

Long-term Administration of Theophylline and Glucose Recovery After Hypoglycaemia in Patients with Type 1 Diabetes Mellitus

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The methylxanthine theophylline increases intrahepatic c-AMP and c-AMP mediates the hepatic glucose response to adrenaline and glucagon. Intravenous theophylline increases glucose recovery during acute insulin-induced hypoglycaemia and caffeine increases hypoglycaemia awareness and glucoregulatory hormone secretion. In this study we tested the hypothesis that long-term administration of theophylline might augment glucose recovery after insulin-induced hypoglycaemia. Eleven healthy subjects and 8 patients with Type 1 diabetes mellitus were made hypoglycaemic by 60 min insulin infusion (40 mU m⁻²) after 2 weeks' oral therapy with Euphyllin Retard (theophylline) or placebo. Plasma glucose nadir was 2.54 (2.31–2.77) mmol l⁻¹ after Euphyllin Retard and 2.27 (2.05–2.48) mmol l⁻¹ after placebo (mean difference 0.26 (0.05–0.58) mmol l⁻¹, $p = 0.09$) for healthy control subjects and 2.56 (2.07–3.04) mmol l⁻¹ and 2.19 (1.37–2.65) mmol l⁻¹ (mean difference 0.38 (0.12–0.63) mmol l⁻¹, $p = 0.01$), respectively, for diabetic patients. The area under the glucose curve was greater after theophylline treatment for healthy control subjects ($p = 0.0292$) and for diabetic patients ($p = 0.0241$) but there were no concomitant significant increases in plasma c-AMP or in endogenous glucose production rate. Whether the increase in glucose recovery is large enough to suggest that chronic theophylline administration will protect against insulin-induced hypoglycaemia remains unsettled. © 1998 John Wiley & Sons, Ltd.

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Introduction

Theophylline, a methylxanthine, modulates intermediate metabolism primarily through two mechanisms: its action as a phosphodiesterase inhibitor increasing c-AMP and its action as an adenosine antagonist. Concomitant administration of glucagon and theophylline¹ increases hepatic glucose production more than glucagon alone and concomitant administration of terbutaline and theophylline increases plasma glucose more than terbutaline alone.² Acute administration of theophylline increases plasma insulin³ and plasma adrenaline,^{4,5} decreases glucagon concentrations⁶ and has variable effects on serum cortisol^{7,8}. These effects are probably mediated through an effect on cyclic AMP. As an adenosine antagonist, theophylline increases plasma free fatty acids and it may modulate intracerebral blood flow like

caffeine.^{9,10} The latter effect could modify the secretion of glucoregulatory hormones.¹⁰

The pharmacological prevention of insulin-induced hypoglycaemia has received increasing interest during recent years. Various compounds have been used to reduce the frequency of hypoglycaemia, improve glucose recovery or increase the blood concentrations of counter-regulatory hormones: acarbose delaying the absorption of carbohydrates from the intestine reduced the frequency of night time hypoglycaemia;¹¹ alanine increasing the secretion of glucagon and terbutaline (a beta adrenoceptor agonist) both improved glucose recovery.¹²

We have previously demonstrated that theophylline improves glucose recovery after acute hypoglycaemia. In a trial involving both healthy subjects and people with Type 1 diabetes mellitus, glucose recovery and plasma c-AMP were increased during the period of most intensive counterregulation. Others¹³ have demonstrated an increase in both physiological responses to and awareness of hypoglycaemia after caffeine, another methylxanthine. However, the ability of methylxanthines to increase plasma adrenaline is known to disappear

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Table 1. Anthropometric data

	Healthy subjects	Patients with Type 1 DM
BMI (kg m ⁻²)	23.4 (22.3–24.4)	23.7 (21.3–26.1)
Age (yr)	28.2 (25.8–30.6)	30.8 (26.2–35.3)
Sex (M/F)	8/3	7/1
Duration of diabetes (yr)	–	3.1 (1.0–5.3)
Haemoglobin A _{1c} (%)	–	8.2 (6.8–9.6)
Insulin dosage (IU)	–	41 (30–51)

Data are given as mean (95 % confidence interval).

with long-term administration of the compound.^{14,15} In the present study we looked at the effect of chronic theophylline administration on glucose recovery after acute hypoglycaemia.

Patients and Methods

Subjects

Eleven healthy subjects and eight patients with Type 1 (insulin-dependent) diabetes mellitus (DM) participated in the study after giving written, informed consent. Anthropometric data are given in Table 1. The study was approved by the Regional Ethics Committee of Copenhagen and by the Danish Health Authorities (J. NR. 2740-84-1992).

Protocol

All subjects participated in two hypoglycaemia experiments in randomized order. Euphyllin Retard (Lundbeck Pharma A/S, Copenhagen, Denmark) or placebo 350 mg twice a day was administered orally for 2 weeks prior to a hypoglycaemia challenge. Euphyllin Retard (Lundbeck Pharma A/S, Copenhagen, Denmark) is a combination drug composed of approximately two-thirds theophylline monohydrate and one-third ethylenediamine and the total theophylline dose is 282 mg. The administration of placebo/Euphyllin Retard was double-blinded to the investigator and the dose was adjusted as necessary by an otherwise non-participating physician to obtain serum levels of 55–85 µM 4 h after the administration of the morning dose of active drug. Some of the subjects had their placebo dose adjusted to ensure double blindness.

The patients with Type 1 DM were admitted at 9.00 pm. They had two intravenous canulae inserted into antecubital veins, one for sampling of mixed venous blood and one for infusion of insulin (Actrapid Human, Novo-Nordisk A/S, Denmark). An overnight infusion of soluble insulin was begun at 10 pm. The rate of the insulin infusion was adjusted to obtain normoglycaemia as early in the night as possible. The infusion of insulin continued until hypoglycaemia was induced the next day.

For all subjects, an intravenous bolus injection of 0.2 µCi kg⁻¹ 3-³H-glucose was administered at 7.00 am,

followed by an infusion of 0.002 µCi kg⁻¹ min⁻¹ 3-³H-glucose. At the same time the subjects took their morning dose of Euphyllin Retard or placebo. At 9.00 am (*t* = 0) hypoglycaemia was induced by infusion of 40 mU m⁻² of soluble insulin. The infusion of insulin was stopped after 60 min and observations continued for 90 min. The control subjects were admitted at 7.00 am after a 12 h fast, having abstained from tobacco in the same period, and were made hypoglycaemic with the same protocol. Samples for analysis of plasma glucose were drawn at –30 min, every 5 min from *t* = 0 to *t* = 90 min and every 10 min thereafter. Additional venous blood samples for measurement of serum theophylline were drawn immediately before induction of hypoglycaemia and for analysis of plasma glucagon, plasma insulin, plasma free insulin, plasma cyclic AMP, and plasma 3-³H-glucose 10–30 min intervals throughout. Samples for analysis of 3-³H-glucose were transferred into tubes containing heparin; for analysis of plasma glucagon, plasma insulin, and plasma free insulin the tubes contained Trasylol (Aprotinin, Novo-Nordisk) and EDTA. For analysis of plasma cyclic AMP the tubes contained heparin, and for analysis of plasma catecholamines the tubes contained EGTA and glutathione. All tubes were kept on ice during the experiments; they were centrifuged for 20 min at 4 °C within 2 h. Plasma and serum were kept at –20 °C until analysis.

Plasma glucose was analysed in duplicate at the bedside using a Beckman Autoanalyzer (Beckman Instruments, Fullerton, California, USA). Analyses for serum theophylline,¹⁶ plasma cyclic AMP,¹⁷ plasma glucagon,¹⁸ plasma catecholamines,¹⁹ plasma insulin,¹⁸ serum growth hormone,²⁰ and plasma 3-³H-glucose²¹ were done as previously described. Serum cortisol was analysed with a commercial kit (Cortisol [¹²⁵I]Radioimmunoassay, Farnos Diagnostica, Orion Corporation, Finland). Plasma was extracted with 75 % ethanol for the analysis of plasma free insulin on the day of analysis. Glucose appearance rate (Ra) and glucose disappearance rate (Rd) were calculated using Steele's equations²² modified by de Bodo.²³

Statistical Analysis

Results are described as mean (95 % confidence interval) or as mean ± SEM as appropriate. Area under the curve

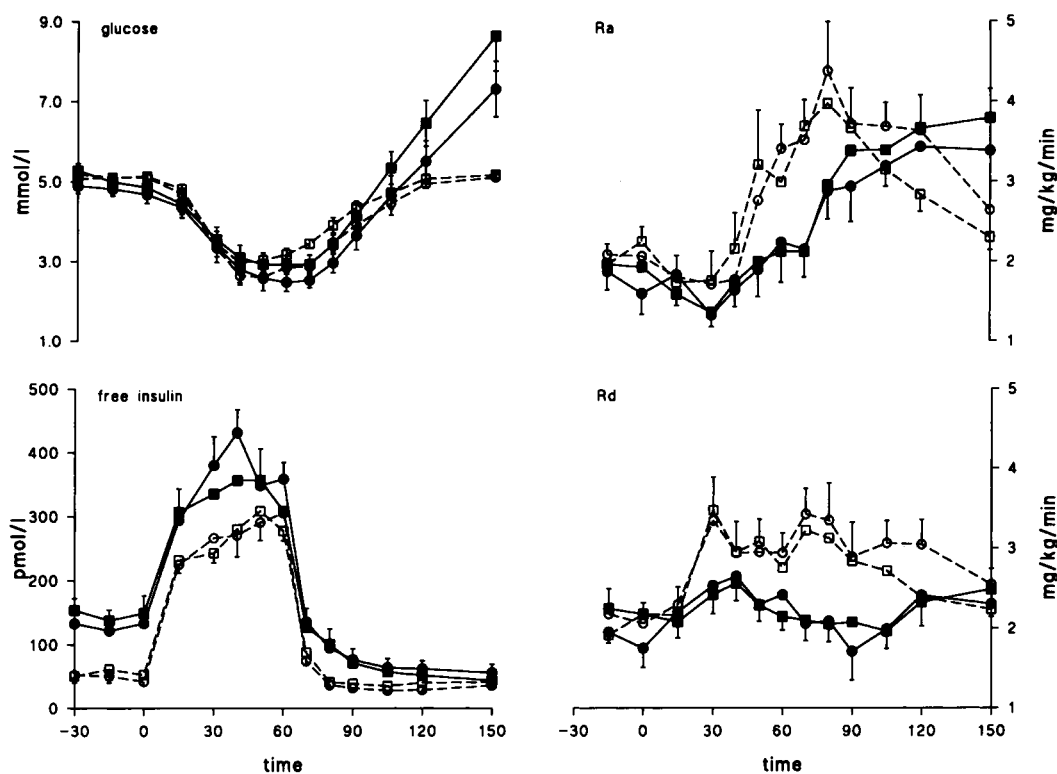


Figure 1. Plasma glucose, plasma insulin, glucose appearance rate (Ra) and glucose disappearance rate (Rd) in 8 patients with diabetes (lines, closed symbols) and in 11 healthy subjects (dotted lines, open symbols) with (squares) and without (circles) long-term theophylline treatment during insulin-induced hypoglycaemia

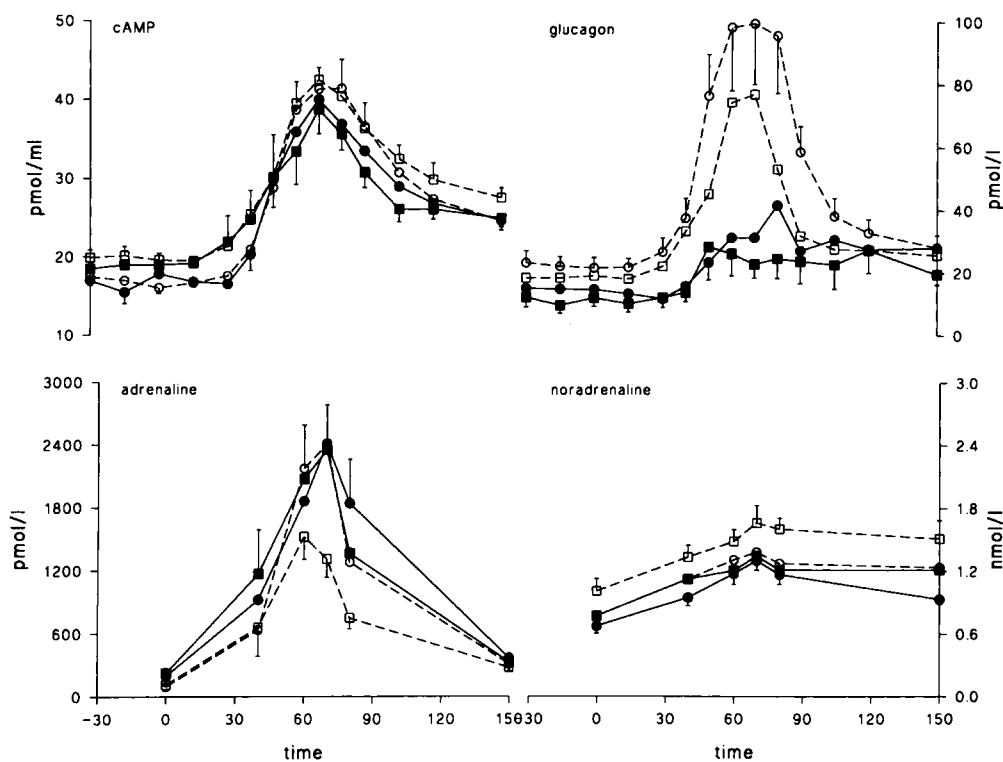


Figure 2. Plasma glucagon, plasma c-AMP, plasma adrenaline, and plasma noradrenaline in 8 patients with diabetes (lines, closed symbols) and in 11 healthy subjects (dotted lines, open symbols) with (squares) and without (circles) long-term theophylline treatment during insulin-induced hypoglycaemia

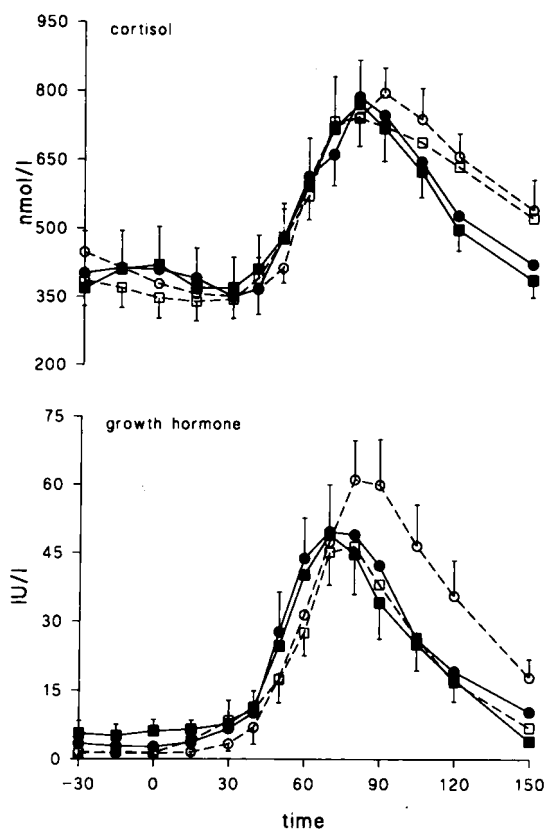


Figure 3. Serum growth hormone and serum cortisol in 8 patients with diabetes (lines, closed symbols) and in 11 healthy subjects (dotted lines, open symbols) with (squares) and without (circles) long-term theophylline treatment during insulin-induced hypoglycaemia

(AUC) was measured using the trapezoid rule. For incremental area under the curve, baseline values were subtracted. Student's *t*-test for paired comparison was used to compare group results; *p* values (two-tailed) less than 0.05 were considered significant.

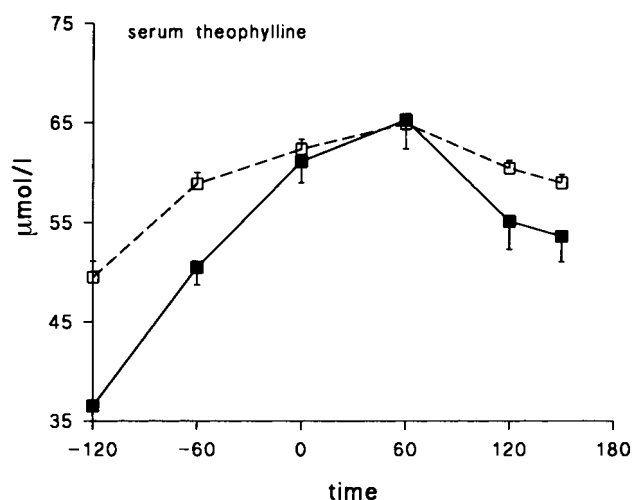


Figure 4. Serum theophylline after long-term theophylline treatment in 8 patients with diabetes (lines, closed symbols) and in 11 healthy subjects (dotted lines, open symbols) during insulin-induced hypoglycaemia

Results

Figures 1 to 4 show changes over time in plasma glucose, plasma c-AMP, glucose turnover, glucoregulatory hormones and serum theophylline. Table 2 shows baseline values and Tables 3 and 4 show area under the curve and mean differences in area under the curve for plasma glucose, plasma c-AMP, glucose turnover, glucoregulatory hormones, and serum theophylline.

Healthy Control Subjects

Baseline values drawn 120 min after the administration of Euphyllin Retard did not differ statistically between the two treatment regimens except for plasma c-AMP (mean difference 3.06 (1.86–4.26) pmol ml⁻¹, *p* = 0.0002) and plasma noradrenaline (mean difference 0.04 (0.009–0.071) pmol l⁻¹, *p* = 0.0163). For plasma glucagon baseline values were highest after placebo (mean difference -3.69 (-7.43–0.03) pmol l⁻¹) but this did not reach statistical significance (*p* = 0.0517).

The plasma glucose nadir was 2.54 (2.31–2.77) mmol l⁻¹ after Euphyllin Retard and 2.27 (2.05–2.48) mmol l⁻¹ after placebo (mean difference 0.26 (0.05–0.58) mmol l⁻¹, *p* = 0.0949). The area under the glucose curve was significantly higher after Euphyllin Retard as compared to placebo (mean difference -37 (-70–6) min*mmol l⁻¹, *p* = 0.0291). The incremental area under the curve was significantly lower after Euphyllin Retard for plasma glucagon (mean difference 1485 (793–2178) min*pmol l⁻¹, *p* = 0.0007). There was no statistically significant differences in incremental area under the curve for plasma c-AMP (*p* = 0.599), serum growth hormone (*p* = 0.143), serum cortisol (*p* = 0.392), plasma insulin (*p* = 0.363), plasma adrenaline (*p* = 0.341), plasma noradrenaline (*p* = 0.648), glucose appearance rate (*p* = 0.157), and glucose disappearance rate (*p* = 0.202).

Diabetic Patients

Baseline values drawn 120 min after the administration of Euphyllin Retard did not differ statistically from control, except that the glucose disappearance rate (mean difference was higher 0.36 (0.015–0.705) mg kg⁻¹ min⁻¹, *p* = 0.0423). Baseline plasma c-AMP was higher (mean difference 2.04 (-0.041–4.12) pmol ml⁻¹) with Euphyllin Retard but this did not reach statistical significance (*p* = 0.0535).

Plasma glucose nadir was significantly higher after Euphyllin Retard (2.56 (2.07–3.04) vs 2.19 (1.37–2.65) mmol l⁻¹ after placebo, mean difference 0.38 (0.12–0.63) mmol l⁻¹, *p* = 0.0112). The area under the glucose curve was also significantly higher after Euphyllin Retard (mean difference -83 (-151–14) min*mmol l⁻¹, *p* = 0.0241). There were no statistical differences for the incremental areas under the curve for c-AMP (*p* = 0.324), serum growth hormone (*p* = 0.205), serum cortisol (*p* = 0.989), plasma glucagon (*p* = 0.993), plasma free insulin

Table 2. Basal values. Eight Type 1 diabetic patients and 11 healthy subjects treated with theophylline p.o. or placebo

	Patients with Type 1 DM		Healthy subjects	
	Theophylline	Placebo	Theophylline	Placebo
Glucose (mmol L ⁻¹)	5.04 (4.72–5.36)	4.79 (4.43–5.15)	5.10 (5.02–5.18)	5.13 (5.02–5.24)
Ra (mg kg ⁻¹ min ⁻¹)	1.94 (1.63–2.26)	1.73 (1.35–2.11)	2.10 (2.01–2.20)	2.07 (1.94–2.20)
Rd (mg kg ⁻¹ min ⁻¹)	2.21 (1.96–2.47)	1.85 (1.52–2.19)	2.05 (1.95–2.15)	2.12 (1.98–2.26)
c-AMP (pmol ml ⁻¹)	18.8 (17.4–20.3)	16.8 (15.3–18.2)	19.9 (19.1–20.8) ^a	16.8 (16.2–17.5) ^a
Glucagon (pmol L ⁻¹)	13.2 (8.2–18.1)	15.2 (9.6–20.8)	18.9 (16.7–21.2)	22.6 (19.4–25.9)
Cortisol (nmol L ⁻¹)	400 (296–531)	408 (264–553)	368 (323–412)	414 (377–451)
Adrenaline (pmol L ⁻¹)	225 (178–272)	198 (153–243)	114 (100–127)	104 (94–113)
Noradrenaline (nmol L ⁻¹)	0.77 (0.57–0.96)	0.67 (0.54–0.81)	1.00 (0.89–1.13) ^a	0.77 (0.70–0.84) ^a
Free insulin (pmol L ⁻¹)	129 (76–181)	131 (83–180)	55 (47–63)	46 (42–50)
Growth hormone (IU L ⁻¹)	5.6 (1.6–9.6)	3.0 (0.6–5.4)	1.43 (0.93–1.94)	1.38 (0.49–2.28)

Data are given as mean (95 % confidence interval).

^aIndicates $p < 0.05$.

Table 3. Area under the curve (plasma glucose) and incremental area under the curve (hormones and glucose metabolism). Eight Type 1 diabetic patients and 11 healthy subjects treated with theophylline p.o. or placebo

	Patients with Type 1 DM		Healthy subjects	
	Theophylline	Placebo	Theophylline	Placebo
Glucose (min*mmol L ⁻¹)	708 (620–796)	625 (527–723)	635 (613–657)	598 (568–628)
Ra (mg kg ⁻¹)	105 (66–144)	118 (54–182)	101 (50–152)	135 (100–170)
Rd (mg kg ⁻¹)	3 (–30–35)	56 (4–108)	99 (48–150)	119 (78–160)
c-AMP (min*pmol ml ⁻¹)	1260 (749–1771)	1540 (860–2220)	1546 (1272–1820)	1668 (1262–2074)
Glucagon (min*pmol L ⁻¹)	1486 (560–2412)	1480 (326–2634)	2513 (1449–3577)	3998 (2772–5224)
Cortisol (min*pmol L ⁻¹)	18.5 (7.7–29.4)	18.6 (3.8–33.4)	27.8 (18.6–37.0)	23.5 (15.9–31.1)
Adrenaline (min*pmol L ⁻¹)	23.1 (14.1–32.1)	25.6 (16.0–35.2)	14.8 (11.7–17.9)	23.2 (14.4–32.0)
Noradrenaline (min*nmol L ⁻¹)	9.5 (7.2–11.8)	8.6 (5.8–11.4)	11.7 (7.7–15.8)	10.4 (5.3–15.5)
Free insulin (min*pmol L ⁻¹)	4448 (–849–9745)	3678 (–1208–8565)	10805 (9467–12 142)	11 669 (9704–13 634)
Growth hormone (min*IU L ⁻¹)	2328 (955–3701)	2990 (1679–4301)	2789 (1763–3814)	4017 (2579–5455)

Data are given as mean (95 % confidence interval).

Mean differences and p values are presented in Table 4.Table 4. Mean differences and p -values for area under the curve (plasma glucose) and incremental area under the curve (hormones and glucose metabolism). Eight Type 1 diabetic patients and 11 healthy subjects treated with theophylline p.o. or placebo

	Patients with Type 1 DM		Healthy subjects	
	Mean difference (95 % CI)	p value	Mean difference (95 % CI)	p value
Glucose (min*mmol L ⁻¹)	–83 (–151–14)	0.0241	–37 (–70–6)	0.0292
Ra (mg kg ⁻¹)	12.4 (–3–87)	0.7068	33.6 (–15.7–82.9)	0.1881
Rd (mg kg ⁻¹)	53 (–37–144)	0.2074	19.8 (–25.8–65.4)	0.3513
c-AMP (min*pmol ml ⁻¹)	280 (–344–904)	0.3241	122.5 (–367–611)	0.5890
Glucagon (min*pmol L ⁻¹)	–5.9 (–1510–1497)	0.9928	1485 (793–2178)	0.0007
Cortisol (min*nmol L ⁻¹)	77 (–12 978–13 133)	0.9892	–4267 (–14 895–6361)	0.3919
Adrenaline (min*pmol L ⁻¹)	2.6 (–9.8–15.0)	0.6350	8.4 (–0.8–17.7)	0.0703
Noradrenaline (min*nmol L ⁻¹)	–0.9 (–6.2–4.4)	0.6973	–1.3 (–7.7–5.0)	0.6483
Free insulin (min*pmol L ⁻¹)	770 (–5165–6705)	0.7678	864 (–1156–2884)	0.3631
Growth hormone (min*IU L ⁻¹)	662 (–458–1782)	0.2049	1228 (–497–2952)	0.1436

Data are given as mean difference (95 % confidence interval).

($p = 0.850$), plasma adrenaline ($p = 0.635$), plasma noradrenaline ($p = 0.697$), glucose appearance rate ($p = 0.707$) or glucose disappearance rate ($p = 0.207$).

Discussion

We have previously demonstrated that glucose recovery increased in insulin-induced hypoglycaemia preceded by infusion of theophylline. The increase in glucose recovery was accompanied by increases in plasma c-AMP and in glucose appearance rate at specific time points during the period of most active counterregulation for healthy subjects. For patients with Type 1 DM there was an increase in incremental area under the curve for plasma c-AMP. These findings were consistent with the hypothesis that theophylline increases glycogenolysis with concomitant increases in plasma c-AMP and hepatic glucose production. Adrenaline and glucagon, secreted in response to hypoglycaemia induce hepatic glycogenolysis using c-AMP as the second messenger in adrenaline and glucagon stimulated hepatic glycogenolysis. The breakdown of c-AMP is catalysed by the enzyme phosphodiesterase and this enzyme is inhibited by theophylline.

It has been shown by others that the effect of methylxanthines on intermediate metabolism vanishes with long-term administration of the compound.^{14,15} We therefore wanted to test the effect of theophylline given chronically on responses to hypoglycaemia. Furthermore, we used an intravenous infusion of insulin to minimize elevations in plasma insulin.^{24,25} The present study shows that there is a small effect of long-term theophylline administration on acute glucose recovery after insulin-induced hypoglycaemia. Glucose nadir was significantly higher after Euphyllin Retard for diabetic patients and the area under the glucose curve was significantly higher with theophylline as compared to without theophylline for both healthy subjects and diabetic patients. There were however no significant increases in the responses of either plasma c-AMP or glucose appearance rate in healthy control subjects or diabetic patients. We conclude that the mechanism for the above increase in glucose recovery is uncertain.

We observed a small increase in incremental area under the curve for plasma c-AMP in patients with Type 1 DM in our previous acute study but this was not reproduced in the present study. Plasma theophylline levels were higher in the present study and the most plausible explanation would be a decrease in the effect of theophylline on plasma c-AMP with long-term administration. According to our hypothesis, a reduction in plasma c-AMP might reduce glucose recovery. Fasting plasma c-AMP was higher with theophylline in healthy subjects with a tendency towards the same in diabetic subjects. It may be that theophylline through the effect on c-AMP increases fasting plasma glucose and fasting hepatic glucose production. Any increases in these parameters were, however, not statistically significant.

A type 2 error may have occurred for the hormone result as the power of our study was based on a change in plasma glucose. On the other hand we do not feel that even a small statistically significant increase in glucose appearance rate or in plasma c-AMP would change our main conclusion, that the increase in glucose recovery is minor and is unlikely to be of major importance in the pharmacological prevention of hypoglycaemia.

In conclusion the present study demonstrated a small but significant increase in glucose recovery after theophylline. This increase was not accompanied by any increase in incremental c-AMP or in incremental glucose appearance rate. Whether the increase in glucose recovery is large enough to be of clinical importance remains to be settled.

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