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Effect of lithium on plasma glucose, insulin and glucagon in normal and streptozotocin-diabetic rats: role of glucagon in the hyperglycaemic response

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1 Lithium salts, used in the treatment of affective disorders, may have adverse effects on glucose tolerance in man, and suppress glucose-stimulated insulin secretion in rats.

2 To study the interaction of these effects with pre-existing diabetes mellitus, plasma glucose and insulin responses to lithium chloride were measured in male Wistar rats made diabetic with intraperitoneal streptozotocin, and in normal controls.

3 In both normal and diabetic anaesthetized rats, intravenous lithium (4 mEq kg^{-1}) caused a rise in plasma glucose. In absolute terms, the rise was greater in diabetic (5.2 mmol l^{-1}) than in normal rats (2.3 mmol l^{-1}).

4 Plasma insulin concentrations were reduced by lithium in normal rats, but the low insulin concentrations measured in the diabetic rats were not significantly changed.

5 After intravenous glucose (0.5 g kg^{-1}), lithium-treated diabetic rats showed a second rise in plasma glucose at 60–90 min without any insulin response, while normal rats showed typically reduced insulin responses and initial glucose disappearance rates.

6 Intravenous glucose reduced plasma glucagon concentrations to a greater extent in normal than in diabetic rats, but lithium induced an equal rise in plasma glucagon in both groups, with a time-course similar to that of the hyperglycaemic effect.

7 The hyperglycaemic action of lithium is greater in the hypoinsulinaemic diabetic rats and appears to involve a stimulation of glucagon secretion in both normal and diabetic animals.

Keywords: Lithium; streptozotocin; diabetes mellitus; glucose; insulin; glucagon

Introduction

Lithium has become widely used in the treatment of mono- and bipolar affective disorders and its cellular mechanisms of action have been the subject of extensive research (Birch, 1991), both in relation to its therapeutic effect and its many unwanted secondary effects. Among the latter are variable effects on glucose tolerance and interactions with pre-existing diabetes mellitus (reviewed by Lazarus, 1986). While there have been several reports of a decrease in glucose tolerance in human patients on lithium, a similar number of studies have shown an increased tolerance; and whereas there are isolated reports of the development or deterioration of diabetes mellitus of type 1 or 2 during lithium treatment, a controlled study of type 2 diabetic patients showed a diminished plasma glucose response to a 50 g carbohydrate breakfast after 1 week of lithium administration (Jones *et al.*, 1983).

In normal rats, lithium administration causes a rise in plasma glucose while plasma insulin concentrations remain unchanged or are slightly suppressed (Fontela *et al.*, 1986). Glucose-stimulated insulin release is inhibited both *in vivo* and *in vitro* (Anderson & Blackard, 1978; Shah & Pishdad, 1980; Fontela *et al.*, 1986; 1987), and these effects appear to be mediated by central opiate and by neural and endocrine catecholamine pathways, the latter acting on the pancreatic β cell through its α_2-adrenoceptors (Fontela *et al.*, 1990; García-Hermida *et al.*, 1991). The aim of the present study was to investigate how this 'diabetogenic' effect of lithium in rats interacts with pre-existing diabetes mellitus, using streptozotocin-induced diabetes as the experimental model. The ultimate aim was to obtain data that might, with due caution, help in the interpretation of the highly disparate findings in human patients.

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Methods

Animals

Male Wistar rats (160–220 g) were kept under standard conditions previously described (Fontela *et al.*, 1990). Randomly chosen rats were injected i.p. with 1 ml kg^{-1} of a solution of streptozotocin (Sigma, U.S.A.) 65 mg ml^{-1} in 0.1 M sodium citrate buffer, pH 4.5, while control rats were injected with the corresponding volume of buffer alone. The streptozotocin-treated rats were separated, and their urine tested after 48 h with BM-Test-Glucose strips (Boehringer Mannheim, Germany). Animals showing 1% glycosuria were kept for a further 12 days before being used. Food was withdrawn the night before each experiment, but free access to water was maintained.

Experimental protocol

To compensate for possible effects arising from performing experiments on different days, each day's experiments were carried out simultaneously on streptozotocin-diabetic and non-diabetic control rats. Animals were anaesthetized with pentobarbitone sodium, 60 mg kg^{-1}, i.p., and the previously described experimental protocol was followed (Fontela *et al.*, 1990). Venous blood samples (0.5 ml) were collected before and at 5, 30, 60, 90 and 120 min after rapid (30 s) i.v. infusion of 0.5 M LiCl or control 0.5 M NaCl (4 mEq kg^{-1}), or after the further i.v. infusion (5 min later) of 0.55 M glucose (0.5 g kg^{-1}) for studies of i.v. glucose tolerance and glucose-stimulated insulin secretion. For glucagon determination, blood samples were collected into tubes containing 20 μg aprotinin (Sigma, U.S.A.), equivalent to 400 kallikrein inhibitory units per ml of blood. Samples were kept at 4°C, centrifuged, and a small aliquot of plasma was removed for immediate glucose determination by the glucose oxidase me-

Table 1 Characteristics of streptozotocin-treated and control rats

	Body wt. (g)		*Plasma glucose* (mmol l^{-1})	*Plasma insulin* (μu ml^{-1})
	Day 0	Day 14	Day 14	Day 14
Control rats	186 ± 17	276 ± 22	8.0 ± 1.3	24 ± 9
Streptozotocin-diabetic rats	178 ± 9	201 ± 28	21.8 ± 4.7	12 ± 4

Male Wistar rats were injected i.p. with streptozotocin 65 mg kg^{-1} or vehicle alone on Day 0 and used on Day 14. Diabetic rats were selected on the basis of persistent glycosuria >1%. Results are given as mean ± s.d., $n = 36$.

thod. The remaining plasma was frozen at − 18°C for the subsequent determination of insulin (Amersham, Bucks) and pancreatic glucagon (Novo-Nordisk, Denmark; antiserum K 5563) by radioimmunoassay following the suppliers' instructions.

Analysis of data

The initial rate of fall of the plasma glucose concentration after i.v. glucose administration (the 'K value') was calculated as the percentage fall in plasma glucose per minute during the period 5 to 30 min after the injection of glucose. Results are expressed as means ± s.d. or s.e.mean. Within-group and between-group differences were analysed by Wilcoxon's rank sum tests for paired and unpaired data respectively.

Results

Characteristics of experimental animals

The streptozotocin-diabetic rats, selected on the criterion of >1% glycosuria, showed a markedly reduced weight-gain and elevated basal plasma glucose (Table 1). The mean basal plasma insulin concentration was reduced to half that of controls.

Effects of lithium on plasma glucose and insulin

The effects of i.v. lithium (4 mEq kg^{-1}) on plasma glucose and insulin concentrations in streptozotocin-diabetic and control rats are shown in Figures 1 and 2. Both groups showed a rise in plasma glucose that reached a maximum at 60 min. In absolute terms, the mean rise in streptozotocin-diabetic rats was greater (5.2 mmol l^{-1}) than that in the non-diabetic controls (2.3 mmol l^{-1}). Because of considerable variation in individual responses the rises were of borderline statistical significance in both groups ($0.1 > P > 0.05$, $n = 8$). The non-diabetic controls showed an early reduction of plasma insulin concentrations (Figure 2), maximal at 5 min ($P < 0.05$) and recovering to control levels by 90 min. The low insulin concentrations recorded in the streptozotocin-diabetic rats were unchanged after lithium.

Effects of lithium on i.v. glucose tolerance

Plasma glucose The effects of i.v. lithium on plasma glucose changes after i.v. glucose tolerance tests in streptozotocin-diabetic and non-diabetic control rats are shown in Figure 3. Whereas the K value for glucose-disappearance was reduced by lithium from 1.52 ± s.e.mean 0.06 to 1.23 ± 0.17 (NS, $n = 7$) in the non-diabetic controls, lithium administration increased the K value in the streptozotocin-diabetic rats from 0.15 ± 0.05 to 0.33 ± 0.07 ($P < 0.05$, $n = 7$ and 11). However, there was a second rise of plasma glucose in the streptozotocin-diabetic rats that reached a maximum at 90 min, whereas the non-diabetic controls showed only a minor reduction in the slope of glucose-disappearance around the 60 min time-point.

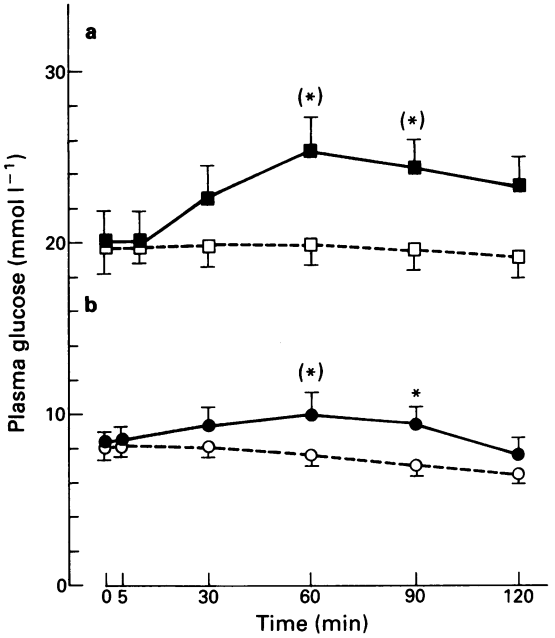

Figure 1 Effect of i.v. LiCl 4 mEq kg^{-1} (closed symbols) or the same dose of NaCl (open symbols) on plasma glucose concentrations in (a) streptozotocin-diabetic (□, ■) and (b) non-diabetic control rats (○, ●). Each result represents the mean ± s.e.mean of 8 rats. Statistical significance of differences from saline controls: (*)$0.1 > P > 0.05$; *$P < 0.05$.

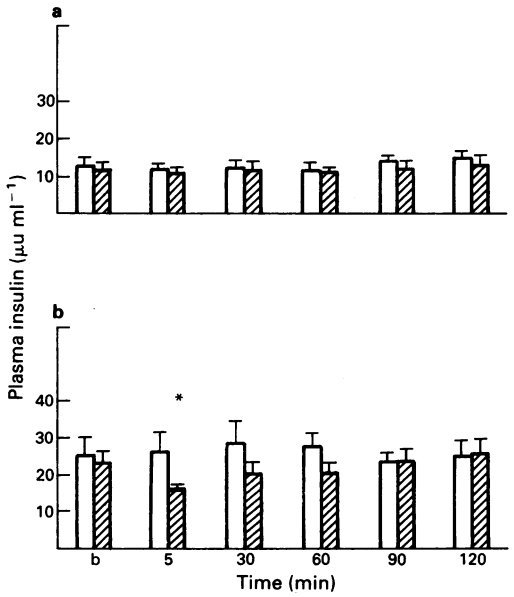

Figure 2 Effect of i.v. LiCl 4 mEq kg^{-1} (hatched columns) or the same dose of NaCl (open columns) on plasma insulin concentrations in streptozotocin-diabetic rats (a) and non-diabetic control rats (b). Each result represents the mean with s.e.mean of 8 rats. Statistical significance of difference from saline controls: *$P < 0.05$.

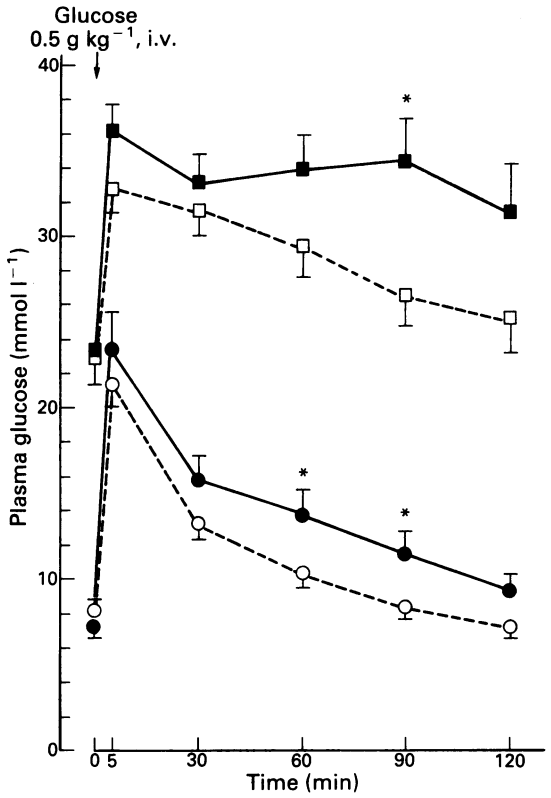

Figure 3 Effect of prior i.v. administration of LiCl 4 mEq kg^{-1} (closed symbols) or the same dose of NaCl (open symbols) on plasma glucose concentrations after i.v. glucose 0.5 g kg^{-1} in streptozotocin-diabetic (■, □) and non-diabetic control rats (●, ○). Each result represents the mean of 7 rats ± s.e.mean (11 for lithium-treated streptozotocin-diabetic rats). Statistical significance of differences from saline controls: *$P<0.05$.

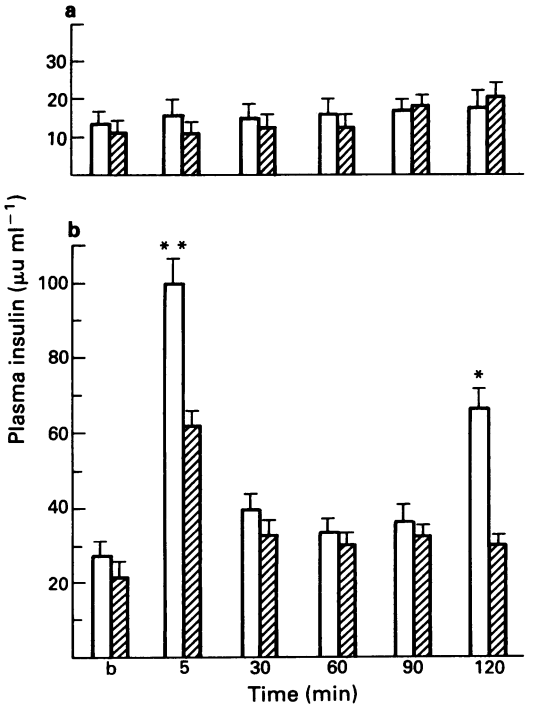

Figure 4 Effect of prior i.v. administration of LiCl 4 mEq kg^{-1} (hatched columns) or the same dose of NaCl (open columns) on plasma insulin concentrations after i.v. glucose 0.5 g kg^{-1} in streptozotocin-diabetic (a) and non-diabetic control rats (b). Each result represents the mean with s.e.mean of 7 rats (11 for lithium-treated streptozotocin-diabetic rats). Statistical significance of differences from saline controls: *$P<0.05$; **$P<0.01$.

Plasma insulin Plasma insulin responses to i.v. glucose are shown in Figure 4. In the non-diabetic controls the insulin response was markedly suppressed by lithium, the mean plasma concentration at 5 min being reduced by 37.6 ± 2.8% s.e.mean ($P<0.01$). A second rise of insulin observed at 120 min in the saline control group was completely absent in the lithium-treated group ($P<0.05$). In the streptozotocin-diabetic rats there was no immediate insulin response to glucose in either lithium-treated or saline control groups, but both groups showed a tendency for plasma insulin concentrations to rise during the 120 min post-glucose period ($P<0.01$ for within-group comparison of 0 and 120 min time-points). There were no significant between-group differences in the plasma insulin levels of the diabetic rats.

Effect of lithium on plasma glucagon

The liberation of endogenous glucose into the circulation in response to lithium, which was particularly apparent at the 60 and 90 min time-points in the diabetic rats, directed attention to the possible role of insulin counter-regulatory hormones. Plasma concentrations of pancreatic glucagon were therefore measured during the i.v. glucose tolerance test, when glucagon secretion would normally be suppressed. The results are shown in Figure 5. In the saline-treated, non-diabetic control rats, mean plasma glucagon fell from 35 ± 10 to 10 ± 1 pmol l^{-1} within 5 min of i.v. glucose ($P<0.05$, $n=6$), remained low at 60 min and then rose slightly by 120 min ($P<0.05$). Lithium treatment superimposed on this a rise in glucagon at 30 and 60 min, reaching a maximum of 14 pmol l^{-1} above corresponding saline control values at 60 min ($P<0.01$). A further rise was observed at 120 min. The saline-treated, streptozotocin-diabetic rats had a slightly lower mean basal glucagon level (25 ± 3 pmol l^{-1}) which was less markedly reduced by i.v. glucose, falling to a minimum of 16 ± 3 pmol l^{-1} at 30 to 60 min, and thereafter again rising. On this response, lithium treatment superimposed a rise in glucagon of the same magnitude and time-course as that seen in the non-diabetic rats, reaching a maximum of 14 pmol l^{-1} at 60 min ($P<0.02$, $n=8$).

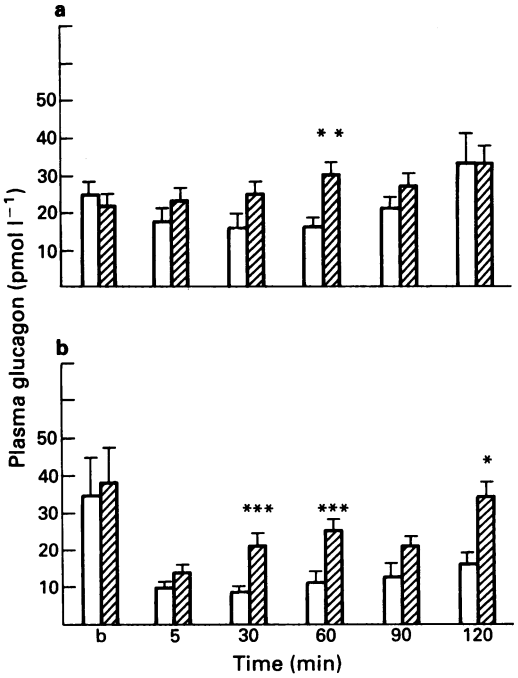

Figure 5 Effect of prior i.v. administration of LiCl 4 mEq kg^{-1} (hatched columns) or the same dose of NaCl (open columns) on plasma concentrations of pancreatic glucagon after i.v. glucose 0.5 g kg^{-1} in streptozotocin-diabetic (a) and non-diabetic control rats (b). Each result represents the mean with s.e.mean of 6 to 8 rats Statistical significance of differences from saline controls: *$P<0.05$; **$P<0.02$; ***$P<0.01$.

Discussion

The results of the present study can be interpreted in the light of the following effects of lithium administration in the intact rat:

(1) Activation of the sympatho-adrenal system

This involves both neural and humoral catecholamine pathways, as shown by the partial reversal of the effects of lithium on plasma glucose and glucose-stimulated insulin secretion by bilateral adrenalectomy (Fontela *et al.*, 1986), the elevation of plasma adrenaline and noradrenaline in response to lithium (Fontela *et al.*, 1991), and the reversal by adrenoceptor antagonists (specifically by the α_2-selective antagonist, yohimbine) of the inhibitory effect of lithium on the second phase of glucose-stimulated insulin secretion from isolated islets (Fontela *et al.*, 1987; 1990). The rise in plasma glucose may thus be attributed to the stimulatory effect of adrenaline and noradrenaline on glycogenolysis, combined with an inhibition of insulin secretion mediated by α_2-adrenoceptors of the pancreatic B cell.

(2) Stimulation of glucose uptake

The 'insulin-like' effect of lithium in promoting glucose uptake by muscle tissue *in vitro* (Bhattacharya, 1959; 1961; Clausen, 1968) is also seen as increased glucose disposal *in vivo*, both in patients on lithium treatment (Vendsborg & Rafaelsen, 1973; Vendsborg, 1979) and in diabetic rats, where lithium can restore insulin-sensitivity and muscle glycogen synthesis (Rossetti, 1989).

The overall results of lithium administration in the intact animal may therefore be expected to reflect the interaction, at different time-courses, of these effects with the capacity of the organism to respond in terms of insulin and its counter-regulatory hormones. Thus, while the adrenergic response to lithium predominates in the intact rat, producing hyperglycaemia, in the adrenalectomized rat the stimulation of glucose disposal produces hypoglycaemia (Fontela *et al.*, 1986). A high incidence of reactive hypoglyceamia during glucose tolerance tests in lithium-treated, non-diabetic patients has also been reported (Shah *et al.*, 1986).

The fact that lithium administration produced a greater rise of plasma glucose in streptozotocin-diabetic rats than in non-diabetic controls may, at least in part, be attributed to their low prevailing insulin levels and lower rates of glucose disposal. In these circumstances the stimulation of glucose disposal by lithium is evidently insufficient to compensate for the liberation of glucose into the circulation that is induced by the stimulation of counter-regulatory hormones.

The results of the i.v. glucose tolerance test show the different balance of effects of lithium on glucose disposal and insulin secretion particularly clearly. In the control rats the potent inhibition of glucose-stimulated insulin secretion outweighed any enhancing effect of lithium on glucose disposal. However, in the diabetic rats, characterized by low insulin levels that are unaffected by lithium, the initial rate of glucose disposal was significantly increased.

While much attention has been directed at the effects of lithium on catecholaminergic systems, there has been relatively little work on its possible effects on other important insulin counter-regulatory hormones. In view of the importance of disturbances in the normal regulation of basal and stimulated glucagon levels in diabetes mellitus (Unger & Orci, 1981), we decided to measure plasma glucagon responses to lithium after i.v. glucose. Under these conditions, glucagon secretion should be suppressed in normal rats, reducing the considerable individual variations in the fasting 'basal' levels seen in anaesthetized and cannulated animals and facilitating the demonstration of secretory responses due to other mechanisms. In diabetic rats, the glucagon-secreting A cells have to some extent escaped from the inhibitory effect of high plasma glucose concentrations. Nevertheless, the basal glucagon levels of the streptozotocin-diabetic rats were slightly lower than those of the non-diabetic controls ($0.1 > P > 0.05$), and were still significantly suppressed by i.v. glucose, although to a much lesser extent than in the controls. Despite these differences, however, the glucagon responses to lithium were identical in magnitude in the diabetic and control rats.

The existence of a glucagon response to lithium, previously suggested by Mellerup *et al.* (1970), has, as far as we know, not hitherto been substantiated by more recent work. In the present state of knowledge, it may be regarded as a consequence of activation of the sympatho-adrenal system. The A cells of the pancreatic islets carry β-adrenoceptors, demonstrated in isolated A cells by Schuit & Pipeleers (1986), and in isolated, perfused rat pancreas by Filipponi *et al.* (1986). The effect of β-adrenoceptor agonists is to stimulate glucagon secretion, and the blockage of this effect by metoprolol indicates that the receptors are of the β_1-type (Gregorio *et al.*, 1986). While the work of Gross *et al.* (1991) showed that A-cell responses to isoprenaline were completely suppressed after 5 weeks of streptozotocin-induced diabetes, the present results demonstrate an intact response to lithium after 2 weeks. The time during which the A cells have been subjected to hyperglycaemia and/or insulin deficiency may be significant in this respect. Our findings suggest that, after lithium administration, the induction of glucagon secretion co-operates with catecholamines in stimulating glycogenolysis.

Any application of these results to the use of lithium in man must be extremely cautious. Chronic treatment with slow-release oral lithium carbonate will not produce rapid changes in plasma lithium concentrations and may therefore have less effect on peripheral catecholamine pathways, whereas the potentiating effect of lithium on glucose disposal seems to persist. Our results suggest that the hyperglycaemic effect of lithium is enhanced when it is acutely administered in animals with low insulin levels and intact insulin counter-regulatory systems, including the glucagon response. This would imply that it is in inadequately treated diabetes that initiation of lithium treatment may contribute to hyperglycaemia.

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