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THE RELATIONSHIP BETWEEN THE TRANSPORT OF GLUCOSE AND CATIONS ACROSS CELL MEMBRANES IN ISOLATED TISSUES

IV. THE 'INSULIN-LIKE' EFFECT OF Li^+

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SUMMARY

1. Li^+, in concentrations down to 5 mM, was shown to promote glucose uptake in isolated rat hemidiaphragm.

2. This effect was accompanied by an increase in glycogen deposition and a decrease in lactate release. In rat diaphragm, glycogen synthesis was also stimulated by Li^+ *in vivo*.

3. In the presence of Li^+, rat hemidiaphragms lost K^+ and accumulated Li^+.

4. This pattern of effects differed both quantitatively and qualitatively from that produced by insulin in the same tissue.

5. Apart from its effect on sugar permeability, Li^+ seems to induce a progressive decrease in glucose catabolism. It is possible that this is the result of a gradual reduction in the energy demand for active cation transport.

INTRODUCTION

A number of conditions and factors are known to imitate the action of insulin in stimulating the glucose uptake in rat hemidiaphragm (anoxia, CN^-, 2,4-dinitrophenol, arsenite, salicylate and hyperosmolarity[1–3]). On the other hand, these factors also promote glucose catabolism[2–5] and reduce the glycogen content[2–6].

In a previous report on the present study it was shown that total replacement of Na^+ with Li^+ led to an increase in glucose uptake and glycogen deposition in isolated rat hemidiaphragm[3]. A similar effect of Li^+ on glucose transport was first described by BHATTACHARYA[7] in 1959. A more recent report by the same author showed that Li^+, even in low concentrations, exerts an insulin-like effect in promoting glucose uptake in diaphragm as well as in epididymal fat pad from rats. It was also demonstrated that Li^+ injections produce hypoglycemia in rats and rabbits[8].

If it were possible to imitate some effects of a hormone, this might provide information about its mode of action. Therefore, it seemed reasonable to study in some detail the qualitative and quantitative similarities and differences between the effects of insulin and Li^+ on carbohydrate metabolism.

METHODS

Experiments with hemidiaphragms performed in vitro

Male albino rats of the Wistar strain, weighing from 110 to 170 g, were fasted for 18 h. The procedure for preparation of tissue, incubation, determination of glucose, lactate, total glycogen, and ^{14}C activity in glycogen have been described in previous reports[3,9,10]. The basic medium was Krebs–Ringer bicarbonate buffer containing 11.1 mM of D-glucose and D-[$^{14}C_6$]glucose. Li^+ was added as substitute for an equimolar amount of Na^+. LiCl (Fisher) was used, and the chloride content of all media was checked by electrometric titration. The difference in chloride concentration between the normal Krebs–Ringer bicarbonate buffer and the Li^+-containing media never exceeded 1 %.

In order to measure the amounts of K^+ and Li^+ in the tissue, this was digested in 0.200 ml 65 % HNO_3 for 5 min on a boiling-water bath. After dilution with water, the cations were determined in an Eppendorf flame photometer. Standards containing both Na^+, K^+ and HNO_3 in the same concentration range as the diluted tissue digest were used as reference values. The results (Table IV) were expressed as μmoles per g of the wet weight at the end of incubation. All the other data from the experiments performed *in vitro* presented in this paper were calculated on the basis of the wet weight before the incubation.

Experiments performed in vivo

These were performed essentially as originally described by RAFAELSEN[11]. Wistar rats (each group consisted of approximately equal numbers of male and female animals), weighing from 80 to 120 g, were fasted for 18 h. 2 ml of a solution containing NaCl or LiCl, 5.6 mM of D-glucose and 0.5 μC of D-[$^{14}C_6$]glucose were injected intraperitoneally. A few rats were injected with 154 mM NaCl to which insulin had been added. 60 min later, the animals were killed under light ether anaesthesia, the diaphragm quickly excised, placed on wet filter paper mounted on an ice block, and washed briefly with ice-cold 154 mM NaCl. Hemidiaphragms were prepared and directly transferred to 30 % KOH. Glycogen was isolated and its ^{14}C activity determined as previously described[3].

RESULTS

From Fig. 1 it appears that Li^+ in concentrations down to 5 mM gave a stimulation of glucose uptake. At 1 mM no significant change could be detected. However, at this concentration, the incorporation of glucose into glycogen was significantly increased (Fig. 2). The stimulation of glycogen deposition seemed to be most pronounced at 60 mM of Li^+ (222 %). In other experiments it was found that total replacement of Na^+ with Li^+ gave a smaller increase, both in ^{14}C activity of glycogen and glucose disappearance.[3]

The effect of Li^+ on glycogen deposition could also be demonstrated *in vivo*. Up to 4-fold increases in ^{14}C activity of diaphragm glycogen were produced when LiCl was injected intraperitoneally. Hyperosmotic solutions of NaCl apparently gave a small, although not significant increase ($P > 0.10$). Under the same experimental conditions, insulin produced an 86-fold increase in ^{14}C activity of glycogen (Fig. 3).

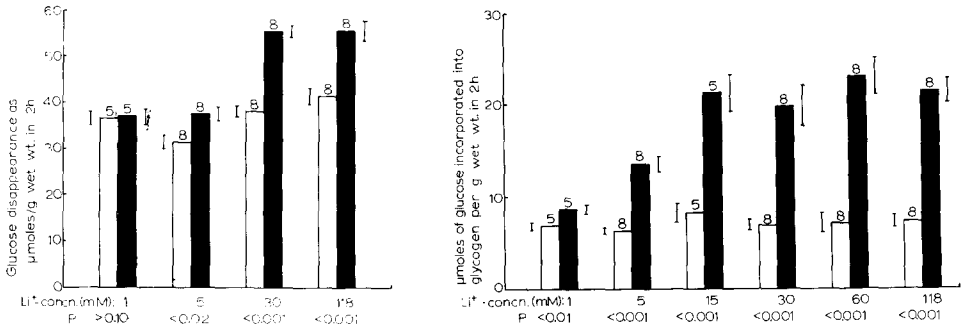

Fig. 1. Effect of various concentrations of Li^+ on glucose uptake in isolated rat hemidiaphragm. The number of tissues is indicated at the top of each column. Open areas, control; black areas, with Li^+; bars, 2 × S.E. of the mean.

Fig. 2. Effect of various concentrations of Li^+ on the ^{14}C activity of glycogen in isolated rat hemidiaphragm. The number of tissues is indicated at the top of each column. Open areas, control; black areas, with Li; bars, 2 × S.E. of the mean.

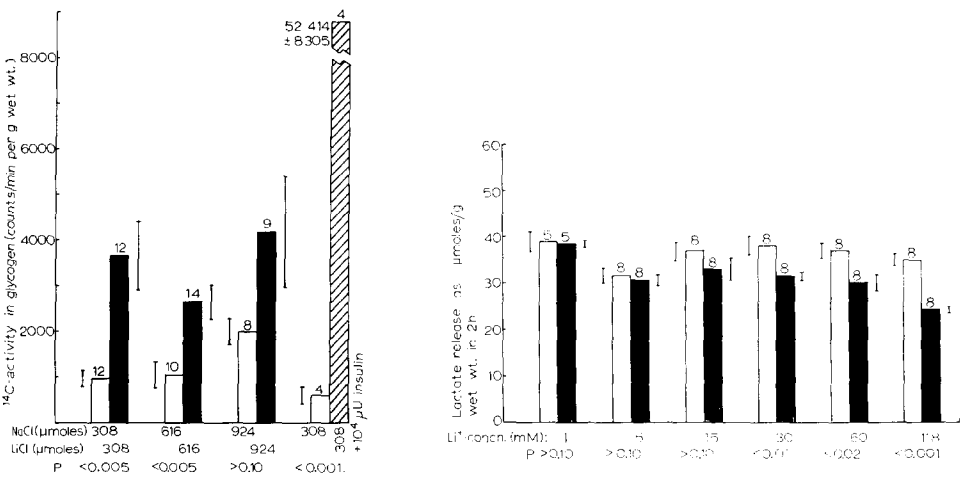

Fig. 3. ^{14}C activity in glycogen of rat diaphragm after intraperitioneal injection of D-[$^{14}C_6$]glucose in solutions of NaCl (open areas), LiCl (black areas), and NaCl with insulin (hatched area). The number of tissues is indicated at the top of each column. Bars, 2 × S.E. of the mean.

Fig. 4. Effect of various concentrations of Li^+ on the release of lactate from isolated rat hemidiaphragm. The number of tissues is indicated at the top of each column. Open areas, control; black areas, with Li^+; bars, 2 × S.E. of the mean.

In vitro, the lactate release showed a small, but significant, inhibition by Li^+ in concentrations down to 30 mM (Fig. 4).

The time course of the Li^+ effects is shown in Tables I and II. It appears that the effect of Li^+ on glucose uptake and ^{14}C activity of glycogen was evident after 30 min of incubation. Chemical determinations of the total glycogen content showed that this was higher after incubation in 118 mM of Li^+ than in the controls. The difference, when expressed in μmoles/g wet weight, is of roughly the same order of magnitude as that found by the ^{14}C measurements (Table II). The total glycogen content of the controls

TABLE I

EFFECT OF Li^+ ON GLUCOSE METABOLISM IN ISOLATED RAT HEMIDIAPHRAGM

30 mM of NaCl in the Krebs–Ringer bicarbonate buffer was replaced with an equimolar amount of LiCl. The results are given as mean ± standard error of the mean with the number of hemidiaphragms in parentheses.

Duration of incubation (min)	*Li^+*	*Glucose disappearance (μmoles/g wet weight)*	*Change (%)*	*Lactate release (μmoles/g wet weight)*	*Change (%)*	*μmoles of glucose incorporated into glycogen/g wet weight*	*Change (%)*
30	0	12.3 ± 0.2 (4)	22 ($P < 0.001$)	12.3 ± 0.8 (4)	10 ($P > 0.10$)	3.51 ± 0.61 (4)	47 ($P > 0.10$)
	+	15.0 ± 0.2 (4)		13.5 ± 1.3 (4)		5.15 ± 0.79 (4)	
60	0	23.5 ± 1.7 (3)	19 ($P > 0.10$)	23.6 ± 2.4 (3)	−14 ($P > 0.10$)	4.83 ± 0.58 (3)	106 ($P < 0.005$)
	+	27.9 ± 1.4 (3)		20.3 ± 1.0 (3)		9.94 ± 0.68 (3)	
90	0	28.0 ± 1.4 (3)	42 ($P < 0.005$)	33.6 ± 1.8 (3)	−9 ($P > 0.10$)	5.79 ± 0.95 (3)	170 ($P < 0.005$)
	+	39.9 ± 0.5 (3)		30.7 ± 0.8 (3)		15.65 ± 0.85 (3)	

TABLE II

EFFECT OF Li^+ ON GLUCOSE METABOLISM IN ISOLATED RAT HEMIDIAPHRAGM

All NaCl (118 mM) in the Krebs–Ringer bicarbonate buffer was replaced with an equimolar amount of LiCl. The results are given as mean ± standard error of the mean with the number of hemidiaphragms in parentheses.

Duration of incubation (min)	*Li^+*	*Glucose disappearance (μmoles/g wet weight)*	*Change (%)*	*Lactate release (μmoles/g wet weight)*	*Change (%)*	*μmoles of glucose incorporated into glycogen/g wet weight*	*Change (%)*	*Total glycogen content (μmoles of glucose/g wet weight)*	*Change (%)*
30	0	9.3 ± 0.9 (4)	32 ($P < 0.05$)	15.8 ± 1.1 (4)	−18 ($P > 0.10$)	2.43 ± 0.24 (4)	61 ($P < 0.02$)	25.0 ± 2.7 (4)	6 ($P < 0.02$)
	+	12.3 ± 0.7 (4)		12.9 ± 1.2 (4)		3.90 ± 0.38 (4)		26.5 ± 2.4 (4)	
60	0	15.6 ± 1.0 (3)	61 ($P < 0.005$)	21.3 ± 0.7 (3)	−2 ($P > 0.10$)	3.86 ± 0.67 (3)	165 ($P < 0.005$)	23.6 ± 1.4 (3)	21 ($P < 0.05$)
	+	25.1 ± 1.0 (3)		20.9 ± 0.8 (3)		10.22 ± 1.09 (3)		28.6 ± 1.1 (3)	
90	0	23.4 ± 2.8 (3)	51 ($P < 0.02$)	33.0 ± 1.2 (3)	−24 ($P > 0.005$)	4.78 ± 1.22 (3)	258 ($P < 0.02$)	20.9 ± 1.7 (3)	57 ($P < 0.005$)
	+	35.3 ± 3.9 (3)		25.0 ± 0.6 (3)		17.12 ± 3.29 (3)		32.9 ± 1.0 (3)	

was slightly lowered with longer duration of the incubation, whereas in the presence of Li^+, there was a net rise. In these experiments, the weak inhibitory effect of Li^+ on lactate release was statistically significant in only one instance (Table II, 90 min). All the effects of Li^+ showed a tendency to become more pronounced with longer duration of the incubation.

Table III shows data from similar experiments with insulin. It appears that in contradistinction to Li^+, the stimulatory effect of insulin, when expressed in %, was decreasing when longer incubation periods were used.

TABLE III

EFFECT OF INSULIN ON THE GLUCOSE METABOLISM OF ISOLATED RAT HEMIDIAPHRAGM IN NORMAL KREBS–RINGER BICARBONATE BUFFER (143 mM OF Na^+)

The results are given as mean ± standard error of the mean with the number of hemidiaphragms in parentheses. In each experiment, the data of the control and the experimental group were obtained using "paired" hemidiaphragms, and all the differences between the pairs are significant ($P < 0.001$).

Duration of incubation (min)	*Insulin (0.1 I.U./ml)*	*Glucose disappearance (μmoles/g wet weight)*	*Change (%)*	*Lactate release (μmoles/g wet weight)*	*Change (%)*	*μmoles of glucose incorporated into glycogen/g wet weight*	*Change (%)*
30	o	8.9 ± 0.8 (8)	179	13.9 ± 0.9 (8)	46	2.89 ± 0.18 (8)	324
	+	24.8 ± 0.7 (8)		20.3 ± 0.6 (8)		12.26 ± 0.42 (8)	
60	o	21.4 ± 0.9 (8)	85	20.4 ± 1.4 (8)	38	6.07 ± 0.51 (8)	197
	+	39.5 ± 1.5 (8)		28.1 ± 1.1 (8)		18.00 ± 1.12 (8)	
120	o	41.6 ± 1.7 (12)	53	40.6 ± 1.6 (11)	32	6.99 ± 0.45 (12)	151
	+	63.6 ± 1.6 (12)		53.6 ± 1.0 (11)		17.53 ± 0.88 (12)	

TABLE IV

EFFECT OF Li^+ ON K^+ LOSS, K^+ CONTENT, AND Li^+ CONTENT IN ISOLATED RAT HEMIDIAPHRAGM

All tissues were incubated for 120 min. The results are given as μmoles per g wet weight ± standard error of the mean with the number of hemidiaphragms in parentheses.

Incubation buffer	*K^+ loss*	*Change (%)*	*K^+ content*	*Change (%)*	*Li^+ content*
Normal Krebs–Ringer bicarbonate	12.8 ± 1.4 (8)	41 ($P < 0.005$)	70.5 ± 1.5 (8)	−9 ($P < 0.02$)	
Krebs–Ringer bicarbonate: 5 mM NaCl replaced with LiCl	18.1 ± 1.6 (8)		64.1 ± 1.5 (8)		7.2 ± 0.1 (8)
Normal Krebs–Ringer bicarbonate	14.1 ± 1.9 (8)	138 ($P < 0.001$)	74.4 ± 2.9 (8)	−32 ($P < 0.001$)	
Krebs–Ringer bicarbonate: 30 mM NaCl replaced with NiCl	33.6 ± 2.9 (8)		50.8 ± 2.4 (8)		44.0 ± 0.6 (8)

It appears from Table IV that Li^+ induces a loss of K^+ to the incubation medium. Correspondingly, the K^+ content of the tissue was lowered at the end of incubation. The tissue took up Li^+ to give a final content per g wet weight which was about 1.45 times higher than the extracellular concentration of Li^+. Control measurements of the Li^+ concentration in the incubation medium showed that the amount of Li^+ found in the tissue was in close agreement with that disappearing from the buffer.

DISCUSSION

It is evident that Li^+ imitates the action of insulin in promoting glucose uptake. The largest increase obtained was, however, somewhat smaller than that produced by insulin, when this was present in a concentration likely to give maximum stimulation of the glucose uptake[12]. In shorter incubation periods, a still larger quantitative difference could be demonstrated (Tables I, II and III).

Li^+ also exerts an insulin-like effect on glycogen deposition. In the 120-min experiments, Li^+ even induces a larger increase in ^{14}C activity of glycogen than insulin (compare Fig. 2 and Table III). In the same test system, however, the glycogenic effect of Li^+ is smaller than that of the hormone, when shorter incubation periods are used. When tested with the technique *in vivo*, it becomes evident that insulin (0.01 I.U. per animal) is a considerably more potent stimulator (Fig. 3).

Whereas insulin augments the lactate release in hemidiaphragm, Li^+ has the opposite effect (Fig. 4).

It must be added that Li^+ induces a loss of K^+ from hemidiaphragms (Table IV), whereas insulin increases the K^+ content in the same preparation[13].

On the basis of these results it seems fairly safe to conclude that Li^+ and insulin differ from each other in their action, not only quantitatively, but also qualitatively.

On the other hand, Li^+ seems to induce a change in the permeability of the cell membrane to glucose (and arabinose[14]), which makes it tempting to deal with the possibility that a certain structural modification of the cell membrane is the common basis for the effect of the ion and that of insulin. Compared with other monovalent cations, Li^+ has a great affinity for water. It can be hypothesized that this may alter the structure of water bound in the cell membrane in a way leading to a lowering of the permeability barrier for water-soluble molecules.

As at the end of incubation the tissue contained more Li^+ per g wet weight than the buffer, it is likely that Li^+ had been accumulated intracellularly. Also in erythrocytes, Li^+ has been shown to accumulate against a concentration gradient[15]. Li^+ seems to displace intracellular K^+. In erythrocytes, Li^+ induces a K^+ loss[16]. Intraperitoneal injections of Li^+ (6 mM/kg) cause a significant rise in plasma K^+ in rats[17]. In cat papillary muscle, Li^+ reduces the K^+ content. This was accompanied by a gradual depolarization[18]. A lowering of the electrical gradient across the cell membrane will probably induce an increased K^+ efflux and a decreased K^+ influx. While it is natural to expect changes in the passive K^+ fluxes, it is still uncertain whether the active K^+ influx is influenced by Li^+. In sartorius muscle and ventricular smooth muscle from frogs, Li^+ was found to inhibit K^+ uptake[19]. In frog sciatic nerve, Li^+ reduced the uptake of ^{42}K (ref. 20). Also in rabbit kidney slices[21] and rat brain slices[22], Li^+ diminished the accumulation of K^+. As little as 1 mM gave a detectable change[22].

In erythrocytes, there is some evidence that Li^+ can influence K^+ uptake by competition[15,23].

In previous reports it was shown that glycogen deposition is favoured by conditions leading to a decrease of active cation transport[9,10]. The inhibition of glycogenolysis found in Li^+-containing media may be an analogous result of decreased energy demand for active cation transport, although the evidence does not allow one to exclude the possibility that other mechanisms are also operative in the experiments with Li^+. Li^+ in itself seems to exert direct effects on a number of enzymatic reactions[24].

Since CADE's discovery[25] of the beneficial effect of Li^+ on certain psychotic states, lithium has been firmly established as valuable in the treatment of manic-depressive psychoses in humans[26]. From Fig. 2 it appears that concentrations in the same range as those prevailing in the serum of psychiatric patients receiving lithium treatment (0.5–2.0 mM)[24], are sufficient to stimulate the glycogen deposition in muscle.

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