Regulation of postabsorptive glucose production in patients with type 2 diabetes mellitus

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CHAPTER 4

Aminophylline stimulates insulin secretion in patients with type 2 diabetes mellitus

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\textit{submitted for publication}
Abstract

In healthy subjects, basal endogenous glucose production is partly regulated by paracrine intrahepatic factors. Administration of pentoxifylline, an adenosine receptor antagonist, inhibited transiently endogenous glucose production in healthy humans without any changes in glucoregulatory hormone concentrations. It is unknown whether similar paracrine factors influence basal endogenous glucose production in type 2 diabetes mellitus. To evaluate the modulatory role of adenosine on endogenous glucose production in type 2 diabetes, aminophylline, a potent adenosine receptor antagonist, was administered intravenously to 5 patients with type 2 diabetes mellitus in a saline controlled study. Endogenous glucose production was measured before and during 6 hours after administration of aminophylline/saline, by primed, continuous infusion of \([6,6-^{2}H]_{2}\)glucose. During both experiments, the decrease in plasma glucose concentration was similar (16 vs 18% from basal, ns). After aminophylline administration, basal endogenous glucose production was transiently inhibited within 15 minutes to 70% from basal, whereas it did not change significantly in the control experiment (p=.02). The inhibition of glucose production coincided with stimulation of insulin secretion to 144% from basal, 90 minutes after the administration of aminophylline (p=.008). In the control experiment insulin secretion decreased gradually by 29% after six hours.

Thus, aminophylline stimulates insulin secretion and inhibits endogenous glucose production in type 2 diabetes.

Introduction

Endogenous glucose production is regulated predominantly by glucoregulatory hormones and substrate supply (5;12;15). In addition to these major regulatory mechanisms, there are indications in healthy adults, that other factors are involved in the modulation of basal endogenous glucose production, a process frequently referred to as autoregulation (15). One of these factors involves the interaction between hepatocytes and Kupffer cells via mediators like adenosine. Adenosine is released in all tissues, including the liver (1;6;17;22) In vitro, adenosine stimulates glycogenolysis in hepatocytes (10;16). In vivo, adenosine antagonists, like pentoxifylline, inhibits basal endogenous glucose production in healthy humans without changes in glucoregulatory hormone concentrations (3;4). These data indicate that mediators like adenosine are involved in the regulation of basal glucose production.
In patients with type 2 diabetes mellitus, basal endogenous glucose production is inappropriately increased, considering the elevated glucose and insulin concentrations. In addition, regulation of endogenous glucose production by glucose per se seems to be impaired in type 2 diabetes mellitus (14). It is currently unknown, whether paracrine factors also influence basal endogenous glucose production in patients with type 2 diabetes mellitus. Therefore, we evaluated the involvement of adenosine in the regulation of basal glucose production in type 2 diabetes, by measuring endogenous glucose production during intravenous administration of aminophylline, a adenosine receptor antagonist, in a saline controlled study, in 5 patients with type 2 diabetes mellitus.

**Materials and Methods**

**Subjects**

Five patients with type 2 diabetes mellitus were studied. Their clinical characteristics are shown in table 1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>BMI (kg/m²)</th>
<th>Glyc Hb (%)</th>
<th>FPG (mmol/L)</th>
<th>FPI (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>m</td>
<td>65</td>
<td>28.4</td>
<td>7.8</td>
<td>8.8</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>f</td>
<td>54</td>
<td>29.1</td>
<td>8.5</td>
<td>11.0</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>f</td>
<td>67</td>
<td>33.2</td>
<td>7.0</td>
<td>8.3</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>m</td>
<td>64</td>
<td>32.4</td>
<td>7.1</td>
<td>8.5</td>
<td>210</td>
</tr>
<tr>
<td>5</td>
<td>m</td>
<td>69</td>
<td>21.3</td>
<td>5.7</td>
<td>7.6</td>
<td>115</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>3/2</td>
<td>63.8 ± 2.6</td>
<td>28.9 ± 2.1</td>
<td>7.2 ± 0.5</td>
<td>8.8 ± 0.6</td>
<td>115 ± 25</td>
</tr>
</tbody>
</table>

*BMI: body mass index; Glyc Hb: glycosylated hemoglobin; FPG: mean fasting plasma glucose concentration after a 17 hour fast; FPI: mean fasting plasma insulin after a 17 hour fast*
Their mean glycosylated hemoglobin level was 7.2 ± 0.5 %. Except for the presence of type 2 diabetes, they were otherwise healthy and taking no other medication known to affect glucose metabolism. None had been treated with insulin. Oral antidiabetic agents were discontinued 72 hours before the start of the study. All consumed a weight-maintaining diet of at least 250 g carbohydrate for 3 days before the study. Written informed consent was obtained from all the patients. The study was approved by the Institutional Ethics and Isotope Committees.

Study design (figure 1)

Each subject served as his or her own control and completed two study protocols separated by at least 2 weeks. On one occasion, the subjects were studied during intravenous administration of aminophylline and, on the other occasion, during intravenous administration of saline (control experiment). The sequence of both studies was determined by random assignment. The subjects were studied in the post-absorptive state, after a 14-hr fast. A 19-Gauge catheter was inserted in a forearm vein for infusion of [6,6-\(^2\)H\(_2\)]glucose. Another 19-gauge catheter was inserted retrogradely into a wrist vein of the contralateral arm and maintained at 60 °C in a thermoregulated plexiglas box for sampling of arterialized venous blood.

Figure 1: study design

![Blood samples diagram]

- Blood samples over time:
  - 8.00
  - 11.00
  - 12.00
  - 13.00
  - 14.00
  - 15.00
  - 16.00
  - 17.00

- Infusions:
  - 8.00: Aminophylline / NaCl 0.9%
  - 9.00: [6,6-\(^2\)H\(_2\)]glucose
Aminophylline in type 2 diabetes

After obtaining a baseline sample for determination of background isotopic enrichment and plasma glucose concentration, a primed, continuous (0.22 μmol/kg/min) infusion of [6,6-²H₂]glucose (99% Isotec, Miamisburg, OH) dissolved in sterile isotonic saline and sterilised by passage of the solution through a Millipore filter (0.2 μm, Minisart; Sartorius, Gottingen, Germany) was started, and continued throughout the study. The priming dose was increased according to the formula derived by Hother-Nielsen et al. (11): adjusted prime = normal prime (17.6 μmol/kg) x [actual plasma glucose concentration (mmol/l) / 5 (= normal plasma glucose)].

Fasting plasma glucose concentration was measured at the bedside using a Precision Q.I.D.™ glucometer (Medisense®, Abbott Laboratories Company, Chicago, III). After 165 minutes of [6,6-²H₂]glucose infusion, three blood samples were collected at 5 minute intervals for determination of the plasma glucose concentration and [6,6-²H₂]glucose enrichment. Blood samples for measurement of plasma concentrations of insulin, counter-regulatory hormones and cytokines (IL-6 and TNF) were also collected after 175 minutes of isotope infusion.

At time 0, after a three hour equilibration period of [6,6-²H₂]glucose infusion, either aminophylline (Euphyllin, Byk, The Netherlands, priming dose 5.6 mg/kg infused during 20 min, followed by 0.45 mg/kg/min), or isotonic saline was administered intravenously. Blood samples for measurement of plasma glucose concentration, [6,6-²H₂]glucose enrichment, glucoregulatory hormones and cytokines were obtained every 15 minutes for the first two hours after the intervention and every hour thereafter until the end of the study. Blood samples for free fatty acids (FFA) were collected at time 0, 45 min and 6 hours after the intervention.

**Assays**

All measurements were performed in duplicate, and all samples from each individual subject were analysed in the same run. The glucose concentration and [6,6-²H₂]glucose enrichment in plasma were measured by gas chromatography/mass spectrometry using selected ion monitoring. The method was adapted from Reinauer et al, using phenyl-β-D-glucose as internal standard (18).

Plasma insulin concentration was measured by commercial RIA (Pharmacia Diagnostics, Upsala, Sweden), C-peptide by ¹²⁵I radio-immunoassay.
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(Byk Santec, Dietzenbach, Germany), plasma cortisol levels by fluorescence polarisation immunoassay on technical device X (Abbot laboratories, Chicago, Ill), glucagon by RIA (Linco Research Inc., St. Charles, MO); glucagon-antiserum elicited in guinea pigs against pancreatic specific glucagon; cross reactivity with glucagon-like substances of intestinal origin less than 0.1%), and plasma epinephrine and norepinephrine by high performance liquid chromatography with fluorescence detection, using a-methyl norepinephrine as internal standard.

Calculations and statistics

EGP was calculated by the non-steady state equations of Steele (21) in their derivative form, since it has been known that in patients with type 2 diabetes the fasting state is not a steady state (11). The effective distribution volume for glucose was assumed to be 165 ml/kg.

Results are reported as the mean ± SEM. Data were analysed by a two-sided non-parametric test for paired samples (Wilcoxon Signed Rank test). Data within the groups were analysed by ANOVA for randomised block design. A p-value of less than 0.05 was considered to represent a statistical significant difference.

Results

Glucose kinetics (fig 2)

Basal plasma glucose concentrations were significantly different between the two experiments (9.4 ± 0.7 mmol/l and 8.2 ± 0.5 mmol/l, aminophylline vs. control). However, in both the control experiment as well as after administration of aminophylline, the decrease in plasma glucose concentration during the six hour observation period was similar (16% and 18% from basal, ns between both studies).

Basal endogenous glucose production was not significantly different between the two experiments (9.4 ± 0.9 μmol/kg/min and 9.9 ± 1.2 μmol/kg/min, aminophylline resp control (ns)). During the control experiment endogenous glucose production did not change significantly. Within 15 minutes after start of the administration of aminophylline, endogenous glucose production was inhibited transiently to 70% from basal (nadir: 6.6 μmol/kg/min)(p = .02). Subsequently, glucose production rose to a maximum of 11.0 ± 1.4 μmol/kg/min, 45 min after the administration of aminophylline (p = .024 vs. control).
Figure 2: plasma glucose concentration and endogenous glucose production during aminophylline (closed circles) and saline (open circles). *represents a statistical significant difference and change between the groups. Data are expressed as mean ± SEM.

Hormone concentrations (fig 3 and 4)

Baseline values of insulin, C-peptide and counterregulatory hormones were not different between the two studies. In the control experiment plasma insulin and C-peptide concentrations decreased gradually in all patients from 111 ± 26 to 79 ± 21
Figure 3: plasma insulin and C-peptide concentrations during aminophylline (closed circles) and saline (open circles). *represents a statistical significant difference between the groups. Data are expressed as mean ± SEM.

pmol/l (or by 28 %)(p = .01) and from 1310 ± 89 to 884 ± 249 pmol/l (or by 32%) (p = .001).
After administration of aminophylline plasma insulin as well as C-peptide concentrations increased in all patients from 117 ± 24 to a maximum at t= 1.5 hours of 169 ± 31 pmol/l (or by 44%) (p= .008 vs. control) and from 1334 ± 244 to 1648 ± 245 pmol/l (or by 24%)(p= .003). At the end of the aminophylline study, plasma insulin concentration was still significantly higher than in the control experiment (114 ± 22 vs. 79 ± 21 pmol/l) (p= .008 at t = 6 h, aminophylline vs. control). Plasma C-peptide concentration declined more rapidly and was not significantly different from the control experiment at the end of the study (1168 ± 214 vs. 884 ± 244 pmol/l)(p= .06 vs. control).

Figure 4: plasma glucagon, cortisol, adrenalin and noradrenalin concentrations during aminophylline (closed circles) and saline (open circles).*represents a statistical significant difference between the groups. Data are expressed as mean ± SEM.
Basal levels of plasma glucagon, cortisol, adrenaline and noradrenaline were not significantly different between the two studies and no significant differences were observed during both experiments.

Basal levels of free fatty acids (FFA) were not different between the two studies (0.78 ±0.03 vs 0.70 ± 0.06 mmol/l, aminophylline vs control). During the control experiment plasma FFA concentrations did not change significantly (0.78 ± 0.03 to 0.88 ± 0.08 mmol/l) whereas during administration of aminophylline plasma FFA concentrations increased with 33% (to 0.93 ± 0.11 mmol/l, p=.034).

Aminophylline serum concentrations were all in the range of 10 - 20 mg/l at t= 30 min, t= 2 h, as well as at t= 6 h.

**Discussion**

Administration of aminophylline to patients with type 2 diabetes mellitus stimulated insulin secretion, reflected in increased insulin and C-peptide levels. This was associated with a transient decrease in endogenous glucose production of 30% without affecting plasma glucose concentrations. Because aminophylline is an adenosine receptor antagonist, these data indicate that adenosine may inhibit postabsorptive insulin secretion in patients with type 2 diabetes mellitus.

The basal values of glucose production and hormone levels were similar in both experiments. Plasma glucose levels, however, were slightly lower in the control experiment. Nonetheless, this does not affect our conclusion with respect to the effect of aminophylline on insulin secretion. During short-term starvation insulin secretion decreases in patients with type 2 diabetes (7;8) like in healthy subjects in contrast to the stimulatory effects of aminophylline in postabsorptive patients with type 2 diabetes mellitus.

In healthy subjects pentoxifylline, another adenosine receptor antagonist, inhibits glucose production without any effect on glucoregulatory hormones. In patients with type 2 diabetes mellitus the inhibitory effect of aminophylline on basal glucose production is associated with increased insulin levels. This inhibitory effect of aminophylline on endogenous glucose production *in vivo* in humans is different from the stimulatory effect on glucose production found in rats *in vivo*. Aminophylline increased hepatic glucose production as well as insulin secretion in rats (20). This difference between humans and rodents suggest interspecies differences with respect to postabsorptive glucoregulation, which have also been
documented with respect to the glucoregulatory effects of another paracrine mediator like prostaglandines.

Aminophylline appears to have multiple effects and inhibits phosphodiesterase, in addition to blocking adenosine receptors. It is unlikely, however, that the inhibition of endogenous glucose production was due merely to inhibition of phosphodiesterase by aminophylline. For instance, Rizza et al. (19) showed that theophylline stimulated rather than inhibited endogenous glucose production in the presence of glucagon in healthy subjects.

The effect of aminophylline on basal insulin secretion *in vivo* in humans has been studied in three other studies, in healthy subjects. Cathcart-Rake et al, studied 13 healthy subjects during administration of aminophylline with similar plasma aminophylline levels compared to the present study (10-20 μg/ml). They observed small increases in plasma glucose levels without any changes in plasma concentrations of insulin or other glucoregulatory hormones (2). In accordance, Jenkins et al. found no short-term effect of low-dose aminophylline (30 min at an infusion rate of 0.2 mg/kg/min) on glucose or insulin concentrations in four healthy volunteers (13). In contrast, Vestal et al. studied six postabsorptive healthy males during four different infusion rates of aminophylline, reaching theophylline concentrations between 4.5 and 20 μg/ml, respectively. They observed dose-related increases in plasma concentrations of glucose and insulin (23). Endogenous glucose production was not measured in any of those studies. Finally, another adenosine receptor antagonist, pentoxifylline, did not alter plasma insulin concentrations during an observation period of 7 hours in healthy subjects (3;4). Thus, in healthy humans the effects of adenosine-receptor antagonists on basal insulin are inconclusive.

In addition to inhibition of insulin secretion, adenosine also influences insulin-stimulated glucose uptake. A recent study showed, that adenosine potentiates insulin-stimulated glucose transport by enhancing the increase in GLUT4 at the cell surface of rat skeletal muscle, a process that could be blocked by administration of adenosine deaminase (9). In our study, despite a significant increase in insulin concentrations and decrease in the production of glucose, plasma glucose concentration declined at a similar rate during aminophylline administration, as during the control experiment. This is in accordance with the above mentioned study in rats that administration of an adenosine receptor antagonist, like aminophylline, can increase peripheral insulin resistance.
In conclusion, aminophylline stimulates insulin secretion, associated with transient inhibition of endogenous glucose production in patients with type 2 diabetes mellitus. This observation indicates that basal insulin secretion is actively inhibited in patients with type 2 diabetes mellitus by mechanisms that involve factors like adenosine.
Reference List


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