Theophylline Improves Hypoglycemia Unawareness in Type 1 Diabetes

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Iatrogenic hypoglycemias and the subsequent occurrence of hypoglycemia unawareness are well-known complications of intensive insulin therapy in type 1 diabetic patients that limit glycemic management. From a pharmacological point of view, the adenosine-receptor antagonist theophylline might be beneficial in the management of hypoglycemia unawareness. Theophylline stimulates the release of catecholamines and reduces cerebral blood flow, thereby facilitating stronger metabolic responses to and a prompter perception of decreasing glucose levels. To test the effect of theophylline on responses to hypoglycemia, we performed paired hyperinsulinemic-hypoglycemic clamp studies in 15 diabetic patients with hypoglycemia unawareness and 15 matched healthy control subjects. In random order, we concurrently infused either theophylline or placebo. Measurements included counterregulatory hormones, symptoms, hemodynamic parameters, and sweat detection using a dew-point electrode. Additionally, middle cerebral artery velocities (V_{MCA}) using transcranial Doppler were monitored as an estimate of cerebral blood flow. When compared with placebo, theophylline significantly enhanced responses of plasma epinephrine, norepinephrine, and cortisol levels in both diabetic patients and control subjects. Because of the theophylline, sweat production started at ~0.3 mmol/l higher glucose levels in both groups ($P < 0.01$), and symptom scores in diabetic patients approached those in control subjects. Theophylline decreased V_{MCA} in both groups ($P < 0.001$), but significantly greater in diabetic patients ($P < 0.01$), and prevented the hypoglycemia-induced increase of V_{MCA} that occurred during the placebo studies. We conclude that theophylline improves counterregulatory responses to and perception of hypoglycemia in diabetic patients with impaired awareness of hypoglycemia. *Diabetes* 51:790–796, 2002

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Received for publication 16 March 2001 and accepted in revised form 11 December 2001.

CBF, cerebral blood flow; CV, coefficient of variation; GH, growth hormone; GIR, glucose infusion rate; HPLC, high performance liquid chromatography; RIA, radioimmunoassay.

In type 1 diabetes, iatrogenic hypoglycemias frequently occur because (relative) insulin excess is accompanied by an insufficient counterregulatory network that is unable to prevent glucose levels from decreasing too much (1). This often results in the loss of characteristic hypoglycemic warning symptoms, so that neuroglycopenia or overt cognitive impairment becomes the first manifestation of hypoglycemia, a phenomenon known as hypoglycemia unawareness (2). Hypoglycemia unawareness thus jeopardizes a patient's ability of self-management and is thought to be the result of cerebral adaptation to recurrent hypoglycemic events (3,4), further increasing the risk for hypoglycemias that require external help. Whereas defects in hormonal counterregulation might be considered as more or less unavoidable consequences of intensive insulin treatment, complete loss of warning symptoms is often thought unacceptable in the pursuit for better glycemic control. Therefore, hypoglycemic risk-reducing strategies are required that reverse the syndrome of hypoglycemia unawareness, but preferably within the boundaries of optimal glycemic control (5).

Currently, meticulous avoidance of hypoglycemias (6) and blood glucose awareness training (7) are the only means to successfully combat hypoglycemia unawareness and defective counterregulation. Recently, it has been suggested that antagonism of central adenosine receptors might be an effective alternative (8,9). Theophylline and the related methylxanthine derivative, caffeine, block central adenosine receptors, leading to increased alertness and enhanced secretion of catecholamines (10). Secondly, by blocking adenosine-induced maintenance of vascular tone, they reduce cerebral blood flow (CBF), enabling faster perception of decreasing plasma glucose levels and earlier initiation of metabolic and symptom responses. The concept of improved hypoglycemia awareness by adenosine-receptor antagonism has already been successfully tested in humans (9,11,12). However, studies in type 1 diabetic patients with proven hypoglycemia unawareness are still lacking. Therefore, we conducted a placebo-controlled cross-over study to investigate the effect of theophylline on metabolic responses to and perception of insulin-induced hypoglycemia in type 1 diabetic patients with hypoglycemia unawareness. Theophylline was chosen over caffeine because it is widely available in various formulations, allowing a controlled pharmacological approach, and because it is a more potent adenosine-receptor antagonist (13).

RESEARCH DESIGN AND METHODS

Written informed consent was obtained from 15 type 1 diabetic patients with hypoglycemia unawareness and 15 age-, sex-, and BMI-matched healthy control subjects. The patients were recruited from the outpatient clinic of our hospital. Hypoglycemia unawareness was assessed on the basis of self-reported failure to accurately perceive low blood glucose levels, a consistent history of hypoglycemic events (i.e., readings <3 mmol/l by self-measurement methods) for at least 1 year, and the identification of principally neuroglycopenic, as opposed to autonomic symptoms, of hypoglycemia on a standardized symptom questionnaire. Although metabolic control was not regarded as a criterion per se, the majority of patients had HbA_{1c} values well below 8.0%. All patients were treated for diabetes for at least 5 years and were free of long-term diabetes complications. Autonomic neuropathy was excluded by normal cardiovascular tests, including heart rate response to Valsalva maneuver, beat-to-beat variation to deep breathing, heart-rate and blood pressure responses to standing up, and blood pressure response to sustained handgrip (14). Patients were otherwise healthy and did not use additional medication other than insulin or oral contraceptives. The study was approved by the medical ethical committee of the University Medical Center Nijmegen.

Hyperinsulinemic-hypoglycemic glucose clamp. All participants completed two experiments performed at least 3 weeks apart. Women were tested at 4- or 8-week intervals to ensure that the experiments took place in corresponding periods of the menstrual cycle. Subjects arrived at the test location at 8.00 A.M after an overnight fast. To exclude the contribution of caffeine, the participants abstained from caffeine-containing substances (coffee, tea, cola, and chocolate) for at least 3 days to render them caffeine-naive. All patients were requested to reduce bedtime insulin dosages by one-half to avoid nocturnal hypoglycemia. They checked their capillary glucose levels at least four times daily the week before the experiments and at 3:00 A.M. the night before the experiments.

The brachial artery of the nondominant arm was cannulated under local anesthesia for blood sampling and continuous blood pressure measurements. An intravenous catheter was inserted in an antecubital vein of the contralateral arm for infusion of insulin, glucose, and theophylline or placebo solutions. Baseline variables were obtained after a resting period of 30 min.

In a randomized double-blind fashion, a loading dose of 2.8 mg/kg theophylline (Euphyllin; Byk, Zwanenburg, the Netherlands) or a comparable volume of placebo solution (NaCl 0.9%) was subsequently administered intravenously over 20 min, followed by a continuous infusion of $0.5\ mg \cdot kg^{-1} \cdot h^{-1}$ for the remainder of the study period (15). A stepped hyperinsulinemic ($60\ mU \cdot m^{-2} \cdot min^{-1}$)-hypoglycemic glucose clamp procedure was initiated thereafter (16). Using a variable infusion of glucose 20%, to which 10 mmol KCl per 500 ml was added, the plasma glucose concentration was sequentially clamped at 5.0, 3.5, and 2.5 mmol/l, based on arterial plasma glucose levels, measured in duplicate at 5-min intervals. Normoglycemia was obtained within 60–70 min in all diabetic patients and continued for 20–30 min, after which plasma glucose was allowed to subsequently decrease to 3.5 and 2.5 mmol/l, respectively, over 30 min and was maintained at these plateaus for another 30 min. At 30-min intervals, arterial blood samples were taken for the measurement of theophylline, insulin, glucagon, epinephrine, norepinephrine, cortisol, and growth hormone (GH). At baseline, blood sampling included caffeine and C-peptide measurements.

Participants were asked to complete semiquantitative symptom questionnaires every 20 min. Symptoms could be scored from 0 (absent) to 6 (severe) and included six neuroglycopenic symptoms (blurred vision, difficulty in speaking, feeling faint, difficulty in thinking, confusion, and dizziness), six autonomic symptoms (cholinergic: tingling, sweating, and feeling hungry) (adrenergic: palpitations, anxiety, and trembling), four general symptoms (dry mouth, weakness, nausea, and headache), and two dummy symptoms (yellow vision and pain in the legs). In addition to this subjective information concerning hypoglycemic symptoms, sweat evaporation rate was measured by a dew-point sweat detection electrode (Evaporimeter EP1; Servomed, Stockholm, Sweden) connected to the inner portion of the forearm (17). Further, blood pressure and heart rate were continuously monitored (Monitor 378341A; Hewlett Packard, Germany).

Middle cerebral artery velocity (V_{MCA}) was monitored using transcranial Doppler (Multidop L; DWL Elektronische Systeme, Sipplingen, Germany) as an indicator for cerebral blood flow. Based on the assumption that caliber changes in the vessels are small, alterations in velocity appear to be closely associated to changes in blood flow (18). Previous studies have shown that the transcranial Doppler technique can be reliably applied to detect blood flow changes during hypoglycemia (9,11).

Analytical methods. Plasma glucose was measured in duplicate by the glucose oxidation method (Glucose Analyzer II; Beckman, Fullerton, CA) in arterial blood samples and immediately centrifuged for 10 s after withdrawal. Blood samples for measurements of catecholamines were collected in pre-chilled tubes containing glutathione (0.2 mol/l) and EGTA (0.25 mol/l) and immediately stored on ice. Blood samples for measurements of glucagon, GH, cortisol, insulin, C-peptide, caffeine, and theophylline were collected in lithium-containing heparin tubes and stored on ice. Plasma insulin was assessed by radioimmunoassay (RIA) using 125I-labeled human insulin and anti-human insulin antiserum raised in guinea pig. Bound and free tracer were separated by sheep anti-guinea pig antiserum; human insulin (Novo Biolabs, Copenhagen, Denmark) was used for standards. The interassay coefficient of variation (CV) for the insulin measurement was 10.3% at a level of 20.7 mU/l. Plasma C-peptide was determined with a commercially available double-antibody RIA (Diagnostic Products, Los Angeles, CA) with an interassey CV of 6.4% at a level of 0.21 nmol/l. Plasma epinephrine and norepinephrine were analyzed by high performance liquid chromatography (HPLC) with a modification of an earlier described laboratory procedure (19). Plasma glucagon was measured by competitive RIA using a commercially available kit (Eurodiagnostica, Malmö, Sweden). The procedure of glucagon determination was modified as follows. To diminish the risk of interference by larger glucagon-like peptides, serum samples were extracted with ethanol and then washed twice with diethylether. Residues were dried and dissolved in assay buffer. In each assay series, six randomly selected samples were spiked with standard for recovery measurements. The average recovery per series was 102 ± 13%, intra-assay CVs were 8% at a level of 80 pmol/l and 11% at 10 pmol/l, and interassay CVs were 12.5 and 13.9%, respectively. Plasma cortisol was assayed using the TDx batch analyzer (Abbott Laboratories), and interassay CVs were 9.1 and 6.6% at plasma levels of 0.22 and 1.06 μmol/l, respectively. GH was measured by direct RIA using the World Health Organization standard for human GH 80/505 (interassay CVs 9.2 and 6.0% at plasma concentrations of 4.7 and 53.5 mU/l, respectively) (20). HbA_{1c} was measured using HPLC (Bio-Rad Laboratories, Veenendaal, the Netherlands) with reference values of 4.8–6.2%. Plasma theophylline and caffeine were determined by fluorescence polarization immunoassay and HPLC (limit of detection 0.2 mg/l), respectively.

Statistical methods. The gradual decrease in glucose levels between the plateau phases enabled us to calculate glycemic thresholds. Glycemic threshold for the detection of sweat production was defined as the plasma glucose level at which dew-point electrode readings showed at least doubling of the individual values recorded at baseline. For hemodynamic parameters, the threshold was defined as the plasma glucose concentration at which the parameter consistently exceeded the 95% CI observed for changes in that parameter during the normoglycemic steady-state period. Statistical analysis was done using repeated measures ANOVA, and differences in means were tested using paired Student's t test. For calculations and statistical analyses, the SPSS personal computer software package was used, and $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics of type 1 diabetic patients and control subjects are shown in Table 1. Patients and control subjects were well matched for age, sex, and BMI. Except for higher fasting glucose, HbA_{1c}, and heart rates in diabetic patients, hemodynamic and metabolic parameters were comparable at baseline between diabetic patients and control subjects and between placebo and theophylline for either group (data not shown). After insulin infusion, plasma insulin levels increased to similar values in both patients (140 ± 14 mU/l) and control subjects (158 ± 8 mU/l) on either occasion. Similarly, plasma glucose levels were equivalent in all study arms from the moment that normoglycemic steady state was achieved onward (Fig. 1). CVs for glucose levels at each plateau were all <5%. Caffeine (and theophylline) levels were below assay sensitivity before the start of either study. After theophylline bolus, plasma theophylline levels increased to 11.7 ± 0.5 mg/l in control subjects and to 11.1 ± 0.6 mg/l in diabetic patients and remained elevated during the maintenance infusion at 8.1 ± 0.3 and 7.5 ± 0.4 mg/l, respectively (Fig. 1).

Metabolic responses to hypoglycemia. In healthy control subjects, glucose infusion rates (GIRs) required to maintain glucose at normoglycemic levels were similar between placebo and theophylline, but at hypoglycemic

TABLE 1
Baseline characteristics

	Type 1 diabetic patients	Healthy control subjects
n (men/women)	15 (8/7)	15 (10/5)
Age (years)	33.6 ± 10.3	31.5 ± 8.4
BMI (kg/m^2)	23.2 ± 2.3	23.2 ± 2.9
Duration of diabetes (years)	15.5 ± 6.9	—
Insulin dose in diabetic patients (units/kg)	0.72 ± 0.26	—
Systolic BP (mmHg)	135 ± 10	128 ± 9
Diastolic BP (mmHg)	79 ± 6	75 ± 7.5
Heart rate (bpm)	71 ± 10*	63 ± 7
HbA_{1c} (%)	7.37 ± 0.60†	5.13 ± 0.33
Glucagon (pmol/l)	13.6 ± 3.4	15.6 ± 4.7
C-peptide (nmol/l)	<0.01†	0.49 ± 0.21
Epinephrine (nmol/l)	0.23 ± 0.10	0.17 ± 0.08
Norepinephrine (nmol/l)	0.76 ± 0.35	0.59 ± 0.28
Cortisol (μmol/l)	0.61 ± 0.31	0.57 ± 0.33
GH (IU/l)	19.1 ± 20.5	10.2 ± 19.9
V_{MCA} (cm/s)	72.2 ± 3.9	70.8 ± 4.8

Data are means ± SD. *$P < 0.05$ vs. control subjects, †$P < 0.01$ vs. control subjects. V_{MCA}, median cerebral artery velocity; BP, blood pressure.

nadir, GIR was significantly lower in the presence of theophylline (2.9 ± 1.1 vs. 9.5 ± 1.5 μmol · kg^{-1} · min^{-1}, $P < 0.01$) (Fig. 1). In diabetic subjects, theophylline tended to reduce GIR during normoglycemia (19.8 ± 2.3 vs. 27.7 ± 2.2 μmol · kg^{-1} · min^{-1}, $P = 0.05$) and significantly reduced rates for the remainder of the study (12.5 ± 2.1 vs. 19.5 ± 1.6 μmol · kg^{-1} · min^{-1} [glucose 3.5 mmol/l] and 4.8 ± 1.7 vs. 13.9 ± 1.4 μmol · kg^{-1} · min^{-1} [glucose 2.5 mmol/l], both $P < 0.01$) (Fig. 1).

Baseline levels of counterregulatory hormones were similar between theophylline and placebo studies in both groups. At the end of the normoglycemic period, theophylline significantly enhanced epinephrine release in both patients (0.35 ± 0.08 vs. 0.10 ± 0.04 nmol/l, $P < 0.01$) and control subjects (0.20 ± 0.04 vs. 0.07 ± 0.04 nmol/l, $P < 0.01$), whereas norepinephrine levels increased significantly more only in control subjects (0.45 ± 0.07 vs. 0.28 ± 0.04 nmol/l, $P < 0.03$) compared with placebo (Fig. 2). Levels of glucagon, cortisol, and GH did not change before hypoglycemia.

During hypoglycemia, diabetic patients had almost absent glucagon responses ($P < 0.001$ vs. control subjects), severely impaired epinephrine responses ($P < 0.001$), and small but significant impairments in norepinephrine ($P < 0.001$) and cortisol ($P < 0.001$) responses compared with healthy control subjects (Fig. 2). In both groups, theophylline significantly augmented hypoglycemia-induced responses of epinephrine (levels at hypoglycemic nadir 8.2 ± 0.7 vs. 6.5 ± 0.5 and 4.8 ± 0.6 vs. 3.4 ± 0.4 nmol/l for control subjects and type 1 diabetic patients, respectively, both $P < 0.05$), norepinephrine (2.9 ± 0.3 vs. 2.2 ± 0.2 and 2.4 ± 0.2 vs. 1.9 ± 0.2 nmol/l for control subjects and type 1 diabetic patients, respectively, both $P < 0.01$), and cortisol (1.2 ± 0.4 vs. 1.1 ± 0.1 and 1.1 ± 0.1 vs. 0.9 ± 0.1 μmol/l for control subjects and type 1 diabetic subjects, respectively, both $P < 0.02$). During theophylline stimulation in diabetic patients, hypoglycemia-induced norepinephrine and cortisol levels reached values similar to nonstimulated levels in control subjects, whereas epinephrine levels remained somewhat lower ($P < 0.05$). Theophylline did not affect GH and glucagon responses to hypoglycemia in either group.

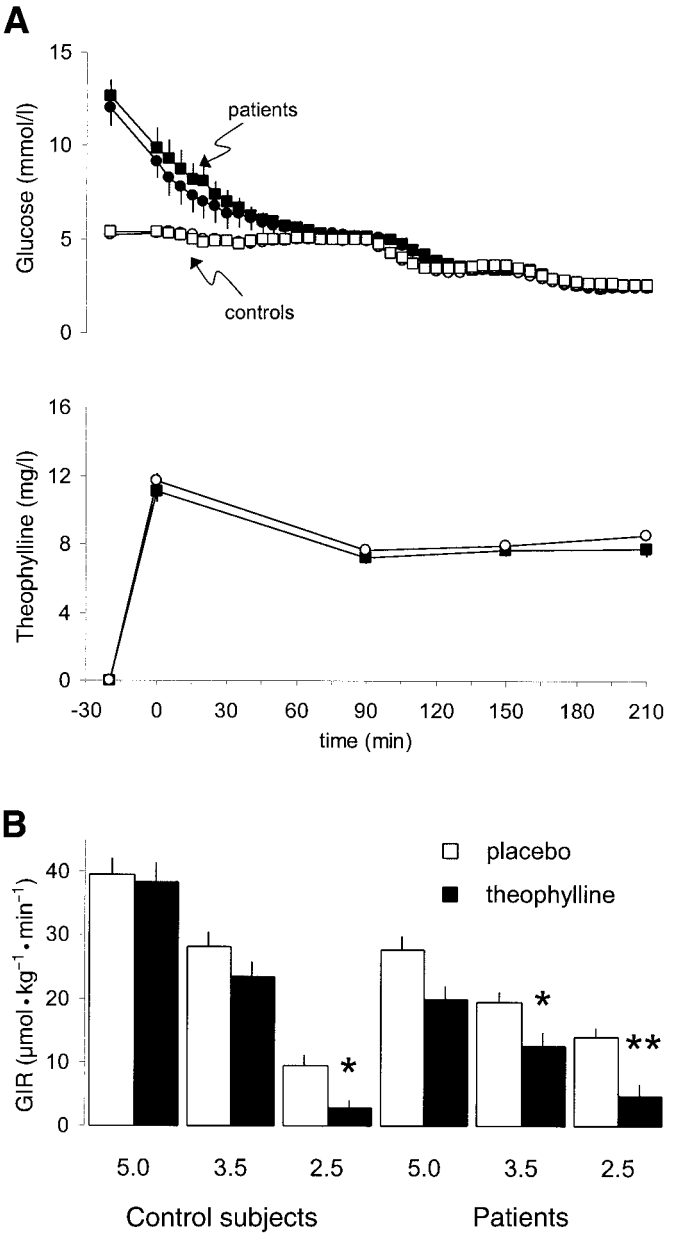

FIG. 1. *A*: Glucose levels during both clamp studies in diabetic (closed symbols) and control (open) subjects and theophylline levels during the theophylline study-arm. *B*: Glucose infusion rates during clamp at 5.0, 3.5, and 2.5 mmol/l, respectively. During hypoglycemia, rates are significantly lower in the presence of theophylline compared with placebo. *$P < 0.01$, **$P < 0.001$ vs. placebo.

Hypoglycemic symptoms. Diabetic patients had reduced symptom responses to hypoglycemia compared with nondiabetic control subjects, both in terms of magnitude ($P = 0.02$) and glycemic thresholds ($P < 0.001$) (Fig. 2, Table 2). In diabetic patients, theophylline shifted glycemic thresholds for symptoms to significantly higher glucose levels compared with placebo (3.37 ± 0.05 vs. 3.00 ± 0.05 mmol/l, $P < 0.001$), and symptom responses tended to be higher ($P = 0.09$). In control subjects, theophylline had no

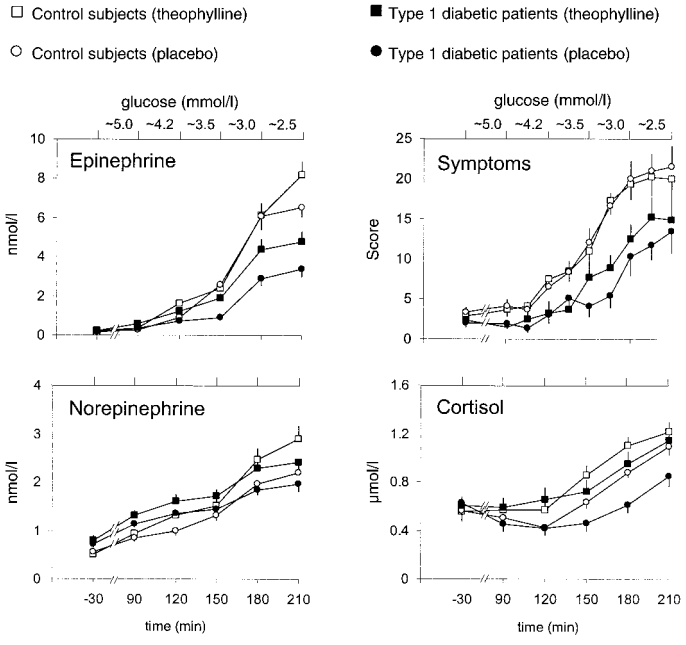

FIG. 2. Responses of hypoglycemic symptoms and counterregulatory hormones to hypoglycemia. Except for symptom responses in control subjects, responses are significantly ($P < 0.05$) higher during theophylline infusion than during placebo.

additional effect on magnitude of symptoms or glycemic threshold.

To assess the appearance of hypoglycemic symptoms more accurately, a dew-point electrode was applied to detect sweating responses. With placebo, sweat responses were registered in all healthy subjects and in 6 of the 15 diabetic patients. These responses occurred at significantly ($P < 0.001$) lower glucose levels in diabetic patients as compared with control subjects (Table 2). Theophylline shifted sweating responses to higher levels of glycemia in both the nondiabetic volunteers ($P < 0.01$) and the aforementioned six diabetic patients ($P < 0.01$). Furthermore, theophylline elicited a response at a mean (±SEM) glucose level of 2.35 ± 0.12 mmol/l in an additional four out of nine patients who had no responses with placebo ($P < 0.03$ by χ^2).

V_{MCA}. Baseline V_{MCA} measurements were comparable between the study arms in both groups. Recordings in diabetic patients appeared higher than in control subjects, but this difference did not attain statistical significance ($P = 0.3$). During the final 30 min in the placebo study, when hypoglycemic nadir was reached, V_{MCA} increased to 4.5 ± 1.5 and 7.6 ± 2.5 cm/s in control subjects ($P < 0.05$) and diabetic patients, respectively ($P < 0.03$). In contrast, theophylline caused immediate decreases in V_{MCA} in control subjects (−7.6 ± 1.4 cm/s, $P < 0.001$) and diabetic patients (−14.5 ± 2.1 cm/s, $P < 0.001$), which remained unchanged for the duration of the studies (Fig. 3). The decrease in V_{MCA} was significantly more pronounced in diabetic patients than control subjects ($P = 0.03$).

Hemodynamic responses. In healthy control subjects, systolic and diastolic blood pressure decreased significantly in response to hypoglycemia, whereas heart rate and pulse pressure increased (Table 2). Maximal responses in diabetic patients were comparable with those in control subjects, except that systolic blood pressure increased instead of decreased ($P < 0.05$ vs. control subjects). Before hypoglycemia, theophylline slightly increased heart rates (3.4 ± 0.8 bpm, $P < 0.01$) in control subjects but did not affect blood pressure in either diabetic patients or control subjects. Theophylline significantly increased heart rate responses to hypoglycemia in both patients ($P < 0.05$) and control subjects ($P < 0.05$) and caused the systolic blood pressure in patients to decrease ($P < 0.05$). In the presence of theophyline, responses of

TABLE 2
Glycemic thresholds for hemodynamic and symptom responses to hypoglycemia

	Type 1 diabetic patients		Healthy control subjects	
	Glycemic threshold (glucose, mmol/l)	Magnitude of response	Glycemic threshold (glucose, mmol/l)	Magnitude of response
Systolic BP (mmHg)				
Placebo	2.40 ± 0.05*	2.5 ± 2.0*	2.57 ± 0.04	−3.4 ± 2.6
Theophylline	2.90 ± 0.07†	−5.9 ± 2.9‡	none	−1.7 ± 2.2
Diastolic BP (mmHg)				
Placebo	3.35 ± 0.05	−5.6 ± 0.9	3.24 ± 0.06	−8.5 ± 1.1
Theophylline	3.86 ± 0.12†	−7.0 ± 1.1	3.43 ± 0.05*	−7.4 ± 1.8
Pulse pressure (mmHg)				
Placebo	2.69 ± 0.05*	4.9 ± 1.3	3.22 ± 0.05	6.5 ± 1.9
Theophylline	3.47 ± 0.05†	2.6 ± 1.5	3.59 ± 0.06†	7.7 ± 1.9
Heart rate (bpm)				
Placebo	3.38 ± 0.05	6.5 ± 3.3	3.22 ± 0.05	10.7 ± 1.9
Theophylline	3.86 ± 0.12†	14.9 ± 2.5‡	3.43 ± 0.06†	17.7 ± 2.9‡
Sweating response (g/m)§				
Placebo	2.38 ± 0.07*	59 ± 11*	2.78 ± 0.10	102 ± 10
Theophylline	2.66 ± 0.08†	71 ± 17	3.05 ± 0.11†	93 ± 9
Hypoglycemic symptoms				
Placebo	3.00 ± 0.05*	13.1 ± 2.8*	3.47 ± 0.05	21.3 ± 2.6
Theophylline	3.37 ± 0.05†	15.9 ± 3.4	3.46 ± 0.06	20.8 ± 2.4

Data are means ± SE. *$P < 0.05$ between placebo tests in diabetic patients versus control subjects; †$P < 0.01$ for differences between placebo and theophylline tests; ‡$P < 0.05$ for differences between placebo and theophylline tests; §values in diabetic patients are derived from six diabetic patients who had responses during both tests.

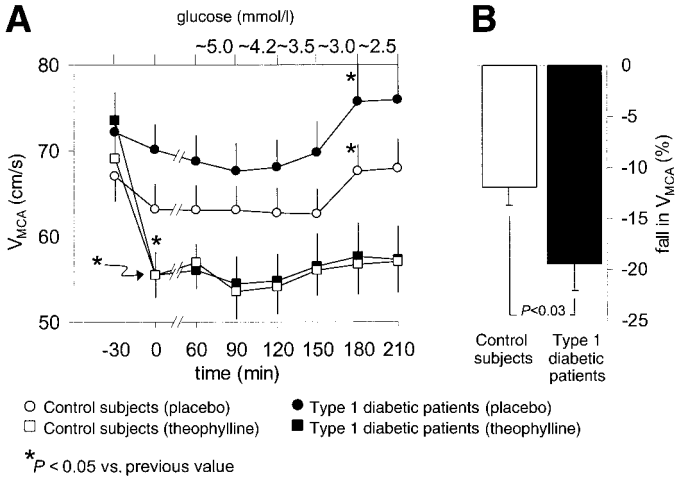

FIG. 3. Responses of V_{MCA} to hypoglycemia. In the placebo studies, V_{MCA} increases significantly in both groups when hypoglycemic nadir is reached (~2.5 mmol/l). Immediately after theophylline (bolus) infusion, V_{MCA} falls in both diabetic patients and control subjects and remains decreased (*A*). The fall in V_{MCA} is significantly higher in diabetic patients than in control subjects (*B*).

diastolic blood pressure ($P < 0.05$), pulse pressure ($P < 0.01$), and heart rate ($P < 0.01$) in both groups and of systolic blood pressure ($P < 0.01$) in diabetic patients were elicited at significantly higher glucose levels. Compared with healthy control subjects, theophylline largely normalized hemodynamic responses to hypoglycemia in diabetic patients (Fig. 4).

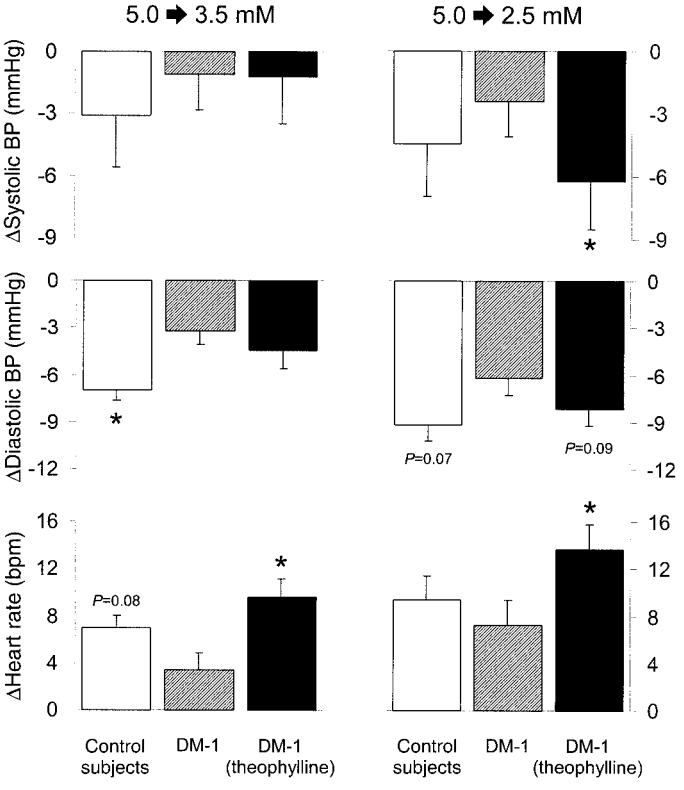

FIG. 4. Change in hemodynamic parameters in response to hypoglycemia in control subjects (□), type 1 diabetic patients (DM-1) with placebo (▨), and type 1 diabetic patients with theophylline (■). Responses in diabetic patients are lower than those in control subjects at both hypoglycemic levels, and theophylline improves the responses in diabetic patients, up to normalization. *$P < 0.05$ vs. DM-1; other *P*-values given are versus DM-1. BP, blood pressure.

DISCUSSION

In the present study, theophylline augmented counterregulatory hormone responses to and perception of insulin-induced hypoglycemia in type 1 diabetic patients who initially had pronounced impairments in glucose counterregulation and in awareness of hypoglycemia. Theophylline almost normalized the glycemic threshold for and magnitude of symptom responses in the diabetic patients and shifted glycemic thresholds for sweating and hemodynamic responses to higher glucose levels in both diabetic patients and control subjects. As a consequence of enhanced counterregulatory hormone release, GIRs were significantly lower with theophylline, reflecting increased endogenous glucose production, diminished glucose uptake, or both. This effect on GIR is in line with earlier findings of enhanced recovery from hypoglycemia in the presence of theophylline (8).

Although theophylline has several well-described modes of action, the beneficial effect on glucose counterregulation most likely reflects its role as an adenosine-receptor antagonist (10,13). In the brain, adenosine antagonism induces cerebral hypoperfusion accompanied by simultaneous increments in glucose utilization, thus uncoupling the relation between (adenosine-maintained) CBF and energy metabolism. In other words, glucose requirements are higher, but delivery is lower. As a consequence, counterregulatory hormone release is stimulated. Further stimulation occurs when cerebral glucose supply is jeopardized by coexisting hypoglycemia, especially beyond a critical glycemic threshold when CBF normally increases to compensate for the decrease in plasma glucose (21,22). Indeed, the finding that theophylline immediately reduced V_{MCA} and prevented hypoglycemia-induced increases in V_{MCA} (Fig. 3) is in accordance with the concept of sensing hypoglycemia and subsequent initiation of counterregulatory responses at higher levels of glycemia.

Our observations of enhanced glucose counterregulation during theophylline treatment appear to be at variance with those of Hvidberg et al. (8), who reported that theophylline did not affect hormonal responses to hypoglycemia. This discrepancy may be explained by significantly higher glucose levels at hypoglycemic nadir during theophylline in their study, which may have masked any effect on counterregulation. Our findings are consistent with those of caffeine reported in diabetic patients without hypoglycemia unawareness, except that caffeine was also found to stimulate GH responses (9). This variance with our data may be explained by differences between theophylline and caffeine on GH regulation and by differences in study design—in our study lower glycemic nadirs were reached and arterial blood was sampled, which may be more reliable than arterialized venous blood sampling (23,24).

While exhibiting pronounced metabolic effects and a clear stimulation of symptoms using objective tools, theophylline appeared to have only a modest effect on symptom responses scored by checklists, especially in control subjects. This apparent discrepancy may be explained, first, by the questionnaire's sensitivity to accurately assess and grade hypoglycemic symptoms, especially in subjects lacking prior "hypoglycemic experience," thus explaining

why the checklist revealed an effect of theophylline in diabetic patients only. Secondly, the hypoglycemic steps may lack sufficient power to detect differences in symptom thresholds. In our study, arterial plasma glucose values decreased from 3.5 to 2.5 mmol/l in 30 min. A study protocol involving more steps to cover the decrease in glucose level or more frequent symptom assessments might have revealed differences in symptom scores between theophylline and placebo (25).

Two observations from the transcranial Doppler recordings deserve some comment (Fig. 3). Firstly, theophylline induced a larger decrease in V_{MCA} in diabetic patients than in control subjects, which is consistent with larger adenosine availability in diabetic patients. This larger adenosine availability may explain the higher CBF reported in type 1 diabetic patients with longstanding disease and frequent hypoglycemic events compared with healthy age-matched control subjects (21). Second, theophylline almost completely suppressed increases in V_{MCA} at hypoglycemic nadir in both diabetic patients and control subjects, indicating that hypoglycemia-induced increases are mediated by adenosine. Indeed, adenosine is involved in the maintenance of cerebral vascular tone and has been found to dramatically increase in rat striatum in response to severe hypoglycemia (26). One might speculate that hypoglycemia stimulates adenosine, which by increasing CBF and hence cerebral glucose availability, initially compensates for low blood glucose but also that the prize of this compensation is reduced awareness of subsequent hypoglycemias. As such, adenosine may be involved in the pathogenesis of hypoglycemia unawareness as a faster-responding component besides the "supposedly slower" upregulation of cerebral glucose transporters (27). Studies showing that avoidance of hypoglycemias for several days partially reverses hypoglycemia unawareness and counterregulatory failure (28), but that full recovery requires at least 3–4 weeks (6,28,29), support this hypothesis. If further research substantiates the role of adenosine in the development of hypoglycemia unawareness, adenosine blockade would be a logical target to reverse this condition.

In summary, we demonstrate that a single dose of theophylline enhances counterregulatory hormone responses to hypoglycemia and partially restores perception of hypoglycemia in diabetic patients with counterregulatory failure and hypoglycemia unawareness. The near normalization of glycemic thresholds allows a more timely behavioral response, i.e., eating something or seeking assistance for recovery. However, these promising results cannot yet be translated into clinical practice because of a number of limitations. Firstly, to obtain stable plasma levels, theophylline was administered intravenously, not orally. Whether oral theophylline is equally effective remains to be demonstrated. Yet, it should be possible to obtain the relatively low plasma levels required to block adenosine receptors (13) by currently available oral theophylline preparations (30). Secondly, our study was not designed to demonstrate sustained effects of theophylline, in terms of reducing the incidence of (severe) asymptomatic hypoglycemic episodes, even though this would be the ultimate clinical goal. Long-term use of theophylline (and related methylxanthine derivatives) is subject to the emergence of tolerance for many of its effects (31). Although there are indications that tolerance remains incomplete under hypoglycemic conditions (12), this issue needs to be further explored. Appropriate studies are therefore required to establish the clinical potential of theophylline in the management of hypoglycemia unawareness.

ACKNOWLEDGMENTS

We are indebted to Wilma Raymann, Lucia Smits, and Rita Westdorp for skilled technical assistance with the transcranial Doppler measurements; to Eugenie Olde Riekerink for performing cardiovascular reflex tests; and to Aarnout Jansen van Rosendaal for help with randomization. We also thank all participants for their cooperation.

REFERENCES

1. Cryer PE: Banting Lecture. Hypoglycemia: the limiting factor in the management of IDDM. *Diabetes* 43:1378–1389, 1994
2. Amiel SA, Tamborlane WV, Simonson DC, Sherwin RS: Defective glucose counterregulation after strict glycemic control of insulin-dependent diabetes mellitus. *N Engl J Med* 316:1376–1383, 1987
3. Biggers DW, Myers SR, Neal D, Stinson R, Cooper NB, Jaspan JB, Williams PE, Cherrington AD, Frizzell RT: Role of brain in counterregulation of insulin-induced hypoglycemia in dogs. *Diabetes* 38:7–16, 1989
4. Boyle PJ, Kempers SF, O'Connor AM, Nagy RJ: Brain glucose uptake and unawareness of hypoglycemia in patients with insulin-dependent diabetes mellitus. *N Engl J Med* 333:1726–1731, 1995
5. The Diabetes Control and Complications Trial Research Group: Hypoglycemia in the Diabetes Control and Complications Trial. *Diabetes* 46:271–286, 1997
6. Fanelli CG, Epifano L, Rambotti AM, Pampanelli S, Di Vincenzo A, Modarelli F, Lepore M, Annibale B, Ciofetta M, Bottini P, Porcellati F, Scionti L, Santeusanio F, Brunetti P, Bolli GB: Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. *Diabetes* 42:1683–1689, 1993
7. Cox D, Gonder-Frederick L, Polonsky W, Schlundt D, Julian D, Clarke W: A multicenter evaluation of blood glucose awareness training-II. *Diabetes Care* 18:523–528, 1995
8. Hvidberg A, Rasmussen MH, Christensen NJ, Hilsted J: Theophylline enhances glucose recovery after hypoglycemia in healthy man and in type I diabetic patients. *Metabolism* 43:776–781, 1994
9. Debrah K, Sherwin RS, Murphy J, Kerr D: Effect of caffeine on recognition of and physiological responses to hypoglycaemia in insulin-dependent diabetes. *Lancet* 347:19–24, 1996
10. Snyder SH, Katims JJ, Annau Z, Bruns RF, Daly JW: Adenosine receptors and behavioral actions of methylxanthines. *Proc Natl Acad Sci U S A* 78:3260–3264, 1981
11. Kerr D, Sherwin RS, Pavalkis F, Fayad PB, Sikorski L, Rife F, Tamborlane WV, During MJ: Effect of caffeine on the recognition of and responses to hypoglycemia in humans. *Ann Intern Med* 119:799–804, 1993
12. Watson JM, Jenkins EJE, Hamilton P, Lunt MJ, Kerr D: Influence of caffeine on the frequency and perception of hypoglycemia in free-living patients with type 1 diabetes. *Diabetes Care* 23:455–459, 2000
13. Fredholm BB: On the mechanism of action of theophylline and caffeine. *Acta Med Scand* 217:149–153, 1985
14. Ewing DJ, Clarke BF: Diagnosis and management of diabetic autonomic neuropathy. *Br Med J* 285:916–918, 1982
15. Mitenko PA, Ogilvie RI: Rational intravenous doses of theophylline. *N Engl J Med* 289:600–603, 1973
16. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
17. Davies AG, Evans S: Sweat detection as an indicator of nocturnal hypoglycaemia. *Lancet* 346:772, 1995
18. Aaslid R, Markwalder TM, Nornes H: Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg* 57:769–774, 1982
19. Willemsen JJ, Ross HA, Jacobs MC, Lenders JW, Thien T, Swinkels LM, Benraad TJ: Highly sensitive and specific HPLC with fluorometric detec-

tion for determination of plasma epinephrine and norepinephrine applied to kinetic studies in humans. *Clin Chem* 41:1455–1460, 1995
20. Span JP, Pieters GF, Sweep CG, Swinkels LM, Smals AG: Plasma IGF-I is a useful marker of growth hormone deficiency in adults. *J Endocrinol Invest* 22:446–450, 1999
21. Tallroth G, Ryding E, Agardh CD: The influence of hypoglycaemia on regional cerebral blood flow and cerebral volume in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 36:530–535, 1993
22. Tallroth G, Ryding E, Agardh CD: Regional cerebral blood flow in normal man during insulin- induced hypoglycemia and in the recovery period following glucose infusion. *Metabolism* 41:717–721, 1992
23. Liu D, Moberg E, Kollind M, Lins PE, Adamson U, MacDonald IA: Arterial, arterialized venous, venous and capillary blood glucose measurements in normal man during hyperinsulinaemic euglycaemia and hypoglycaemia. *Diabetologia* 35:287–290, 1992
24. Best JD, Halter JB: Release and clearance rates of epinephrine in man: importance of arterial measurements. *J Clin Endocrinol Metab* 55:263–268, 1982
25. Mitrakou A, Mokan M, Ryan C, Veneman T, Cryer P, Gerich J: Influence of plasma glucose rate of decrease on hierarchy of responses to hypoglycemia. *J Clin Endocrinol Metab* 76:462–465, 1993
26. Butcher SP, Hagberg H, Sandberg M, Hamberger A: Extracellular purine catabolite and amino acid levels in the rat striatum during sever hypoglycemia: effects of 2-amino-5-phosphonovalerate. *Neurochem Int* 11:95–99, 1987
27. Kumagai AK, Kang YS, Boado RJ, Pardridge WM: Upregulation of blood-brain barrier GLUT1 glucose transporter protein and mRNA in experimental chronic hypoglycemia. *Diabetes* 44:1399–1404, 1995
28. Dagogo Jack S, Rattarasarn C, Cryer PE: Reversal of hypoglycemia unawareness, but not defective glucose counterregulation, in IDDM. *Diabetes* 43:1426–1434, 1994
29. Cranston I, Lomas J, Maran A, Macdonald I, Amiel SA: Restoration of hypoglycaemia awareness in patients with long- duration insulin-dependent diabetes. *Lancet* 344:283–287, 1994
30. Mitenko PA, Ogilvie RI: Bioavailability and efficacy of a sustained-release theophylline tablet. *Clin Pharmacol Ther* 16:720–726, 1974
31. Robertson D, Wade D, Workman R, Woosley RL, Oates JA: Tolerance to the humoral and hemodynamic effects of caffeine in man. *J Clin Invest* 67:1111–1117, 1981