EFFECT OF LITHIUM ON GLYCOGEN SYNTHESIS IN HEPATOCYTES FROM RAT LIVER

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Received 11 October 1979

1. Introduction

Lithium is used as a drug in the treatment of cyclophrenic disorders; the literature on the biological effects of this cation is therefore very large (reviewed [1–4]). As far as liver glycogen metabolism is concerned, administration of Li-Cl in vivo resulted in either a glycogen depletion [5], a glycogen increase [6], or no change was observed [7]. These findings, however, cannot be directly compared because the experimental conditions differed. Furthermore, it has been reported that administration of lithium led to a decreased insulin secretion and an enhanced glucagon level in serum [8], and therefore some of the observed effects of lithium on glycogen levels might be secondary to changes in hormone levels. In in vitro liver systems, very little information is available on the action of this cation on carbohydrate metabolism. In the perfused liver, total substitution of perfusate Na^+ with Li^+ was reported to result in a membrane depolarization, and in an inhibition of gluconeogenesis [9].

Here we show that as low as 1 mM lithium stimulated net glycogen production from glucose in isolated hepatocytes from 24 h fasted rats. Maximal stimulation was observed with 10–20 mM lithium, whereas at higher concentrations glycogen production decreased. Furthermore, it was found that the stimulatory effect of potassium on glycogen production was additive to that of maximally effective lithium concentrations.

2. Materials and methods

Hepatocytes from 24 h fasted rats were prepared as in [10]. Cell viability, as checked by trypan blue exclusion and by measurement of lactate dehydrogenase leakage, was ~90%. Preincubations (20 min) and subsequent incubations (60 min) were carried out with 10–25 × 10^6 cells in stoppered 25 ml Erlenmeyer flasks which were gassed with O_2 + CO_2 (95:5, v/v) and shaken at 37°C. Unless otherwise indicated, the medium for the preincubation (2.5 ml, pH 7.4) consisted of 113.4 mM NaCl, 6.48 mM KCl, 0.95 mM $MgSO_4$, 0.95 mM NaH_2PO_4, 30 mM $NaHCO_3$, 1.56 mM $CaCl_2$ and 4% albumin. After the preincubation, the incubations were started by the addition of isotonic solutions of salts and glucose as indicated in the tables. Isotonic NaCl solution was added to give 3 ml final incubation vol. The final incubation medium corresponded to a Krebs-Ringer bicarbonate buffer in which some of the Na^+ and Cl^- were substituted by other ions or glucose. Glycogen [11], lactate [12], pyruvate [13] and the activity of glycogen synthase *a* [14,15] were determined according to standard procedures. All results are expressed per gram liver wet weight, and a value of 125 × 10^6 cells/gram liver wet wt was used.

3. Results

Addition of lithium to the incubation medium led to a highly significant increase of net glycogen production from both 10 and 30 mM glucose as substrate (table 1). As shown in fig.1, it took 30 min before an effect of lithium on glycogen deposition could be observed. However, glycogen synthase *a* activity reached its maximal level already after 5 min. In 7 analogous experiments, 5 mM Li^+ stimulated the activity of glycogen synthase *a*, measured after 30 min incubation, by 223 ± 50%.

Table 1
Stimulation of net glycogen synthesis by lithium in hepatocytes

Additions		Glycogen deposition
Glucose (mM)	LiCl (mM)	(% control ± SEM)
10	1	137 ± 9[a]
10	5	233 ± 14[a]
30	1	111 ± 2[a]
30	5	134 ± 4[a]

[a] p values (paired data) were < 0.001

Control values of glycogen synthesis (without Li^+) from 10 mM glucose, 2.11 ± 0.37 μmol glucose . g^{-1} . h^{-1} ; from 30 mM glucose, 16.54 ± 2.23 μmol glucose . g^{-1} . h^{-1} . n = number of experiments with hepatocytes from different cell preparations

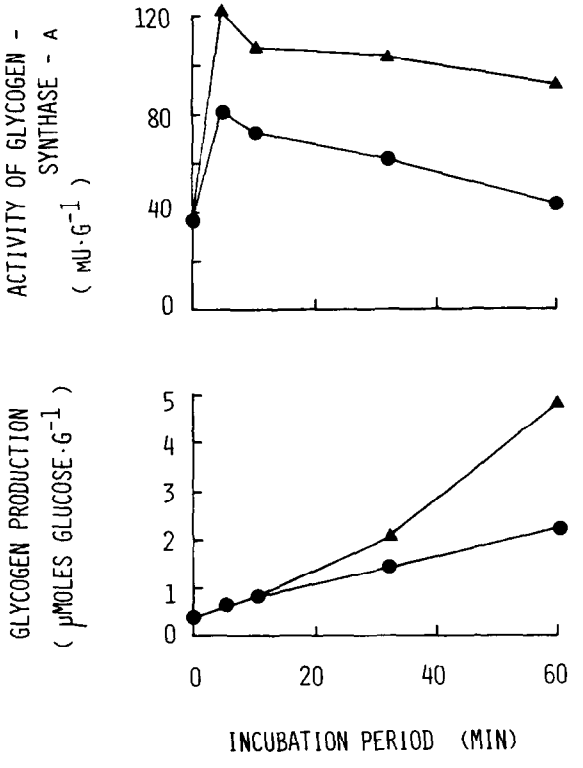

Fig.1. Time course of the lithium effect on net glycogen synthesis and on activity of glycogen synthase *a*. Incubations were carried out in the presence of 10 mM glucose (●-●) or 10 mM glucose + 5 mM Li^+ (▲-▲). n = 3 incubations of the same cell preparation.

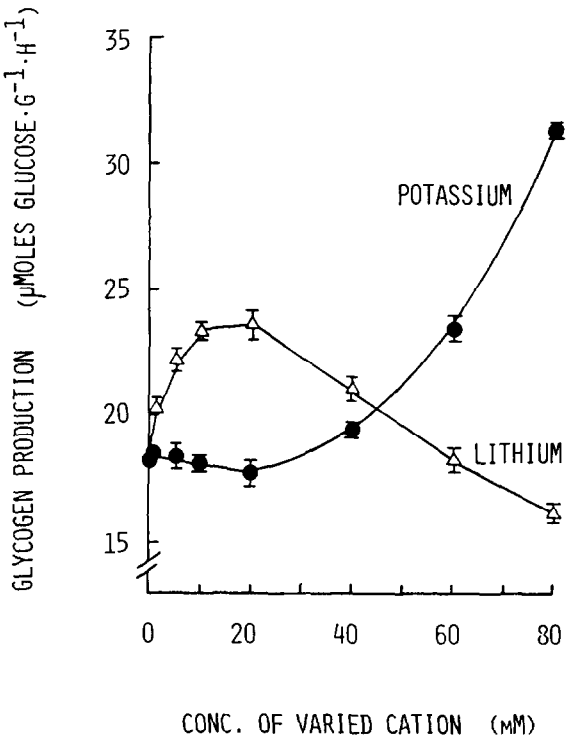

Fig.2. Effect of various concentrations of lithium or potassium on net glycogen synthesis in hepatocytes. The hepatocytes were preincubated for 20 min in Krebs-Ringer bicarbonate buffer, centrifuged and resuspended in fresh Krebs-Ringer buffer in which part of the Na^+ was substituted by the indicated amounts of K^+ or Li^+. Substrate was 10 mM glucose. Incubation time 60 min. Results are mean ± SEM of 4 incubations of the same cell preparation.

Replacement of sodium by potassium is known to stimulate glycogen deposition in liver tissue [15,16]. As shown in fig.2, this effect of potassium was confirmed in our system. Lithium similarly stimulated glycogen deposition, but the effects of the two cations showed a different pattern. At 1 mM the stimulation by lithium was already significant, and it reached a maximal value at 10–20 mM. At higher concentrations, the stimulatory effect decreased and at concentrations > 50 mM, glycogen deposition as compared to control values was even partly inhibited. Potassium, on the other hand, showed the same pattern as observed [15], being effective only at > 40 mM. Addition of potassium to the maximal effective concentration of lithium (20 mM) showed an additive effect (table 2).

Table 2
Effects of lithium and potassium on net glycogen synthesis

Additions	Net glycogen synthesis ± SEM (μmol glucose . g^{-1} . h^{-1})
20 mM Glucose	3.65 ± 0.15
20 mM Glucose 20 mM Li^+	7.97 ± 0.13[a]
20 mM Glucose 90 mM K^+	13.90 ± 0.16[a]
20 mM Glucose 20 mM Li^+ 90 mM K^+	16.50 ± 0.07[a]

[a] p value (unpaired data) were < 0.001

Incubation procedure as in legend of fig.2.
n = 4 incubations with the same cell preparation

4. Discussion

In these experiments, lithium stimulated net glycogen production from glucose and glycogen synthase activity in hepatocytes from 24 h fasted rats. This effect is consistent with observations made with isolated muscle tissue, where lithium was shown to stimulate glucose uptake [17,18], glycogen production [19] and the activity of glycogen synthase I [20]. The results support the general hypothesis that lithium pulls glucose-6-phosphate away from glycolysis and diverts it towards glycogen synthesis by effecting glycogen synthase or (and) some other enzymes of glycogen metabolism.

As seen in fig.2, we could observe a stimulation of net glycogen production by both potassium and lithium. Obviously lithium can stimulate glycogen production in liver as well as in muscle tissue. Potassium, however, which increases glycogen synthesis in liver tissue, has an opposite effect in muscle, where it has been shown to decrease glucose uptake [17], glycogen deposition [18] as well as glycogen synthase I levels [20]. Further experiments are needed to locate the site(s) of action of lithium and to study the effects of lithium in relation to those of potassium, glucose and various hormones on glycogen metabolism in more detail.

Acknowlegements

The excellent technical assistance of Miss B. Kissling, Mrs A. -B. Hentzler and Miss J. Kellerhals is gratefully acknowledged.

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