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

Indomethacin decreases insulin secretion in patients with type 2 diabetes mellitus

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Abstract

In healthy subjects, basal endogenous glucose production is partly regulated by paracrine intrahepatic factors. Administration of indomethacin, a prostaglandin synthesis inhibitor, resulted in a transient stimulation of endogenous glucose production without changes in glucoregulatory hormone concentrations. It is unknown whether similar paracrine factors influence basal endogenous glucose production in type 2 diabetes mellitus. The effects of 150 mg indomethacin, a non-endocrine stimulator of glucose production in healthy adults, and placebo, on endogenous glucose production were measured in a randomized placebo controlled study in patients with type 2 diabetes mellitus (3 men and 3 women, mean age 58.5 yrs and mean BMI 28.6 kg.m\(^2\)). Endogenous glucose production was measured before and during 6 hours after administration of placebo/indomethacin, by primed, continuous infusion of [6,6-\(^2\)H\(_2\)]glucose. After indomethacin, plasma glucose concentration and endogenous glucose production increased in all subjects by 14\% (p<0.05) and 48\% (p<0.05), respectively. In the control experiment, plasma glucose concentration and endogenous glucose production declined gradually in all subjects by 22\% (p<0.001) and 17\% (p=0.004), respectively. The stimulation of glucose production coincided with inhibition of insulin secretion by 52\% within one hour after administration of indomethacin (p<0.001). In the control experiment insulin secretion decreased gradually by 18\% after six hours (p<0.001). Thus, indomethacin inhibits insulin secretion and stimulates endogenous glucose production in type 2 diabetes.

Introduction

In type 2 diabetes mellitus hyperglycemia is attributed to both increased endogenous glucose production (EGP) and impaired glucose uptake (GU) by peripheral tissues (1;7). There is a close correlation between the degree of elevation of EGP and the severity of fasting hyperglycemia in type 2 diabetes mellitus (10;13). The impairment of adequate suppression of EGP in view of the present hyperglycemia and hyperinsulinemia is associated with increased gluconeogenesis (GNG) by enhanced delivery of gluconeogenic substrates and increased efficiency of intrahepatic substrate conversion (4). In addition, regulation of EGP by glucose per se seems to be impaired in type 2 diabetes mellitus (19). In healthy adults, there are indications that besides regulation of glucose production by the classic hormones, other, probably intrahepatic, mechanisms must be operative in maintaining basal endogenous glucose production, a process frequently referred to
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Potential mediators of this process are Kupffer cell products. In the liver, there is intensive interaction between Kupffer cells and hepatocytes, and in vitro animal data suggest that products of these Kupffer cells influence glucose production by hepatocytes. For instance, stimulated Kupffer cells produce prostaglandins (6), cytokines (6;17), and nitric oxide (NO) (2;17), and all these mediators can affect glucose production (2;12). Indomethacin influences the secretion of all these mediators: prostaglandins, cytokines, as well as NO. Administration of indomethacin in our previous study stimulated EGP in healthy adults without any influences in the plasma levels of glucoregulatory hormones, insulin as well as C-peptide (5). These data suggest that intrahepatic produced paracrine mechanisms could influence EGP. The influence of these paracrine factors on EGP was further confirmed in patients with uncomplicated falciparum malaria, in which the already increased basal EGP could be increased even more by indomethacin without any change in plasma glucoregulatory hormones or circulating cytokines (8). This lead us to conclude that in healthy adults as well as in patients with certain infectious diseases, basal EGP is not maximally stimulated, but is partially inhibited, possibly by paracrine factors like prostaglandins, cytokines and/or NO. It is currently unknown if these paracrine factors also influence basal EGP in other conditions with increased EGP like type 2 diabetes mellitus and if so, if dysregulation of paracrine regulation is an important co-factor in maintaining increased EGP in type 2 diabetes mellitus.

To evaluate the effects of indomethacin on EGP in type 2 diabetes mellitus, we measured endogenous glucose production in a placebo controlled crossover study by infusion of [6,6-²H₂]glucose before and after administration of 150 mg indomethacin in patients with type 2 diabetes mellitus.

Subjects and Methods

Subjects

Six patients with type 2 diabetes mellitus were studied. Their clinical characteristics are shown in table 1. Their mean glycosylated hemoglobin level was 8.5% (range 7.0-10.5%), 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 of the 6 patients with type 2 diabetes mellitus

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FPG, FPI, FPC-pept: mean fasting plasma glucose, insulin and C-peptide concentrations at the start of the two experiments (indomethacin vs placebo) after a 17 hour fast

Study design (figure 1)

Each subject served as his or her own control and completed two study protocols separated by at least 8 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 \([6,6-^{2}\text{H}_2]\)glucose. Another 19-gauge catheter was inserted retrogradely into a wrist vein of the contralateral arm and maintained at 60 °C in a thermoregulated plexiglass box for sampling of arterialized venous blood.

After obtaining a baseline sample for determination of background isotopic enrichment and plasma glucose concentration, a primed, continuous (0.22 \(\mu\) mol/kg/min) infusion of \([6,6-^{2}\text{H}_2]\)glucose (99% Isotec, Miamisburg, OH) dissolved in sterile isotonic saline and sterilized by passage of the solution through a millipore filter (0.2 \(\mu\)m, 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 (13): 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, Ill). 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, counterregulatory hormones and cytokines (IL-6 and TNF) were also collected after 175 minutes.

At time 0, after a three hour equilibration period of [6,6-2H₂]glucose infusion, either 150 mg of indomethacin or placebo was administered. 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 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 (21).

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 immunooassay on technical device X (Abbot laboratories, Chicago, Ill), Growth hormone by chemiluminescence immunometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA), 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 α-methyl norepinephrine as internal standard.

Cytokine assays. TNF concentrations were measured by an enzyme-amplified sensitivity immunooassay (EASIA; Medgenix, Amersfoort, the Netherlands) with a detection limit of 5 pg/mL. Plasma concentrations of IL-6 were measured by an enzyme-linked immunosorbent assay (CLB, Amsterdam, the Netherlands), with a detection limit of 2 pg/mL.

Calculations and statistics

EGP was calculated by the non-steady state equations of Steele (27) in their derivative form, since it has been known that in patients with Type 2 Diabetes the fasting state is not a steady state (13). 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, and by Fisher’s least-significant difference test for multiple comparisons when indicated. A p-value of less than 0.05 was considered to represent a statistical significant difference.
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Results

Plasma glucose concentration and endogenous glucose production (fig 2)

Mean baseline plasma concentrations of glucose were not significantly different between the two experiments (10.3 ± 1.6 mmol/L and 11.2 ± 1.7 mmol/L, control vs indomethacin).

In the control experiment, plasma glucose concentration and endogenous glucose production decreased *gradually* in all subjects, by 22% (p<0.001) and 17% (p<0.004), respectively, during the 6 hour observation period.

After administration of indomethacin, plasma glucose concentration and endogenous glucose production increased *transiently* in all subjects. Plasma glucose concentration increased from 11.2 ± 1.7 to a maximum of 12.8 ± 1.7 mmol/L (or by 14%) (p<0.05 vs control). Glucose production increased from 12.0 ± 1.7 to a maximum of 17.8 ± 1.9 μmol/kg/min (or by 48%) (p<0.05 vs control).

Hormone and cytokine concentrations (fig 3 and 4)

Baseline values of insulin, C-peptide and counterregulatory hormones were not different between the two studies (figure 2 and 3). In the control experiment plasma insulin and C-peptide concentrations decreased *gradually* in all patients from 88 ± 15 to 72 ± 17 pmol/L (or by 18%) (p<0.001) and from 952 ± 134 to 720 ± 88 pmol/L (or by 22%) (p<0.001).

After administration of indomethacin plasma insulin and C-peptide concentrations decreased *transiently* in all subjects from 78 ± 11 to a nadir of 38 ± 5 pmol/L (or by 52%) at t=1.75 hours (p<0.05 vs control) and from 992 ± 120 to a nadir of 497 ± 75 at t = 1.5 hours (p<0.05).

Basal levels of plasma glucagon, cortisol, adrenaline and noradrenaline levels were not significantly different between the two studies and remained similar throughout the study. Basal levels of growth hormone were not different between the two studies, but a statistical significant rise in growth hormone levels was noticed 2 and 3 hours after administration of indomethacin, reaching basal levels again at 4 hours.
Figure 2: plasma glucose concentration and endogenous glucose production (EGP) after administration of indomethacin (closed circles) or placebo (open circles). The X axis represents time (hours) * represents a statistical significant difference between the groups (p<0.05)
Figure 3: Plasma insulin, C-peptide, and glucagon concentrations after administration of indomethacin (closed circles) or placebo (open circles). The x-axes represents time (hours); * represents a statistical significant difference between the groups ($p<0.05$).
Basal plasma levels of free fatty acids (FFA) were elevated but not statistically different between the two studies. At the end of the study plasma levels of FFA were lower in the indomethacin experiment (p<0.05). Basal levels of TNF were below the detection limit of the assay during both experiments and remained unchanged (separate data not shown). Basal levels IL-6 were not elevated and not statistically different between the two studies. The plasma levels did not change significantly during both experiments.

Figure 4: plasma cortisol, growth hormone (gh), adrenalin, noradrenalin and interleukin-6 (IL-6) concentrations after administration of indomethacin (closed circles) or placebo (open circles).
Discussion

Administration of the prostaglandin synthesis inhibitor indomethacin to patients with type 2 diabetes mellitus resulted in inhibition of insulin secretion, reflected in decreased insulin and C-peptide levels. This was accompanied by a transient increase of 48% in glucose production and an increase in plasma glucose concentrations. This inhibitory effect of indomethacin on insulin secretion and associated stimulation of endogenous glucose production occurred without any changes in counterregulatory hormone, except growth hormone, or cytokine concentrations.

Indomethacin increased endogenous glucose production to a similar extent in patients with type 2 diabetes compared to the effects in healthy volunteers (~6 μmol/kg/min vs 5-7 μmol/kg/min from basal) (5) (8). Nonetheless, the increase in plasma glucose concentration was much higher in the diabetics (3.5 mmol/L vs 1.5-2 mmol/L from basal). The combination of the same increase in endogenous glucose production and a difference in the change in glucose concentration must be due to a decrease in glucose clearance. A good explanation for this difference between healthy volunteers and patients with type 2 diabetes is the finding that insulin secretion was significantly reduced by indomethacin in type 2 diabetes but not in healthy volunteers. A statistical significant rise in growth hormone levels was measured 2 and 3 h after administration of indomethacin. Growth hormone itself can stimulate endogenous glucose production (15) but it is unlikely that endogenous glucose production was driven by growth hormone or vice versa. The changes in endogenous glucose production and insulin concentrations occurred within 45 minutes after administration of indomethacin, whereas plasma growth hormone concentrations started to rise more than 90 minutes after administration of indomethacin. Moreover, if growth hormone was driven by the rise in endogenous glucose production, an inhibition rather than a stimulation of growth hormone secretion would be expected (29). At the end of the indomethacin experiments FFA concentrations were somewhat lower than in the control experiments. This can be due to the rebound in insulin concentration after initial inhibition.

In our study the increase in glucose concentration and glucose production coincided with the decrease in peripheral C-peptide levels and insulin concentrations. Sindelar et al, recently published data on the relationship of portal vein insulin concentration and basal hepatic glucose production in overnight fasted conscious dogs. Within 15 minutes, after a selective fall of portal insulin concentration from 150 to 30 pmol/L, basal hepatic glucose production increased to
a maximum of 22 μmol/kg/min above basal, and after 3 hours hepatic glucose production was still significantly increased by 6 μmol/kg/min above basal (26). Therefore, unlike in healthy volunteers, in type 2 diabetes the observed stimulatory effect of indomethacin on endogenous glucose production is likely to be the result of inhibition of pancreatic insulin secretion.

The effect of a single oral dose of indomethacin on basal insulin levels in humans has been investigated in only four studies to our knowledge (5;8;16;28). In three of these four, basal insulin levels remained unaffected, whereas in the fourth a small, but significant fall from 9.5 to 6.4 μU/ml was observed 1 hour after 50 mg of indomethacin vs 8.0 to 6.9 μU/ml after placebo (28).

The effect of indomethacin on glucose-induced acute insulin secretion is different from that under basal circumstances. All studies, but one (28), in humans showing an inhibitory effect of indomethacin on insulin secretion (for review, see ref (22)) were done in experimental settings involving glucose infusions. Therefore, the effect of indomethacin on peripheral insulin levels differs depending on basal versus glucose-stimulated conditions. In our type 2 diabetic patients insulin secretion was stimulated, as can be deducted from the two- to threefold increase in basal insulin levels compared to normal values (5). It can thus be postulated that the effect of indomethacin under conditions were insulin secretion is stimulated chronically, like in the present study, resembles the situation of acute glucose stimulated insulin secretion. Indomethacin has no effect on insulin secretion under basal conditions, as is reflected by our experiments in healthy humans (5).

Although indomethacin is a prostaglandin synthesis inhibitor, it is unlikely however that the effect of indomethacin on the beta cell is due to inhibition of prostaglandin synthesis. Prostaglandin E2, synthesized by the pancreatic islet, inhibits glucose-induced insulin secretion (23). Thus inhibition of prostaglandin synthesis would result in stimulation rather than inhibition of insulin secretion. However, besides inhibition of prostaglandin synthesis indomethacin also stimulates cytokine production. In healthy humans indomethacin is a potent stimulator of interleukin (IL)-1-beta, both in vitro as well as in vivo (9). 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. The effect of IL-1 can be either directly by increasing gene expression of COX-2 mRNA, or indirectly through production of nitric oxide (NO) (18). Thus, stimulation of IL-1 by indomethacin could result in inhibition of insulin secretion,
through stimulation of COX-2. A similar IL-1 mediated effect by indomethacin can stimulate growth hormone release (24;25), through stimulation of growth hormone releasing hormone (GHRH) by IL-1 (11). Growth hormone secretion can thus be stimulated directly by indomethacin, independently of endogenous glucose production.

Another possibility for inhibition of insulin secretion by indomethacin is its ability to affect the insulin receptor itself, by inhibiting autophosphorylation of the beta subunit of the insulin receptor (3). Very recent publications indicate that that a functional insulin receptor is a prerequisite for a normal glucose-stimulated insulin secretion (14). Insulin stimulates its own release by a positive feedback loop through binding to its own receptor in the beta cell. Impairment of the function of the insulin receptor by indomethacin by inhibiting autophosphorylation of the beta subunit could lead to inhibition of insulin secretion. The dose of 150 mg of indomethacin used in this study is equivalent to the daily therapeutic recommended dose as antiflogistic or anti inflammatory agent. Our data suggest that this dose can influence glucoregulation in patients with type 2 diabetes mellitus.

In conclusion, in patients with type 2 diabetes mellitus, indomethacin blocks insulin secretion and stimulates endogenous glucose production.

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