concentration which may occur in patients treated with this agent, can alter the acetycholine level in the CNS of the rat.

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The effect of lithium on liver glycogen concentration in the rat

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Lithium salts are currently used in the treatment and prophylaxis of manic depressive psychosis [1]. The precise mechanism of action of this cation is unknown. Several diverse side effects, for example, its action on the kidney, thyroid and body water metabolism have been reviewed by Davis and Fann [2]. Patients receiving lithium tend to increase in weight [3], a feature which has been ascribed to an effect of the cation on carbohydrate metabolism [4]. Lithium increases glucose uptake in rat hemidiaphragm [5] this effect being accompanied by alterations in tissue glycogen distribution. Data on liver glycogen concentrations in response to lithium administration are contradictory. For example, Krulic and Zvolsky [6] reported an increase in rat liver glycogen following lithium administration whereas the converse had been found by Plenge *et al.* [7]. Glucagon is known to stimulate hepatic glycogenolysis and has been considered to be of importance in the mechanism of lithium induced changes in hepatic glycogen concentration [8].

In this paper the effect of varying doses of lithium on liver and skeletal muscle glycogen has been studied in rats of differing weights and measurements of plasma glucagon have been made.

METHODS

Sixty-nine female Wistar rats were studied. They were divided into three groups according to weight. Animals in group *A* weighed 90–110 g, those in group *B* 130–150 g and those in group *C* 175–195 g. The animals were fed a normal laboratory diet with water *ad lib.* All experiments were performed in the morning and food was withdrawn during this time. The appropriate dose of Lithium chloride (Analar) was made up to an injection vol of 1·0 ml with deionised water and physiological saline was used as the control injection. All injections were administered intraperitoneally. Animals were killed 3 hr after injection by a blow on the head. Venous blood was obtained from the jugular veins; the liver and the skeletal muscle from the hind leg was removed and placed in ice-cold physiological saline.

Serum lithium was estimated by flame photometry. Liver and muscle glycogen was estimated as follows: 1 g of tissue was homogenised in 10 ml physiological saline. Sodium acetate buffer (pH 4·8) was prepared containing the glycogen debranching enzymes. 50 μg/ml glucosidase and 100 μg/ml amylo-α-1,4-α-1,6-glucosidase (EC 3.2.1).

Incubation of 100 μl homogenate and 50 μl buffer was carried out at 30° for 60 min. Glucose was then estimated by the hexokinase method [9] and tissue glycogen expressed as mg/g wet tissue wt. *N*-terminal plasma glucagon (total plasma glucagon) was measured by radio-immunoassay [10] in pooled plasma samples from each dose range.

The statistical test used was Student's *t*-test.

RESULTS

Serum lithium varied between 0·2 and 0·4 m-mole/l. following the 200 μmole dose, 0·6–1·8 m-mole/l. following the 500 μmole injection and between 1·9 and 2·3 m-mole/l. at higher dose levels of injected lithium chloride. The effect of injected LiCl on liver glycogen levels in the different groups is shown in Table 1.

In all groups there was a significant fall of liver glycogen concentration 3 hr after injection of 500 μmoles of LiCl

Table 1.

Dose of lithium chloride μmoles	Group A (90–110 g) Dose of lithium chloride μmoles/kg body wt	Liver glycogen (mg/g wet liver wt)	Plasma glucagon pg/ml	Group B (130–150 g) Dose of lithium chloride μmoles/kg body wt	Liver glycogen (mg/g wet liver wt)	Plasma glucagon pg/ml	Group C (175–195 g) Dose of lithium chloride μmoles/kg body wt	Liver glycogen (mg/g wet liver wt)	Plasma glucagon pg/ml
0		18·5 ± 0·4 (8)	150		24·9 ± 0·3 (6)	220		24·8 ± 2·2 (6)	1040
200	2·0	13·0 (2)	180	1·43	14·8 ± 0·4 (3)	140			
500	5·0	*7·8 ± 2·0 (8)	200	3·56	†15·2 ± 1·3 (6)	160	2·7	‡18·6 ± 1·8 (6)	140
750	7·5	12·6 ± 1·8 (2)	100						
1000	10·0	18·9 ± 0·8 (8)	340	7·1	23·9 ± 2·3 (4)	110	5·4	§16·8 ± 2·3 (6)	200
1500				10·7	27·6 (2)		8·1	21·9 (2)	

Liver glycogen (mg/g wet liver wt) 3 hr after intraperitoneal injection of varying doses of lithium chloride to rats of different weights. Values are shown as mean ± 1 S.E. Numbers in brackets refer to number of rats used.

P values refer to significant differences in liver glycogen from control values.

--- = not studied.

Differences in plasma glucagon of more than 18 pg/ml are significant at a level of $P < 0.05$.

* $P < 0.0025$; † $P < 0.005$; ‡ $P < 0.05$; § $P < 0.025$.

as compared to the control group. In groups *A* and *B* a dose of 200 μmoles LiCl also produced a substantial fall in liver glycogen.

When a higher dose of LiCl was injected (1000 μmoles) liver glycogen levels in groups *A* and *B* did not differ from the control values and were significantly higher than those found after a dose of 500 μmoles. In contrast, liver glycogen was significantly lowered in the heaviest animals following 1000 μmoles of LiCl and was not different from the level found after injection of 500 μmoles LiCl. When the results were expressed in relation to the dose of administered LiCl per kg body wt the reduction in liver glycogen observed in this group was still present.

Skeletal muscle glycogen levels were not related to body wt. Mean muscle glycogen was 2·40 mg/g wet muscle wt ± 0·37 (S.E.) in animals given control injections of saline. Following 500 μmoles of LiCl mean muscle glycogen rose significantly ($P < 0.0125$) to 4·40 ± 0·23 mg/g wet muscle wt. A further significant rise in this value to 5·40 ± 0·32 was noted in animals receiving 1000 μmoles LiCl ($P < 0.0125$).

Apart from a high level of 1040 pg/ml seen in the control animals in group *C* plasma glucagon levels ranged between 100 and 340 pg/ml (Table 1). There was no correlation between plasma glucagon and either liver glycogen or the percentage fall in liver glycogen in any of the groups or for all the groups taken together.

DISCUSSION

Acute administration of 500 μmoles LiCl led to a significant fall in liver glycogen in all three groups of animals in 3 hr. Similar results were found in 100 g rats 5 hr after receiving 200–600 μmoles LiCl by Plenge *et al.* [11]. Mellerup *et al.* [8] also documented a significant fall in liver glycogen in similar animals receiving 900 μmoles LiCl.

In contrast Krulik and Zvolsky [6] found an increase in liver glycogen following administration of LiCl (1–2 m-moles/kg per day) to 170-g rats for 10 days. Furthermore, in the present study an administered dose of 1000 μmoles LiCl in the two lighter groups of animals did not lead to a fall in liver glycogen whereas a pronounced decrease was noted with this dose by Plenge *et al.* [11] in animals of similar weight. The time difference is unlikely to explain this as these authors found a similar fall in liver glycogen 3 hr after administration of 900 μmoles LiCl.

The percentage fall of liver glycogen was not related to total body wt. However, the possibility remains that the reduction in hepatic glycogen concentration is related to total liver wt as this was not measured.

Mellerup *et al.* [8] found that total glucagon like activity was increased in the plasma after lithium administration and suggested that this (together with an increase in liver phosphorylase activity) accounted for the observed decrease in liver glycogen. The values of plasma *N*-terminal reactive glucagon (total glucagon like activity) found in the present study tended to decrease following lithium administration in groups *B* and *C* although this decrease was not statistically significant. Thus, no definite relationship between plasma glucagon and liver glycogen has been found in this study.

The rise in muscle glycogen in all groups of animals following lithium administration is in agreement with results of others [5, 11, 12]. Glucose uptake into rat diaphragm is stimulated by lithium [13] and it has been suggested that lithium had an 'insulin like effect' [13].

Lithium, therefore, has different effects on skeletal muscle glycogen compared to liver glycogen. This divergent action has not been explained and points to the possibility of two separate actions of lithium on carbohydrate metabolism. The results of this investigation have shown that the action of lithium on liver glycogen is complex and is clearly not just related to an effect of the ion on plasma glucagon concentration.

In addition to *N*-terminal plasma glucagon measurements of plasma gastrin [14], secretin [10] and *C*-terminal reactive glucagon [10] were also made in this study. They are not reported in detail since no clear relationship between any of the values and changes in liver glycogen were observed.

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Effects of chronically administered morphine on rat liver tyrosine aminotransferase

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Morphine, as well as other opiates, has a dual action on corticosteroid secretion. Single doses of narcotic generally stimulate adrenal cortical responses whereas prolonged administration produces depression of the basal levels of corticosteroid secretion. These effects have been clearly ascertained in the rat by measuring the classical parameters of adrenal activity, namely, the plasma corticosterone and the adrenal ascorbic acid levels [1, 2]. Paroli and Melchiorri [3] have also found an initial increase in corticosteroid urinary excretion in rats treated with morphine, followed by a decreased steroid excretion in a successive stage of the narcotic administration. Taken together, the data indicate a tendency toward the development of a tolerance to the hormonal effect of morphine, like that which is obtained for some of its other pharmacological effects, above all for analgesia.

In previous papers we showed that an acute administration of morphine, as well as of many other drugs acting on the CNS [4, 5], results in a rise of liver tyrosine aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5). Since the levels of this enzyme are regulated either by its substrate or by circulating corticosteroids [6], we tried to ascertain whether continual morphine administration normalizes the liver enzyme levels, in the rat.

Female albino rats of the Sprague Dawley strain with an initial weight of 150 ± 20 g were used. They were fed a standard diet and water *ad lib*. The environmental conditions were standardized (22 ± 2 : 12 hr artificial lighting per day). The rats were randomized into five groups of 10 animals each. The first group of rats received 20 mg/kg morphine (HCl, C. Erba), subcutaneously, at 8.00 hrs; 6 hr after the administration, the rats were killed. The second, third, fourth and fifth groups of rats were treated with morphine, 20 mg/kg/s.c., at 8.00 hrs daily for 7, 11, 14 and 18 days, respectively; the rats were killed 6 hr after the last administration. Controls animals received saline only.

Tyrosine aminotransferase (TAT) activity was determined in whole liver homogenate, in the presence of pyridoxal-5-phosphate, by the method described by Kenney [7] and expressed as μmoles of *p*-hydroxyphenyl-pyruvate/100 mg/hr. P values were calculated by the Student's *t*-test.

Morphine, acutely administered, increased the levels of tyrosine aminotransferase (TAT) in liver (Table 1), confirming our previous results [4]. Rats treated for 7 days with morphine still had high TAT levels, but from the 11th day the enzymatic stimulation was reduced and completely disappeared by the 14th day, when the enzyme levels were similar to those of the control groups. Normalization of tyrosine aminotransferase levels supports, therefore, the hypothesis that the continual administration of morphine might resut in a development of a tolerance to the enzymatic effect of the narcotic. Our findings agree with those of others who have described a depressed adrenal response

Table 1. Liver tyrosine aminotransferase (TAT) after acute or chronic treatment with morphine

No. of rats	Treatment	Dose (mg/kg per day)	Treatment period (days)	TAT (μmoles *p*-hydroxyphenylpyruvate/100 mg per 1 hr)*	% increase
10	Saline	—	1	13·68 ± 0·65	
10	Morphine	20	1	26·08 ± 0·57†	+ 90·6
10	Saline		7	14·17 ± 1·16	
10	Morphine	20	7	26·39 ± 1·30†	+ 86·2
10	Saline		11	16·80 ± 0·93	
10	Morphine	20	11	26·06 ± 1·87†	+ 55·1
10	Saline	—	14	13·01 ± 0·93	
10	Morphine	20	14	13·48 ± 1·87†	− 3·6
10	Saline		18	14·57 ± 1·81	
10	Morphine	20	18	14·26 ± 1·40	− 2·1

* 6 hr after last morphine administration. Results are expressed as mean ± S.E.M.
† $P < 0.05$ compared with respective controls.