Br. J. Pharmacol. (1991), **103**, 1309–1312

Effect of *pertussis* pretreatment on plasma glucose and insulin responses to lithium in rats

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1 Administration of lithium to rats causes a rise in plasma glucose and suppresses glucose-stimulated insulin secretion. These effects are blocked by the α_2-adrenoceptor antagonist, yohimbine.

2 Pretreatment of rats with *Bordetella pertussis* toxin resulted in a reversal of the usual plasma glucose and insulin responses to intravenously administered lithium (4 mEq kg^{-1}). There was a slow fall in plasma glucose, while plasma insulin rose to 267 ± 42% (±s.e.mean) of control values at 30 min. The effect of lithium on glucose-stimulated insulin secretion was also reversed; there was a marked increase in the insulin response which contrasted with the suppression seen in normal controls.

3 In perifused islets of Langerhans isolated from *pertussis* pretreated rats, the previously described inhibition by lithium of the second phase of glucose-stimulated insulin secretion from normal islets was almost completely abolished.

4 The results are consistent with the hypothesis that these effects of lithium are mediated by the influence of catecholamines on the islets. When the inhibitory effect of α_2-adrenoceptors is abolished by *pertussis* treatment, which blocks the action of the inhibitory guanine nucleotide-binding protein G_i, effects of β-adrenoceptor stimulation predominate, leading to an increased secretion of insulin.

Keywords: Lithium; *pertussis* toxin; adrenoceptors; glucose; insulin; islets of Langerhans

Introduction

Administration of lithium to rats leads to a rise in plasma glucose, while the rise in plasma insulin that would normally accompany this is abolished. Correspondingly it has been shown that glucose-stimulated insulin release is inhibited by lithium, both *in vivo* and *in vitro* (Anderson & Blackard, 1978; Shah & Pishdad, 1980). These effects are blocked by pretreatment of rats with the non-selective α-adrenoceptor antagonist, dihydroergotamine (Fontela *et al.*, 1986; 1987) and the selective α_2-adrenoceptor antagonist, yohimbine (Fontela *et al.*, 1990). It has therefore been suggested that this action of lithium is mediated via catecholaminergic stimulation, acting predominantly on the α_2-adrenoceptors of the pancreatic β cell. Activation of these receptors is associated with an inhibition of adenylate cyclase, brought about by the action of the inhibitory guanine nucleotide-binding protein G_i (Yamazaki et al., 1982). *Pertussis* toxin (islet activating protein) blocks the action of G_i by catalysing its ADP-ribosylation, which prevents its interaction with the receptor (Okajima *et al.*, 1985). The toxin is highly specific, in that the stimulatory protein G_s, which interacts with the β-adrenoceptors, is not affected (Murayama & Ui, 1983). Indirect confirmation of the involvement of catecholamines in the effects of lithium on insulin secretion can thus be obtained by the use of *pertussis* toxin to abolish the inhibitory action of the α_2-adrenoceptors while leaving the stimulatory effects of β-receptors intact. If the actions of lithium are mediated by catecholamines acting predominantly via α_2-adrenoceptors in the normal rat, they should be reversed in *pertussis*-treated animals, in which β-adrenoceptor effects predominate. We have therefore studied the effects of lithium on plasma glucose and insulin, and on glucose-stimulated insulin secretion *in vivo* and *in vitro*, in rats previously exposed to *pertussis* toxin.

Methods

Animals

Male Wistar rats (250–300 g) were kept under standard conditions previously described (Fontela *et al.*, 1990). They were injected i.p. with 0.125 ml of a killed liquid culture of *Bordetella pertussis* (8×10^{11} organisms ml^{-1}), containing active *pertussis* toxin (Swiss Serum and Vaccine Institute, Berne, Switzerland), or with an equal volume of vehicle. Experiments were carried out 5 days later. Food was withdrawn the night before each experiment, but free access to water was maintained.

In vivo *experiments*

Rats were anaesthetized with pentobarbitone sodium 20 mg kg^{-1} i.p., and the previously described experimental protocol was followed (Fontela *et al.*, 1990). Venous blood samples 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 glucose-induced insulin secretion. Plasma glucose was measured immediately by the glucose oxidase method and the remaining plasma was frozen at −18°C for determination of immunoreactive insulin (IRI) by radioimmunoassay (Amersham, UK).

Islet experiments

Pancreatic islets were isolated from control and *pertussis* pretreated rats by the collagenase digestion technique (Lacy & Kostianovsky, 1967). The islets were perifused at 0.9–1.0 ml min^{-1} in four parallel Millipore chambers, one pair containing 50 islets each from control rats, and the other pair containing the same number of islets from *pertussis* pretreated rats, as previously described (Fontela *et al.*, 1990). Two perifusion media were employed: a standard medium of normal ionic composition (Na^+ 139, K^+ 5, Ca^{2+} 2, Mg^{2+} 2, Cl^- 124 and HCO_3^- 24 mEq l^{-1}, pH 7.4) used for the first 30 min period and throughout the experimental time-period in the control chamber of each pair; and an otherwise identical lithium medium containing Na^+ 134 and Li^+ 5 mEq l^{-1}, used for the last 60 min time period in the experimental chamber of each pair. Both media were supplemented with bovine serum albumin 5 mg ml^{-1} and with glucose 3.2 mmol l^{-1} for the first 30 min and 16.7 mmol l^{-1} for the final 60 min of perifusion. The media were gassed with 95% O_2 and 5% CO_2, and were collected in graduated tubes at 1 or 5 min intervals. The volumes collected were recorded, and aliquots were removed

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and frozen at −18°C for subsequent radioimmunoassay of IRI.

Analysis of data

The magnitude of the *in vivo* insulin response (total integrated response) was calculated as the area between straight lines joining adjacent points and the baseline, from time 0 to 120 min. For isolated islets the total insulin secreted into the medium per islet during 60 min of perifusion with glucose 16.7 mmol l^{-1} was calculated. The 'K value' (rate of fall of plasma glucose concentration after i.v. glucose administration) was calculated as the percentage fall in plasma glucose per minute during the period 5 to 30 min after the injection of glucose. The normal 'K value' is around 1.7 and lower values are indicative of glucose intolerance. Results are expressed as means ± s.e.mean, and differences between means of experimental and contemporaneous control values were analysed by the two-tailed Student's *t* test for unpaired data.

Results

In vivo *studies*

Effects of lithium on plasma glucose and insulin concentrations in control and pertussis *pretreated rats* Pretreatment of rats with *pertussis* toxin reversed the effects of lithium seen in control rats (Figure 1). Whereas acute lithium administration caused a rise in plasma glucose without a corresponding rise in plasma insulin in controls (Figure 1a and c), in the *pertussis*-treated animals there was a marked rise in plasma insulin to 267 ± 42% ($P < 0.005$) of control values at 30 min (Figure 1b), and a slow fall in plasma glucose to 79 ± 2% ($P < 0.001$) of control values at 120 min (Figure 1d).

Effects of lithium on glucose tolerance and glucose-induced insulin release in control and pertussis *pretreated rats* A similar reversal of lithium effects was seen in studies of i.v. glucose tolerance (Figure 2). Whereas lithium administration reduced glucose-stimulated insulin release in control rats (the total integrated response was 4799 ± 473 min · μu ml^{-1} as compared with 6426 ± 350 min · μu ml^{-1} in saline-injected rats, $P < 0.05$) and lowered the 'K value' of glucose disappearance from 1.85 ± 0.05% to 1.57 ± 0.02% ($P < 0.001$), in *pertussis*-treated rats the integrated insulin response rose from saline control values of 5950 ± 190 min · μu ml^{-1} to 8850 ± 280 min · μu ml^{-1} ($P < 0.001$) and the 'K value' from 1.64 ± 0.11% to 2.16 ± 0.08% ($P < 0.005$).

In vitro *studies*

Effects of lithium on glucose-induced insulin release from perifused islets of control and pertussis *pretreated rats* As shown in Figure 3, 16.7 mmol l^{-1} glucose elicits a biphasic release of IRI. The concurrent administration of Li^+ 5 mmol l^{-1} inhibits the second phase of IRI release, reducing total release from 143 ± 9 to 78 ± 9 μu per islet ($P < 0.005$), equivalent to a fall of 45 ± 6%. By contrast, in islets from *pertussis* pretreated rats, the inhibition of second-phase IRI release by lithium was greatly diminished, total IRI release falling from 140 ± 7 to 123 ± 8 μu per islet, a reduction of only 12 ± 6% that was not statistically significant ($P > 0.1$). It will be noted that *pertussis* pretreatment by itself did not significantly alter glucose-stimulated IRI-release in the absence of lithium.

Discussion

The extensive studies of Ui and his colleages (Ui, 1984) have established that *pertussis* toxin catalyses an ADP-ribosylation of the inhibitory guanine nucleotide-binding protein G_i that interacts with α-adrenoceptors, muscarinic ACh and certain

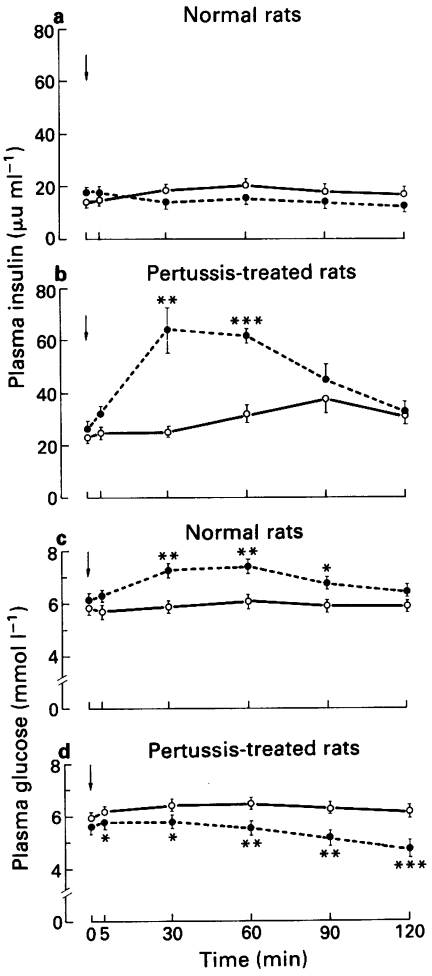

Figure 1 Effect of *pertussis* pretreatment on responses of plasma glucose and insulin to i.v. LiCl 4 mEq kg^{-1} in rats. (○) Results for NaCl-injected animals; (●) results for LiCl-injected animals. Time of LiCl or NaCl injection is marked by an arrow. Upper panels (a, b) show plasma insulin concentrations, and lower panels (c, d) plasma glucose concentrations in (a, c) untreated controls, and (b, d) *pertussis* pretreated rats. Each result represents the mean of five to eight rats and vertical bars show s.e.mean. Asterisks indicate the statistical significance of differences from the corresponding saline control values: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

other receptors, and is responsible for the negative signal transduction that leads to an inhibition of adenylate cyclase. ADP-ribosylation of G_i prevents its interaction with the receptor (Okajima *et al.*, 1985) and thus releases adenylate cyclase from G_i-mediated inhibition. At the same time, adenosine 3′ : 5′-cyclic monophosphate (cyclic AMP) production resulting from ligand-activation of stimulatory receptors is enhanced in the intact cell (Katada *et al.*, 1982), indicating a functional association between inhibitory and stimulatory guanine nucleotide-binding (G_s) proteins in the normal signal transduction from these receptors (Murayama & Ui, 1983; Asano *et al.*, 1984).

Previous studies (Fontela *et al.*, 1986; 1987; 1990) have suggested that the effect of lithium in raising plasma glucose levels and inhibiting glucose-stimulated insulin secretion is mediated by catecholaminergic influences on the pancreatic islets, acting principally via α_2-adrenoceptors. In the normal rat pancreas, α-adrenoceptors predominate over β-adrenoceptors, so that the overall effect of catecholamines is to inhibit insulin secretion (Katada & Ui, 1977). Treatment with *pertussis* toxin blocks the effects of α-adrenoceptor activation and thus exposes the unopposed effects of β-adrenoceptor activation. If lithium acts via a catecholamine related mechanism, its effects on insulin secretion should

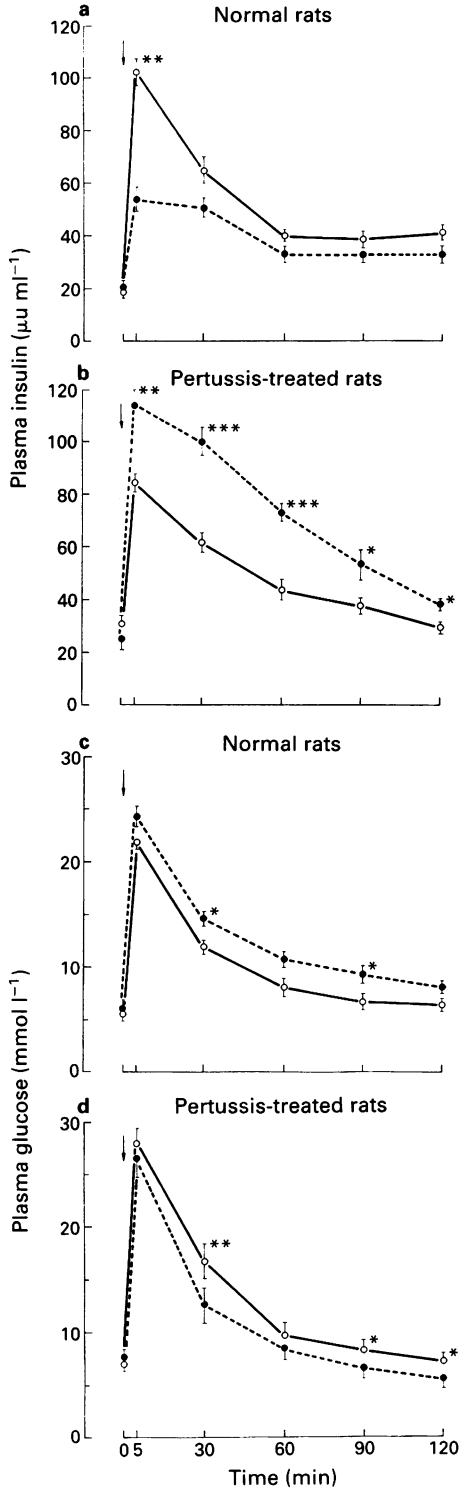

Figure 2 Effect of *pertussis* pretreatment on lithium-modified responses of plasma glucose and insulin after i.v. glucose 0.5 g kg^{-1} in rats. Results are shown for animals injected with NaCl (○) or LiCl 4 mEq kg^{-1} (●) 5 min before the injection of glucose, marked by an arrow. Upper panels (a, b) show plasma insulin concentrations, and lower panels (c, d) plasma glucose concentrations in (a, c) untreated controls, and (b, d) *pertussis* pretreated rats. Each result represents the mean of four to seven rats and vertical bars show s.e.mean. Asterisks indicate the statistical significance of differences from the corresponding saline control values: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

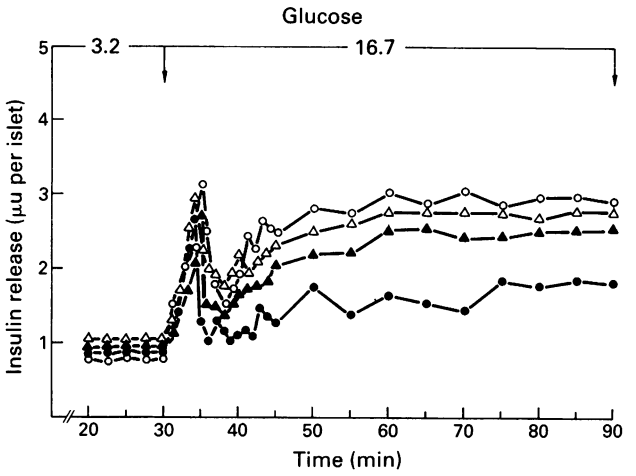

Figure 3 Effect of *pertussis* pretreatment on the inhibition by lithium of glucose-stimulated insulin release from perifused isolated islets. After isolation, islets were perifused for 30 min with 3.2 mmol l^{-1} glucose, and then exposed for a further 60 min to 16.7 mmol l^{-1} glucose either in a medium of normal ionic composition (control islets (○) and islets from *pertussis* pretreated rats (△)) or in a medium containing Li^+ 5 mmol l^{-1} (control islets (●) and islets from *pertussis* pretreated rats (▲)). Results are shown from one of seven similar experiments.

therefore, like those of adrenaline, be reversed in *pertussis*-treated animals. The results of the present study show that this reversal does in fact take place, so that lithium raises plasma insulin levels after an overnight fast, and causes a dramatic enhancement of glucose-stimulated insulin secretion in the intact animal. Thus far, the results are consistent with the pothesis that lithium is acting on the islets via catecholamine effects.

The effect of lithium in reducing plasma glucose levels in the fasted, *pertussis*-treated animal is susceptible of two explanations: first, it may clearly be attributed to the rise in plasma insulin, but secondly, there may be an additive effect from the action of lithium potentiating peripheral glucose uptake (Clausen, 1968). The latter effect is normally outweighed by the hyperglycaemic action of the catecholaminergic response in untreated animals, but may apply in the therapeutic situation where diabetic patients whose insulin secretion is already impaired have shown an improved glucose tolerance on lithium treatment (Jones *et al.*, 1983).

In the perifused rat islet preparation, the fact that pretreatment of the rats with non-selective α- or selective α_2-adrenoceptor antagonists almost fully blocks the inhibitory action of lithium on the second phase of glucose-stimulated insulin release (Fontela et al., 1987; 1990) suggests that this effect of lithium depends on the release within the isolated islets of noradrenaline from nerve terminals that persist around and within the islets. Other, more complex hypotheses to explain the effect, such as interactions of lithium with the unblocked receptor and signal transduction mechanisms, are also possible, but would presuppose actions for which there is no direct evidence. The finding that *pertussis* pretreatment partially reverses the inhibitory effect of lithium is consistent with the prevention of negative signal transduction from the α_2-adrenoceptors, but the absence in these circumstances of a stimulation of insulin secretion via β-adrenoceptors contrasts with the results *in vivo*. The *in vivo* stimulation of insulin secretion by lithium in *pertussis*-treated rats clearly depends on nervous and humoral mechanisms that cannot act on the isolated islets. These mechanisms would include a greater intensity of noradrenergic effect from the intact sympathetic system, the effect of circulating adrenaline, as inferred from the partial blockade of lithium action by bilateral adrenalectomy (Fontela *et al.*, 1986), and the effects of a number of neuropeptides and circulating peptide hormones that may stimulate insulin secretion via G_s-linked receptors. The significance of these *in vivo* effects that cannot act on the isolated islets is clearly demonstrated by the fact that the inhibition of glucose-stimulated insulin secretion by lithium is more profound *in vivo* than in the isolated islets (Fontela *et al.*, 1990). The fact

that the present and earlier results point to a major role for catecholamines in mediating the effect of lithium on insulin secretion does not exclude the possibility of significant co-operative effects mediated by other substances, such as neuropeptides and peptide hormones.

We are grateful to the Swiss Serum and Vaccine Institute and Laboratorios Berna, Madrid, Spain, for their gift of *B. pertussis* suspension. This work was supported by Grant PM-89-0005 from the Dirección General de Investigación Científica y Técnica, and is dedicated to the memory of Dr J. Gómez-Acebo, who died after its inception.

References

ANDERSON, J.H. JR. & BLACKARD, W.G. (1978). Effect of lithium on pancreatic islet insulin release. *Endocrinology*, **102**, 291–295.

ASANO, T., KATADA, T., GILMAN, A.G. & ROSS, E.M. (1984). Activation of the inhibitory GTP-binding protein of adenylate cyclase, G_i, by β-adrenergic receptors in reconstituted phospholipid vesicles. *J. Biol. Chem.*, **259**, 9351–9354.

CLAUSEN, T. (1968). The relationship between the transport of glucose and cations across cell membranes in isolated tissues. IV. The "insulin-like" effect of Li^+. *Biochim. Biophys. Acta*, **150**, 66–72.

FONTELA, T., GARCIA HERMIDA, O. & GOMEZ-ACEBO, J. (1986). Blocking effect of naloxone, dihydroergotamine and adrenalectomy on lithium-induced hyperglycaemia and glucose intolerance in the rat. *Acta Endocinol.*, **111**, 342–348.

FONTELA, T., GARCIA HERMIDA, O. & GOMEZ-ACEBO, J. (1987). Dihydroergotamine, but not naloxone, counteracts lithium as an inhibitor of glucose-induced insulin release in isolated rat islets *in vitro*. *Diabetologia*, **30**, 183–187.

FONTELA, T., GARCIA HERMIDA, O. & GOMEZ-ACEBO, J. (1990). Role of adrenoceptors *in vitro* and *in vivo* in the effects of lithium on blood glucose levels and insulin secretion in the rat. *Br. J. Pharmacol.*, **100**, 283–288.

JONES, G.R., LAZARUS, J.H., DAVIES, C.J. & GREENWOOD, R.H. (1983). The effect of short term lithium carbonate in Type II diabetes mellitus. *Horm. Metab. Res.*, **15**, 422–424.

KATADA, T. & UI, M. (1977). Perfusion of the pancreas isolated from pertussis-sensitized rats: potentiation of insulin secretory responses due to β-adrenergic stimulation. *Endocrinology*, **101**, 1247–1255.

KATADA, T., AMANO, T. & UI, M. (1982). Modulation by islet-activating protein of adenylate cyclase activity in C6 glioma cells. *J. Biol. Chem.*, **257**, 3739–3746.

LACY, P.E. & KOSTIANOVSKY, M. (1967). Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes*, **16**, 35–39.

MURAYAMA, T. & UI, M. (1983). Loss of the inhibitory function of the guanine nucleotide regulatory component of adenylate cyclase due to its ADP-ribosylation by islet-activating protein, pertussis toxin, in adipocyte membranes. *J. Biol. Chem.*, **258**, 3319–3326.

OKAJIMA, F., KATADA, T. & UI, M. (1985). Coupling of the guanine nucleotide regulatory protein to chemotactic peptide receptors in neutrophil membranes and its uncoupling by islet-activating protein, pertussis toxin. *J. Biol. Chem.*, **260**, 6761–6768.

SHAH, J.H. & PISHDAD, G. (1980). The effect of lithium on glucose- and tolbutamide-induced insulin release and glucose tolerance in the intact rat. *Endocrinology*, **107**, 1300–1304.

UI, M. (1984). Islet-activating protein, pertussis toxin: a probe for functions of the inhibitory guanine nucleotide regulatory component of adenylate cyclase. *Trends Pharmacol. Sci.*, **6**, 277–279.

YAMAZAKI, S., KATADA, T. & UI, M. (1982). α_2-adrenergic inhibition of insulin secretion via interference with cyclic AMP generation in rat pancreatic islets. *Mol. Pharmacol.*, **21**, 648–653.

(Received October 2, 1990
Revised January 22, 1991
Accepted January 23, 1991)