^+ content was increased in low-Na^+, K^+-free and hyperosmolar media, but was decreased by insulin. The addition of 20 mM Li^+ to low-Na^+ medium (125 mM) further increased Na^+ content, but 50 mM Li^+ added to 95 mM Na^+ medium decreased Na^+ content. Both 20 and 50 mM Li^+ decreased K^+ content. Thus, the addition of Li^+ to low Na^+ medium generally decreased the intracellular K^+ content, but showed no consistent effect on the Na^+ content.

Rat soleus muscle

Table IV shows the efflux of 3-*O*-methyl-D-glucose and ^{45}Ca from rat soleus muscle. Data are shown for a representative efflux period 10–20 min after change of washout solution to one containing Li^+ (i.e., 100–110 min after start of washout).

50 mM Li^+ stimulated 3-*O*-methyl-D-glucose and ^{45}Ca efflux in parallel in the presence and absence of extracellular Ca^{2+}. The Ca^{2+} ionophore A23187 and insulin significantly stimulated 3-*O*-

TABLE II

EFFECT OF STIMULATORS OF GLUCOSE TRANSPORT ON 3-*O*-[^{14}C]METHYL-D-GLUCOSE AND $^{45}CaCl_2$ UPTAKE IN INTACT ISOLATED RAT HEMIDIAPHRAGM

Insulin (0.25 mU/ml) was added only to the incubation medium. Na^+ was used to replace K^+ in K^+-free medium. The addition of 100 mM mannitol to normal (145 mM Na^+) Krebs-Henseleit bicarbonate buffer was used to produce hyperosmolar conditions.

Control (C)	μmol/ml per min	Treatment (T)	μmol/ml per min	T/C
(A) 3-*O*-[^{14}C]Methyl-D-glucose				
145 Na^+	27±2 (4)	145 Na^+, ins	70±6 (4) [c]	2.59
95 Na^+, 50 Li^+	76±7 (8)	95 Na^+, 50 Li^+, ins	119±5 (8) [c]	1.56
145 Na^+	35±4 (7)	145 Na^+, $-K^+$	48±7 (7) [b]	1.37
95 Na^+, 50 Li^+	37±3(16)	95 Na^+, 50 Li^+, $-K^+$	49±5(16) [c]	1.32
145 Na^+	26±5 (4)	145 Na^+, 100 mann	67±11(4) [c]	2.58
95 Na^+, 50 Li^+	51±3 (4)	95 Na^+, 50 Li^+, 100 mann	110±9 (4) [c]	2.16
(B) ^{45}Ca				
145 Na^+	29±3(16)	145 Na^+, ins	38±4(16) [b]	1.31
95 Na^+, 50 Li^+	34±3(23)	95 Na^+, 50 Li^+, ins	47±4(23) [b]	1.38
145 Na^+	34±4 (7)	145 Na^+, $-K^+$	38±4 (7)	1.12
95 Na^+, 50 Li^+	36±3(15)	95 Na^+, 50 Li^+, $-K^+$	43±4(15) [b]	1.19
145 Na^+	31±3 (6)	145 Na^+, 100 mann	42±3 (6) [c]	1.35
95 Na^+, 50 Li^+	35±3(18)	95 Na^+, 50 Li^+, 100 mann	44±3(18) [c]	1.26

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

TABLE III

EFFECT OF Li^+ AND OTHER FACTORS ON Na^+ AND K^+ CONTENTS IN INTACT ISOLATED RAT HEMIDIAPHRAGM

Data represent millimolar concentrations of Na^+ and K^+ in the intracellular water.

Control (C)		Treatment (T)		T/C
(A) Na^+				
145 Na^+	22.6± 2.7 (4)	125 Na^+, 40 mann	29.2± 2.8 (4) [b]	1.29
125 Na^+, 40 mann	23.6± 3.1 (4)	125 Na^+, 20 Li^+	32.5± 3.8 (4) [b]	1.38
145 Na^+	43.4± 3.5 (5)	95 Na^+, 100 mann	55.8± 3.5 (5) [b]	1.29
95 Na^+, 100 mann	38.6± 2.7(20)	95 Na^+, 50 Li^+	30.3± 2.2(20) [c]	0.78
95 Na^+, 100 mann, $-Ca^{2+}$	16.4± 2.5 (5)	95 Na^+, 50 Li^+, $-Ca^{2+}$	23.6± 4.8 (5) [a]	1.44
145 Na^+	59.2± 6.5(12)	145 Na^+, ins	50.1± 5.9(12) [a]	0.85
95 Na^+, 50 Li^+	42.4± 4.9(14)	95 Na^+, 50 Li^+, ins	56.2± 6.6(14) [b]	1.33
145 Na^+	41.9± 3.3 (9)	145 Na^+, $-K^+$	75.8± 4.5 (9) [c]	1.80
95 Na^+, 50 Li^+	22.4± 4.1(20)	95 Na^+, 50 Li^+, $-K^+$	33.1± 3.8(20) [c]	1.48
145 Na^+	38.0± 4.8 (8)	145 Na^+, 100 mann	60.3± 6.7 (8) [c]	1.59
95 Na^+, 50 Li^+	24.8± 2.1(18)	95 Na^+, 50 Li^+, 100 mann	38.9± 3.7(18) [c]	1.57
(B) K^+				
145 Na^+	163.5± 3.4 (3)	125 Na^+, 40 mann	147.4± 7.0 (3) [a]	0.9
125 Na^+, 40 mann	157.4± 3.0 (6)	125 Na^+, 20 Li^+	140.4± 3.8 (6) [c]	0.89
145 Na^+	153.6± 3.0 (8)	95 Na^+, 100 mann	170.9± 3.5 (8) [c]	1.11
95 Na^+, 100 mann	179.1± 3.7(22)	95 Na^+, 50 Li^+	143.6± 2.3(22) [c]	0.80
95 Na^+, 100 mann, $-Ca^{2+}$	151.0± 6.7 (8)	95 Na^+, 50 Li^+, $-Ca^{2+}$	125.4±20.5 (7) [a]	1.0
145 Na^+	164.4±20.5 (7)	145 Na^+, ins	172.0±21.9 (7)	1.05
95 Na^+, 50 Li^+	130.9± 9.6(13)	95 Na^+, 50 Li^+, ins	142.4±10.0(13)	1.09
145 Na^+	144.7± 9.4(11)	145 Na^+, $-K^+$	112.3± 5.3(11) [c]	0.78
95 Na^+, 50 Li^+	129.5± 5.2(25)	95 Na^+, 50 Li^+, K^+	110.3± 5.1(25) [c]	0.85
145 Na^+	135.8± 6.5 (7)	145 Na^+, 100 mann	176.2±14.3 (7) [c]	1.30
95 Na^+, 50 Li^+	126.7± 2.7(17)	95 Na^+, 50 Li^+, 100 mann	156.2± 4.4(17) [c]	1.24

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

TABLE IV

EFFECT OF Li^+ AND OTHER FACTORS ON THE EFFLUX OF 3-*O*-[^{14}C]METHYL-D-GLUCOSE AND $^{45}CaCl_2$ FROM RAT SOLEUS MUSCLES

Data represent mean fractional loss (% per min)±S.E. for the 10–20 min period after change of washout solution (see text). *P* values refer to paired comparisons.

Conditions	3-*O*-[^{14}C]-Methyl-D-glucose	$^{45}CaCl_2$
95 Na^+, 100 mann	1.09 ±0.24 (4)	1.12±0.10(4)
95 Na^+, 50 Li^+	1.74 ±0.05 (7) [c]	1.41±0.08(9) [c]
95 Na^+, 100 mann, $-Ca^{2+}$	1.31 ±0.16 (5)	1.14±0.08(6)
95 Na^+, 50 Li^+, $-Ca^{2+}$	1.92 ±0.11 (7) [c]	1.70±0.13(7) [c]
145 Na^+, $-Ca^{2+}$	1.207±0.003(3)	1.48±0.10(4)
145 Na^+, $-Ca^{2+}$, ins	1.74 ±0.02 (3) [c]	1.73±).001(4) [b]
95 Na^+, 50 Li^+	1.68 ±0.04 (3)	1.39±0.11(8)
95 Na^+, 50 Li^+, ins	2.40 ±0.05 (3) [a]	1.38±0.07(8)
95 Na^+, 50 Li^+, $-Ca^{2+}$	1.92 ±0.05 (2)	1.46±0.05(6)
95 Na^+, 50 Li^+, $-Ca^{2+}$, ins	2.52 ±0.20 (2) [a]	1.58±0.09(6) [a]
145 Na^+, $-Ca^{2+}$	1.50 ±0.08 (7)	1.57±0.07(7)
145 Na^+, A23187, $-Ca^{2+}$	1.71 ±0.08 (5) [b]	1.98±0.11(7) [c]

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

methyl-D-glucose and ^{45}Ca efflux in Ca^{2+}-free 145 mM Na^+ medium. The stimulatory effects of insulin on sugar and ^{45}Ca efflux were maintained in Ca^{2+}-free low-Na^+ medium (95 mM) with 50 mM Li^+, but in Ca^{2+}-containing medium only sugar efflux was stimulated by insulin in the presence of 50 mM Li^+. These results show that the stimulation of sugar transport by Li^+, insulin and the Ca^{2+} ionophore A23187 may be associated with the release of Ca^{2+} from intracellular storage sites.

Rat ventricular muscle slices

The role of mitochondrial Ca^{2+} transport in the stimulation of sugar transport by Li^+ was investigated in preparations of cardiac muscle. Table V shows 3-*O*-methyl-D-glucose uptake and mitochondrial Ca^{2+} content of rat ventricular muscle slices incubated in a low-Na^+, Li^+-containing medium in the presence and absence of extracellular Ca^{2+}. In rat ventricular muscle slices, the presence of many damaged cells may give rise to a large nonspecific component of uptake. Transport via these nonspecific pathways was corrected by subtracting the nonspecific entry measured in the presence of a maximal concentration of cytochalasin B, a competitive inhibitor of specific carrier-mediated glucose transport [26]. Thus, in Ta-

TABLE V

EFFECT OF Li^+ ON 3-*O*-[^{14}C]METHYL-D-GLUCOSE UPTAKE AND MITOCHONDRIAL Ca^{2+} CONTENT IN RAT CARDIAC MUSCLE

In ventricular muscle slices, 3-*O*-methyl-D-glucose uptake was corrected for nonspecific transport using 10 μg/ml cytochalasin B; mitochondria were isolated from cardiac muscle slices. In intact cardiac muscle, sugar transport was measured in left atria, and mitochondria were isolated from ventricular muscle of perfused rat hearts. 3-*O*-Methyl-D-glucose uptake was expressed as μmol/ml per min, and mitochondrial (mito) Ca^{2+} content as nmol/mg protein.

	Ventricular muscle slices		Perifused left atria, uptake of [^{14}C]methylglucose	Perfused ventricular muscle, mito Ca^{2+} content
	uptake of [^{14}C]methylglucose	mito Ca^{2+} content		
95 Na^+, 100 mann			81± 8(8)	47.8±2.6(18)
95 Na^+, 50 Li^+			73± 7(8)	52.8±4.2 (7) [a]
70 Na^+, 150 mann	44±5(4)	87.8±14.7(4)	75± 4(8)	109.8±2.4 (4)
70 Na^+, 75 Li^+	69±7(4) [b]	57.9±10.9(4) [a]	122±15(6) [c]	117.3±8.0 (4)
95 Na^+, 100 mann, $-Ca^{2+}$			86± 7(5)	46.9±3.7(32)
95 Na^+, 50 Li^+, $-Ca^{2+}$			109± 9(5) [b]	45.8±3.7(20)
70 Na^+, 150 mann, $-Ca^{2+}$	55±3(4)	37.1± 6.3(7)	84± 9(5)	94.0±8.4 (4)
70 Na^+, 75 Li^+, $-Ca^{2+}$	64±1(3) [b]	20.7± 1.7(7) [c]	92± 9(5)	91.6±11.3 (4)

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

ble V, values obtained in rat ventricular muscle slices represent only that portion of total transport that was inhibited by 10 μg/ml cytochalasin B. The data show that 75 mM Li^+ significantly increased 3-*O*-methyl-D-glucose transport in rat ventricular muscle slices. At the same time, the Ca^{2+} content of mitochondria prepared from Li^+-treated slices was significantly decreased. These effects persisted in a Ca^{2+}-free medium. In addition, it may be noted that the Ca^{2+} content of mitochondria from slices exposed to Ca^{2+}-free medium (with or without Li^+) was much lower than that in the presence of Ca^{2+}.

Intact cardiac muscle

Intact cardiac muscle refers to preparations of rat cardiac muscle in which the muscle fibers remain relatively undamaged. Sugar transport in intact cardiac muscle was measured using the isolated rat left atrial preparation which is fully 'resting' [16], thereby excluding the stimulating effect of contraction on sugar transport. As found earlier in skeletal muscle [27], reduction of $[Na^+]_o$ in this preparation was associated with the stimulation of sugar transport. In the presence of Ca^{2+}, basal 3-*O*-methyl-D-glucose uptake was 36 ± 3 (6) μmol/ml per min in 145 mM Na^+ medium. This was significantly increased with the reduction of extracellular Na^+ from 145 mM to 95 mM ($P < 0.001$) or 70 mM ($P < 0.001$) Na^+ (Table V). In Ca^{2+}-free media, basal 3-*O*-methyl-D-glucose uptake was 75 ± 5 (5) μmol/ml per min in 145 mM Na^+. This was significantly increased with the reduction of extracellular Na^+ to 95 mM Na^+ ($P < 0.05$). Table V also shows the effects of Li^+ on 3-*O*-methyl-D-glucose uptake. There was significant stimulation by 75 mM Li^+ in the presence of extracellular Ca^{2+} and by 50 mM Li^+ in Ca^{2+}-free medium.

Mitochondrial Ca^{2+} content of intact cardiac muscle was measured using the ventricular muscle portion of Langendorff-perfused rat hearts. As shown in Table V, 50 mM Li^+ significantly increased ($P < 0.05$) mitochondrial Ca^{2+} content in the presence of extracellular Ca^{2+}, but had no effect in Ca^{2+}-free medium. 75 mM Li^+ did not significantly alter the mitochondrial Ca^{2+} levels in the presence and absence of extracellular Ca^{2+}. The results show that Li^+ did not cause a consistent change in the Ca^{2+} content of mitochondria

TABLE VI

EFFECT OF Li^+ ON THE OXIDATIVE PHOSPHORYLATION PARAMETERS OF MITOCHONDRIA ISOLATED FROM RAT CARDIAC MUSCLE

Mitochondria were isolated either from ventricular slices or the ventricular portion of intact perfusion hearts, as described in Methods. The reaction mixture contained 0.23 M mannitol, 0.07 M sucrose, 0.02 M Tris-HCl, 0.02 M EDTA, 0.005 M potassium phosphate (pH 7.2), 1–1.5 mg mitochondrial protein and 0.5 M pyruvate/0.1 M malate as substrate. Q_{O_2} was expressed as μmol O_2/min per g protein, RCI, respiratory control index; Q_{O_2}, oxygen consumption rate.

	RCI	ADP:O	Q_{O_2}
(A) Ventricular muscle slices			
70 Na^+, 150 mann	1.5±0.2 (3)	4.5±1.4 (4)	32±10 (4)
70 Na^+, 75 Li^+	1.3±0.01 (2)	5.5±1.8 (2)	15± 5 (2)
70 Na^+, 150 mann, $-Ca^{2+}$	1.4±0.2 (6)	1.9±0.4 (6)	41±11 (6)
70 Na^+, 75 Li^+, $-Ca^{2+}$	1.5±0.2 (5)	1.8±0.7 (5)	20± 5 (5) [b]
(B) Intact heart			
95 Na^+, 100 mann	5.0±0.3 (17)	3.2±0.3 (7)	106± 6(17)
95 Na^+, 50 Li^+	5.2±0.5 (7)	3.5±0.1 (7)	117± 4 (7) [a]
70 Na^+, 150 mann	8.5±1.5 (4)	2.8±0.1 (4)	190±12 (4)
70 Na^+, 75 Li^+	5.4±0.8 (4) [a]	3.0±0.1 (4) [a]	169±19 (4)
95 Na^+, 100 mann, $-Ca^{2+}$	8.0±0.7 (23)	3.4±0.1(23)	125± 9(23)
95 Na^+, 50 Li^+, $-Ca^{2+}$	5.4±0.4 (18) [c]	3.4±0.1(18)	100± 5(18) [c]
70 Na^+, 150 mann, $-Ca^{2+}$	4.2±0.4 (4)	2.6±0.1 (4)	160±20 (4)
70Na^+, 75 Li^+, $-Ca^{2+}$	3.9±0.7 (4)	3.1±0.1 (4)	143± 5 (4)

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

isolated from ventricular muscle of perfused rat hearts.

Unlike cardiac muscle slices, Li^+ stimulation of sugar transport in rat left atria was not correlated with a consistent decrease in the Ca^{2+} content of mitochondria isolated from ventricular muscle of perfused rat hearts. This disparity may be attributed to differences in the mitochondrial function of the two different preparations. Table VI summarizes the oxidative phosphorylation parameters of mitochondria isolated from rat ventricular muscle slices and from ventricular muscle of rat hearts perfused with the same media as used in Table V. Mitochondria from cardiac slices exhibited values for the respiratory control index (RCI), ADP:O ratio, and oxygen consumption rate (Q_{O_2}) which were generally lower than the corresponding values for similar buffer conditions obtained with mitochondria from ventricular muscle of perfused rat hearts. The result suggest that the mitochondrial function is significantly depressed in cardiac muscle slices as compared to mitochondrial function in vascularly perfused ventricular muscle.

Total Ca^{2+} content in hemidiaphragm muscles

Table VII shows the total Ca^{2+} content (nmol per mg tissue) corrected for the extracellular Ca^{2+}. Tissue Ca^{2+} content was increased significantly with the reduction of extracellular Na^+ from 145 mM to 95 mM. The addition of Li^+ (50 mM) to 95 mM Na^+ medium was associated with a further significant increase in Ca^{2+} content. K^+-free medium also caused a significant increase, while omission of extracellular Ca^{2+} decreased Ca^{2+} content.

Discussion

In several studies, activation of sugar transport was correlated in terms of time and direction of changes with increased Ca^{2+} influx or decreased Ca^{2+} efflux across the sarcolemma, and as well, with the release of Ca^{2+} from intracellular stores such as the sarcoplasmic reticulum, inner face of the sarcolemma, and mitochondria [13]. Presumably these processes may lead to increased availability of free cytoplasmic Ca^{2+}.

Net sarcolemmal Ca^{2+} influx would contribute to a rise in cytosolic Ca^{2+}. Sarcolemmal Ca^{2+} influx in muscle [28] and nerve [29] is mediated to a large extent by the bidirectional Na^+-Ca^{2+} exchange system. If extracellular Na^+ is reduced, Ca^{2+} will more successfully compete for the extracellular carrier site, and net Ca^{2+} influx will occur. As shown in Table I, reduction in extracellular Na^+ from 145 mM to 95 mM Na^+ was associated with an increase in Ca^{2+} influx and stimulation of sugar transport. Likewise, if intracellular Na^+ is increased, the equilibrium of Na^+-Ca^{2+} exchange will also be shifted towards greater net Ca^{2+} uptake. Conditions which inhibit the Na^+ pump lead to accumulation of intracellular Na^+, and should thus increase Ca^{2+} influx via

TABLE VII

EFFECT OF LOW Na^+, Li^+ AND OTHER FACTORS ON TOTAL Ca^{2+} CONTENT (nmol/mg tissue) IN RAT HEMIDIAPHRAGMS

Hemidiaphragms were incubated for 50 min. Tissue Ca^{2+} content was corrected for the extracellular space.

Incubated in	145 Na^+	95 Na^+	95 Na^+, 50 Li^+
Fresh tissue	$0.78 \pm 0.13(8)$ [a]		
Buffer	$0.94 \pm 0.08(9)$	$1.18 \pm 0.07(5)$ [a]	$1.56 \pm 0.08(9)$ [b]
+ Insulin	$1.09 \pm 0.04(6)$		$1.56 \pm 0.17(6)$
K^+-free buffer	$1.36 \pm 0.14(8)$ [a]		$1.73 \pm 0.13(8)$
Ca^{2+}-free buffer	$0.62 \pm 0.05(8)$	$0.75 \pm 0.02(8)$ [c,d]	$0.81 \pm 0.05(9)$ [c]

[a] $P < 0.05$ compared to 145 Na^+ buffer.
[b] $P < 0.01$ compared to 95 Na^+ buffer.
[c] $P < 0.001$ compared to respective Ca^{2+} containing buffer.
[d] $P < 0.05$ compared to 145 Na^+, Ca^{2+}-free buffer.

Na^+-Ca^{2+} exchange. As shown in Table II, K^+-free medium increased cellular Na^+, ^{45}Ca uptake, and 3-*O*-methyl-D-glucose transport.

Unlike factors which inhibit the Na^+ pump, insulin stimulates both the Na^+ pump (Table III) and sugar transport (Table II). This suggests that interaction between the Na^+ pump and Na^+-Ca^{2+} exchange described above may be overshadowed in the case of insulin by other mechanisms. A parallel relationship between insulin stimulation of sugar transport and sarcolemmal Ca^{2+} fluxes has not been consistently demonstrated. Several studies in cardiac and skeletal muscles have shown that insulin stimulation of sugar transport was depressed in the absence of extracellular Ca^{2+} [30–32], or with the Ca^{2+} chelator, EGTA [32], in the presence of heavy metal antagonists, La^{3+}, Ni^{2+} and Zn^{2+} [30], or with the Ca^{2+} antagonistic drug, D-600 [31,32]. Schudt et al. [33] showed that in developing muscle cells from chick embryo breast muscle (in culture), insulin increased Ca^{2+} uptake, the mitochondrial Ca^{2+} pool, and the apparent rate constant for Ca^{2+} efflux. Based on these observations, it was suggested that insulin may increase the cytoplasmic Ca^{2+} concentration, which may serve as the intracellular signal for stimulation of glucose transport. In agreement with this concept, the results in Table II show that insulin increases Ca^{2+} influx in parallel with sugar transport in rat hemidiaphragms. However, others have observed insulin effects independent of extracellular Ca^{2+} [34]. As shown in Table IV, under certain conditions (95 mM Na^+, 50 mM Li^+, Ca^{2+} present) insulin stimulated 3-*O*-methyl-D-glucose efflux from rat soleus muscle, but had no significant effect on ^{45}Ca efflux.

Li^+ may increase internal Na^+ levels by at least two mechanisms. In Li^+-treated excitable cells, Li^+ efflux is mediated by Na^+-Li^+ countertransport [35]. Thus, if Li^+ is present intracellularly, net Na^+ influx could occur and hence intracellular Na^+ would increase. A second mechanism for Li^+ to increase intracellular Na^+ would be through interference with Na^+ efflux via the Na^+ pump. Because the intracellular Na^+ content is affected by several transport mechanisms (($Na^+ + K^+$)-ATPase, Na^+-Ca^{2+} exchange, Na^+-Li^+ countertransport), the net effect of Li^+ on the Na^+ content may not be easily predictable. However, the consistent loss of K^+ in the presence of Li^+ may reflect Li^+ interference with the ($Na^+ + K^+$)-ATPase (Table III). In the arterially perfused interventricular septum of the rabbit, substitution of Li^+ for Na^+ in K^+-free medium (0 mM K^+, 36 mM Na^+, 110 mM Li^+) increased the ratio of intracellular Na^+ to extracellular Na^+, resulting in increased Ca^{2+} uptake, which reached a plateau within 30 min and initiated muscular contracture [10]. Replacement of extracellular Na^+ by Li^+ prevented relaxation of contracted frog skeletal muscle in the presence and absence of extracellular Ca^{2+}. It was suggested that the increased level of cytosolic Ca^{2+} was responsible for maintaining contracture [9]. Results from the present study show that in the isolated intact rat hemidiaphragm, Li^+ causes an increase in ^{45}Ca influx which is correlated with an increase in 3-*O*-methyl-D-glucose uptake (Table I).

Increased sarcolemmal Ca^{2+} influx was associated with the stimulation of sugar transport by K^+ depolarization-induced contractures [36] and by anoxia and metabolic inhibitors [37]. In agreement with these findings, the results of this study show that in intact rat hemidiaphragm, insulin, K^+-free medium, hyperosmolarity, and Li^+ stimulated 3-*O*-methyl-D-glucose transport and ^{45}Ca uptake in parallel (Tables I and II). This correlation is consistent with the concept discussed above that changes in Ca^{2+} distribution leading to a rise in cytosolic Ca^{2+} may be involved in the activation of sugar transport. However, Li^+ maintained its stimulatory effect on sugar transport in Ca^{2+}-free medium (Table I). Thus, in addition to Li^+-induced increase in sarcolemmal Ca^{2+} influx, there exist other mechanisms, one of which may be to increase cytosolic Ca^{2+} levels through releasing Ca^{2+} from intracellular storage sites.

Measurements of ^{45}Ca efflux indicate the availability of cytosolic Ca^{2+} for efflux [38]. If external Ca^{2+} is present and sarcolemmal Ca^{2+} influx is increased, then Ca^{2+} efflux will also be increased. In nominally Ca^{2+}-free medium, there should be only minimal sarcolemmal influx, and any rise in Ca^{2+} efflux reflecting a rise in cytosolic Ca^{2+} should be due largely to release of Ca^{2+} from intracellular stores. ^{45}Ca efflux studies can thus demonstrate the ability of some regulators of sugar transport to release intracellular stores of Ca^{2+}. In

soleus muscle, Ca^{2+} is released from the sarcoplasmic reticulum by electrical stimulation, K^+ depolarization, caffeine or veratrine. 2,4-DNP, H_2O_2, salicylate, and cyanide may also induce a rapid loss of Ca^{2+} from the mitochondria. In all instances, there was a rapid rise in the efflux of ^{45}Ca preceding or coinciding with the increased efflux of sugar [39]. Vanadate inhibits the Ca^{2+}-activated ATPase of isolated sarcoplasmic reticulum [40] and sarcolemma [41] and thus interferes with Ca^{2+} clearance from the cytoplasm. In whole epididymal fat pads, preloaded fat cells, extensor digitorum longus, and soleus muscles of the rat, vanadate stimulated the efflux of sugar, an effect preceded by an increase in ^{45}Ca efflux [40]. In agreement with these findings, the present study shows that in rat soleus muscle, Li^+ increased 3-*O*-methyl-D-glucose and ^{45}Ca efflux in the absence as well as in the presence of extracellular Ca^{2+}. This effect was additive to stimulation by other factors, including insulin, K^+-free or low-Na^+ medium (Table IV).

The role of mitochondrial Ca^{2+} in the stimulatory effect of Li^+ on sugar transport was also examined. The mitochondria are capable of storing large amounts of Ca^{2+} and provide the largest proportion (87%) of the total membrane area available for Ca^{2+} transport within cardiac muscle. Thus mitochondria could represent a major component of Ca^{2+} release from intracellular storage sites [42]. In addition, Li^+ was shown to cause release of mitochondrial Ca^{2+} stores by direct activation of mitochondrial Na^+-Ca^{2+} exchange. The maximum velocity of Ca^{2+} efflux with Li^+ is about one-third of that with Na^+, and the K_m for Li^+ is about 15 mM compared with 8 mM for Na^+ [9]. In addition, Li^+ may indirectly cause the release of mitochondrial Ca^{2+} stores by increasing intracellular Na^+ levels which affect mitochondrial membrane Na^+-Ca^{2+} exchange, the major pathway for Ca^{2+} efflux from mitochondria.

Net release of Ca^{2+} stores from mitochondria depends on the delicate balance between active Ca^{2+} uptake and passive Ca^{2+} efflux systems present in the mitochondrial membrane. This balance depends, among other things, on the functional integrity of the mitochondria, since mitochondrial function, as expressed by the parameters of oxidative phosphorylation, determines the efficiency of the active Ca^{2+} uptake process in the mitochondrial membrane. When mitochondrial function is maintained (intact cardiac muscle preparations, Table VI), the mitochondria operate in a homeostatic role and function to buffer any rise in the cytosolic Ca^{2+} levels. An increase in cytosolic Ca^{2+} levels will activate Ca^{2+} uptake into mitochondria [43]. For example, low $[Na^+]_o$ and Li^+ increase sarcolemmal Ca^{2+} influx (rat hemidiaphragms, Table I) and stimulate mitochondrial Ca^{2+} uptake (intact cardiac muscle, Table V). Conversely, in Ca^{2+}-free medium, there is a net outward flux of Ca^{2+}, and mitochondrial Ca^{2+} content also drops (Table V). As shown in Table V, omission of Ca^{2+} resulted in mitochondrial Ca^{2+} levels slightly decreased or unchanged as compared to corresponding values in Ca^{2+}-containing medium.

Thus, in their homeostatic role, mitochondria do not appear to significantly contribute to the availability of cytosolic Ca^{2+} required for the stimulation of sugar transport. As shown in Table V, an increased rate of sugar transport was not correlated with a specific and consistent change in mitochondrial Ca^{2+} levels in intact rat cardiac muscle. The stimulation of sugar transport in intact cardiac muscle preparations may be mediated by Ca^{2+} released from intracellular stores other than mitochondria. Lithium was shown to cause release of Ca^{2+} accumulated by the sarcoplasmic reticulum of rabbit hearts [10]. In skeletal muscle microsomes, Li^+ inhibited Ca^{2+} uptake more than Na^+ or K^+ [11], and uncoupled ATP hydrolysis from Ca^{2+} uptake [12].

In cardiac muscle slices, impaired mitochondrial function is evident by the decreased parameters of oxidative phosphorylation (Table VI). When mitochondria lose their functional integrity, active mitochondrial Ca^{2+} uptake is diminished, and net Ca^{2+} efflux may occur. Ca^{2+} levels were lower in mitochondria isolated from cardiac muscle slices than in those from intact cardiac muscle (Table V). A less efficient Ca^{2+} uptake system may be unable to compensate fully for increases in cytosolic Ca^{2+} levels and the ability of Li^+ to release Ca^{2+} from mitochondria (Table V) is unmasked. Under these conditions, an increased rate of sugar transport was observed (Table V). This correlation, in terms of direction of change between sugar transport and mitochondrial Ca^{2+} content

(i.e., decreased levels indicating mitochondrial Ca^{2+} release), is consistent with the concept that changes in Ca^{2+} distribution may be involved in the regulation of sugar transport.

In conclusion, we have found that Li^{+} causes changes in cellular Ca^{2+} distribution which would be expected to increase the cytosolic level of Ca^{2+} (i.e., increased sarcolemmal Ca^{2+} influx, release of intracellular stores of Ca^{2+}). These changes were associated with stimulation of sugar transport. The data represent additional indirect evidence favouring the hypothesis that increased availability of Ca^{2+} for binding to a specific regulatory site may activate sugar transport [13]. They also show that the effect of Li^{+} involves release of Ca^{2+} from intracellular stores; however, mitochondrial Ca^{2+} release is unlikely to contribute to this effect unless the functional integrity of mitochondria is impaired.

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