Regulation of postabsorptive glucose production in patients with type 2 diabetes mellitus
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CHAPTER 3

Somatostatin inhibits the stimulatory effect of indomethacin on glucose production in type 2 diabetes mellitus

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Abstract

In patients with type 2 diabetes mellitus, indomethacin stimulates endogenous glucose production and inhibits insulin secretion. To evaluate whether this stimulatory effect on glucose production is solely attributable to inhibition of insulin secretion, indomethacin was administered in a placebo controlled study to 5 patients with type 2 diabetes during continuous infusion of somatostatin, in order to block endogenous insulin and glucagon secretion, and infusion of basal concentrations of insulin and glucagon. Endogenous glucose production was measured 3 hours after the start of the somatostatin, insulin and glucagon infusion, for 4 hours after administration of placebo/indomethacin, by primed, continuous infusion of [6,6-$^2$H$_2$]glucose. At the time of administration of placebo or indomethacin, plasma glucose concentrations and endogenous glucose production rates were not significantly different between the two experiments (16.4 ± 2.09 mmol/l vs 16.6 ± 1.34 mmol/l and 17.7 ± 1.05 micromol/kg/min and 17.0 ± 1.06 micromol/kg/min), control vs indomethacin). In the four hours after administration of indomethacin or placebo plasma glucose concentration did not change significantly. There was no difference in the decrease in endogenous glucose production between both experiments after placebo or indomethacin. Mean plasma C-peptide concentrations were all below the detection limit of the assay as a reflection of adequate suppression of endogenous insulin secretion by somatostatin. Plasma concentrations of insulin (76 ± 5 vs 74 ± 4 pmol/l) and glucagon (69 ± 8 vs 71 ± 6 ng/l) were not different between the studies and remained unchanged in both experiments. Plasma concentrations of cortisol, epinephrine and norepinephrine were not different between the two studies and did not change significantly. We conclude that indomethacin stimulates endogenous glucose production in patients with type 2 diabetes mellitus by inhibition of insulin secretion.

Introduction

In healthy subjects, basal endogenous glucose production is partly regulated by paracrine intrahepatic factors. For instance, administration of indomethacin, a prostaglandin synthesis inhibitor, resulted in a transient stimulation of endogenous glucose production without changes in glucoregulatory hormone concentrations (2). A similar transient stimulatory effect of indomethacin on glucose production was also observed in patients with type 2 diabetes mellitus.
However, in contrast with the absence of an effect of indomethacin on insulin secretion in healthy volunteers, indomethacin inhibited insulin secretion in patients with type 2 diabetes. Since a lowering of portal insulin concentrations stimulate glucose production (11), a possible direct hepatic effect of indomethacin can not be discriminated from the effect on insulin secretion. Therefore, we evaluated, whether the effects of indomethacin on endogenous glucose production in type 2 diabetes were solely attributed to its inhibitory effect on insulin secretion, by measuring endogenous glucose production in a placebo-controlled crossover study, before and after administration of 150 mg indomethacin in patients with type 2 diabetes mellitus. Endogenous glucose production was measured by infusion of [6,6-\textsuperscript{2}H\textsubscript{2}]glucose. The effect of indomethacin on insulin secretion was blocked by infusion of somatostatin to block endogenous secretion of insulin and glucagon, and infusion of exogenous insulin and glucagon to maintain plasma concentrations at basal levels.

**Subjects and methods**

**Subjects**

Five patients with type 2 diabetes mellitus were studied. Their clinical characteristics are shown in table 1. Their mean glycosylated hemoglobin level was 7.7% (range 6.5-8.6%), and except for the presence type 2 diabetes, they were otherwise healthy and were taking no other medication known to affect glucose metabolism. None had been treated with insulin. Oral antidiabetics 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 studies were approved by the Institutional Ethics and Isotope Committees.
Table 1: clinical characteristics

<table>
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<th>patient</th>
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<th>age (yr)</th>
<th>BMI (kg/m²)</th>
<th>Glyc Hb (%)</th>
<th>FPG (mmol/L)</th>
</tr>
</thead>
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<tr>
<td>2</td>
<td>m</td>
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<td>28.0</td>
<td>7.1</td>
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<tr>
<td>3</td>
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<td>27.4</td>
<td>8.3</td>
<td>14.3</td>
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<tr>
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<td>f</td>
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<td>26.0</td>
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<tr>
<td>5</td>
<td>m</td>
<td>45</td>
<td>23.8</td>
<td>8.6</td>
<td>20.0</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>4 / 1</td>
<td>62 ± 0.6</td>
<td>26.5 ± 0.7</td>
<td>7.7 ± 0.4</td>
<td>13.4 ± 1.8</td>
</tr>
</tbody>
</table>

BMI: body mass index; Glyc Hb: glycosylated hemoglobin; FPG: mean fasting plasma glucose concentration on both occasions at 8.00 a.m. after a 14 hour fast.

Study design (figure 1)

Each subject served as his or her own control and completed two study protocols separated by 2 to 3 weeks. On one occasion, the subjects were studied after taking indomethacin 150 mg orally and on the other occasion after taking placebo (control experiment). The sequence of both studies was determined by random assignment. The subjects were studied in the postabsorptive state, after a 14-hr fast. A 19-Gauge catheter was inserted in a forearm vein for infusion of [6,6-²H₂]glucose, somatostatin, insulin, and glucagon. Another 19-gauge catheter was inserted retrogradely into a wrist vein of the contralateral arm and maintained at 60 °C in a thermoregulated plexiglass box for sampling of arterialized venous blood.

At 8.00 a.m., 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 sterilized 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. (4): adjusted prime = normal prime (17.6 μmol/kg) x [actual plasma glucose concentration (mmol/L) / 5 (= normal plasma glucose)].

At the same time, a continuous infusion of somatostatin (250 μmol/h, UCB Pharma, Breda, the Netherlands), insulin (0.15 mU/kg/min⁻¹ (Actrapid, Novo Nordisk A/S Bagsvaerd, Denmark), and glucagon (0.75 ng/kg/min⁻¹, GlucaGen, Novo Nordisk A/S Bagsvaerd, Denmark) was started, which were also continued throughout the study. This infusion was prepared with 200 mg/ml human albumin diluted in saline. Fasting plasma glucose concentration at the bedside was measured using a glucose analyser (Beckman Instruments, CA) every 20 min during the first 3 hours of the study (the equilibration period), and every 15 minutes thereafter until the end of the study. After 170 minutes of [6,6-²H₂]glucose, somatostatin, insulin and glucagon 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, glucagon, cortisol and catecholamines were also collected after 175 minutes.

At 11.00 a.m. (time = 0), after a three hour equilibration period of infusion of [6,6-$^2$H$_2$]glucose, somatostatin, insulin and glucagon infusion, either 150 mg of indomethacin or placebo was administered orally. Blood samples for measurement of plasma glucose concentration, [6,6-$^2$H$_2$]glucose enrichment, C-peptide, and glucoregulatory hormones were obtained every 15 minutes for the first three hours after the intervention and every 30 minutes for the last hour. Hourly blood samples were collected for free fatty acids (FFA) at time 0, 1, 2, 3 and 4 hours after the intervention. Blood samples for measurement of plasma concentrations of indomethacin were obtained at time 0, and 30 and 60 minutes after administration of indomethacin/placebo.

Assays

All measurements were performed in duplicate, and all samples from each individual subject were analyzed in the same run. The glucose concentration and [6,6-$^2$H$_2$]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 (8).

Plasma insulin concentration was measured by commercial RIA (Pharmacia Diagnostics, Upsala, Sweden), C-peptide by $^{125}$I radio-immunoassay (Byk Santec, Dietzenbach, Germany), plasma cortisol levels by fluorescence polarization 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 (12) in their derivative form, since it has been known that in patients with type 2 diabetes the fasting state is not a steady state (4). The effective distribution volume for
glucose was assumed to be 165 mL/kg. Results are reported as the mean ± SEM. Data were analyzed by a two-sided non-parametric test for paired samples (Wilcoxon Signed Rank test). Data within the groups were analyzed by ANOVA for randomized block design. A p-value of less than 0.05 was considered to represent a statistical significant difference.

Results

Plasma glucose concentration and endogenous glucose production (fig 2)

Before the start of the hormone infusions, (8:00 am) plasma concentrations of glucose were not significantly different between the two experiments (13.13 ± 1.78 mmol/l and 13.61 ± 1.82 mmol/l, control vs indomethacin). At 11.00 am (t = 0), 3 hours after the start of the somatostatin, insulin and glucagon infusion, plasma glucose concentrations were not different between the two experiments (16.40 ± 1.62 mmol/l and 16.60 ± 1.34 mmol/l, control vs indomethacin). In the subsequent four hours after administration of indomethacin/placebo, plasma glucose concentration did not change significantly.

The rates of endogenous glucose production at t = 0 were not significantly different between the two experiments (17.7 ± 1.05 mmol/l and 17.0 ± 1.06 mmol/l, control vs indomethacin). In the subsequent four hours after administration of indomethacin/placebo a gradual decrease in the rates of glucose production occurred in both experiments by 18% vs 27%, respectively (ns, control vs indomethacin). At the end of the experiments, the rates of endogenous glucose production were not significantly different between the two studies.

Plasma hormone concentrations (fig 3)

At 8.00 a.m. plasma concentrations of insulin, C-peptide and glucagon were not different between the two experiments. At t = 0 hours (11.00 a.m.), 3 hours after the start of the combined infusion of somatostatin, insulin and glucagon, plasma insulin and glucagon concentrations were 76 ± 5 vs 74 ± 4 pmol/l and 69 ± 8 vs 71 ± 6 ng/l, respectively (ns, control vs indomethacin), and were, except of time points 12.30 and 14.00 hours, not significantly different between both experiments.
Figure 2: mean plasma glucose concentration and endogenous glucose production four hours after administration of indomethacin (closed circles) vs placebo (open circles)

All plasma C-peptide concentrations were below the detection limit of our assay (<100 pmol/l) at t=0, and remained suppressed until the end in both experiments.
Figure 3: mean plasma insulin and glucagon concentration four hours after administration of indomethacin (closed circles) vs placebo (open circles)

At t=0, plasma concentrations of cortisol (358 ± 89 and 390 ± 101 nmol/l), epinephrine (0.11 ± 0.04 and 0.05 ± 0.02 nmol/l) and norepinephrine (1.09 ± 0.10 and 1.58 ± 0.14) were not significantly different between the two experiments.
Indomethacin concentrations

Serum indomethacin concentrations were detectable 30 minutes after ingestion and were all within the therapeutic range 60 minutes after the oral administration of indomethacin (mean 1.25 mg/l; therapeutic range 0.8 – 2.5 mg/l).

Discussion

Administration of the prostaglandin synthesis inhibitor indomethacin to patients with type 2 diabetes mellitus, in the presence of stable insulin concentrations during somatostatin and insulin infusion, did not influence endogenous glucose production.

Paracrine factors influence basal glucose production in healthy subjects, since administration of indomethacin stimulates endogenous glucose production by ~50% from basal in healthy subjects without any changes in glucoregulatory hormone concentrations (2). Subsequently, a disturbance in the paracrine regulation of basal endogenous glucose production was explored as a possible mechanism for increased glucose production in type 2 diabetes mellitus. Administration of indomethacin induced the same increase in glucose production in type 2 diabetics as in healthy subjects, but this increase in glucose production coincided with inhibition of insulin secretion (7). If prostaglandins are representative for this modulatory action of mediators produced by the Kupffer cells, it must be concluded that overproduction of mediators in this paracrine system is not the pathophysiological mechanism behind the increased glucose production in type 2 diabetes mellitus, since there were no effects of indomethacin in the presence of stable insulin concentrations. Therefore, the effect of indomethacin on glucose production in type 2 diabetes mellitus observed in our previous study must have been caused by decreased insulin secretion.

Theoretically, indomethacin can affect insulin secretion in three ways: first, by inhibiting prostaglandin synthesis. Under physiological conditions the beta-cell tonically synthesizes prostaglandin E2, a process known to be stimulated by glucose (13). On the other hand, prostaglandin E2 inhibits glucose-induced insulin secretion (10). Thus, it appears unlikely, that the effect of indomethacin on beta cells is due to inhibition of prostaglandin synthesis, because that would result in stimulation rather than inhibition of insulin secretion. Second, indomethacin can inhibit insulin secretion by stimulation of cytokine production. In healthy humans indomethacin is a potent stimulator of interleukin (IL)-1-beta, both in vitro as well
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as in vivo (3). IL-1 beta stimulates the generation of the inducible form of cyclooxygenase (COX-2), the enzyme responsible for generation of prostaglandin E2 from arachidonic acid (9), either directly by increasing gene expression of COX-2 mRNA, or indirectly through production of nitric oxide (NO)(6). Thus, stimulation of IL-1 by indomethacin can result, through stimulation of COX-2, in inhibition of insulin secretion. The third possibility is by affecting the signal-transduction pathways of the insulin receptor itself. For instance, mice with tissue-specific knockout of the insulin receptor in beta-cells, but not elsewhere in the body, develop insulin secretory defects similar to those in type 2 diabetes (5). A functional insulin receptor on beta-cells thus is a prerequisite for a normal glucose-stimulated insulin secretion. Apparently, insulin stimulates its own release by a positive feedback loop through binding to its own receptor in the beta cell. Interestingly, indomethacin can inhibit autophosphorylation of the beta subunit of the insulin receptor (1). Therefore, impairment of the function of the insulin receptor by indomethacin by inhibiting autophosphorylation of the beta subunit could lead to inhibition of insulin secretion.

In the present study basal endogenous glucose production was ~45% higher than in the previous study without somatostatin. After administration of indomethacin without somatostatin endogenous glucose production increased from 12.0 μmol.kg.min⁻¹ to a maximum value of 17.8 μmol.kg.min⁻¹ (7), whereas basal endogenous glucose production in our experiments with somatostatin was 16 μmol.kg.min⁻¹, and insulin and glucagon concentrations were comparable. The patients in the somatostatin study thus seem to be more insulin resistant. If the modulatory role of paracrine factors is limited to situations where endogenous glucose production is only mildly deranged, no effect of indomethacin could be expected in the somatostatin study, however, this consideration does not affect our conclusion with respect to the inhibitory effects of indomethacin on insulin secretion in type 2 diabetes.

In conclusion, indomethacin deranges basal endogenous glucose production in patients with type 2 diabetes by inhibition of insulin secretion. From a clinical perspective, the use of indomethacin should be discouraged in patients with type 2 diabetes mellitus.
Reference List


