Indomethacin Decreases Insulin Secretion in Patients With Type 2 Diabetes Mellitus

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In healthy subjects, basal endogenous glucose production (EGP) is partly regulated by paracrine intrahepatic factors. Administration of indomethacin, an inhibitor of prostaglandin synthesis, resulted in a transient stimulation of EGP without changes in glucoregulatory hormone concentrations. It is unknown whether similar paracrine factors influence basal EGP in type 2 diabetes mellitus. The effects of 150 mg indomethacin, a nonendocrine stimulator of glucose production in healthy adults, and placebo on EGP were measured in a randomized placebo-controlled study in patients with type 2 diabetes mellitus (3 men and 3 women; mean age, 58.5 years; mean body mass index, 28.6 kg · m^{-2}). EGP was measured before and for 6 hours after administration of placebo/indomethacin, by a primed, continuous infusion of [6,6-$^2H_2$]glucose. After indomethacin, plasma glucose and EGP increased in all subjects by 14% ($P < .05$) and 48% ($P < .05$), respectively. In the control experiment, plasma glucose and EGP declined gradually in all subjects by 22% ($P < .001$) and 17% ($P = .004$), respectively. The stimulation of glucose production coincided with the inhibition of insulin secretion by 52% within 1 hour after administration of indomethacin ($P < .001$). In the control experiment, insulin secretion decreased gradually by 18% after 6 hours ($P < .001$). Thus, indomethacin inhibits insulin secretion and stimulates EGP in type 2 diabetes.

IN TYPE 2 DIABETES MELLITUS, hyperglycemia is attributed to both increased endogenous glucose production (EGP) and impaired glucose uptake by peripheral tissues.[1,2] There is a close correlation between the degree of elevation of EGP and the severity of fasting hyperglycemia in type 2 diabetes mellitus.[3,4] The impairment of adequate suppression of EGP in view of the present hyperglycemia and hyperinsulinemia is associated with increased gluconeogenesis by enhanced delivery of gluconeogenetic substrates and increased efficiency of intrahepatic substrate conversion.[5] In addition, regulation of EGP by glucose per se seems to be impaired in type 2 diabetes mellitus.[6]

In healthy adults, there are indications that besides the regulation of glucose production by the classic hormones, other, probably intrahepatic mechanisms must be operative in maintaining basal EGP, a process frequently referred to as autoregulation.[7] 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,[8] cytokines,[8,9] and nitric oxide (NO),[9,10] and all of these mediators can affect glucose production.[10,11] Indomethacin influences the secretion of all of these mediators. Administration of indomethacin in our previous study stimulated EGP in healthy adults without any influence on the plasma level of glucoregulatory hormones, insulin as well as C-peptide.[12] These data suggest that intrahepatically 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 elevated basal EGP could be increased even more by indomethacin without any change in plasma glucoregulatory hormones or circulating cytokines.[13] This led us to conclude that in healthy adults, as well as 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 such as type 2 diabetes mellitus and, if so, if paracrine dysregulation is an important cofactor in maintaining increased EGP in type 2 diabetes mellitus.

To evaluate the effects of indomethacin on EGP in type 2 diabetes mellitus, we measured EGP in a placebo-controlled crossover study by infusion of [6,6-$^2H_2$]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. The mean glycosylated hemoglobin level was 8.5% (range, 7.0% to 10.5%), and except for the presence of type 2 diabetes, they were otherwise healthy and using 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 subjects consumed a weight-maintaining diet of at least 250 g carbohydrate for 3 days before the study. Written informed consent was obtained from all patients, and the studies were approved by the Institutional Ethics and Isotope Committees.

Study Design

Each subject served as his or her own control and completed 2 study protocols separated by at least 8 weeks (Fig 1). On one occasion, the subjects were studied after administration of indomethacin 150 mg orally, and on the other occasion after placebo (control experiment). The

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Table 1. Clinical Characteristics of Six Patients With Type 2 Diabetes Mellitus

Patient No.	Sex/Age (yr)	BMI ($kg \cdot m^{-2}$)	FPG (mmol/L)	FPI (pmol/L)	FPC-pept (pmol/L)
1	F/51	34.7	7.9	105	1,122
2	M/65	28.4	10.0	80	835
3	F/54	29.1	12.5	100	1,050
4	F/67	33.2	8.7	70	1,320
5	M/37	24.7	12.6	50	600
6	M/57	21.7	17.8	65	655

Abbreviations: M, male; F, female; BMI, body mass index; FPG, FPI, and FPC-pept, mean fasting plasma glucose, insulin, and C-peptide at the start of the 2 experiments (indomethacin *v* placebo) after a 17-hour fast.

sequence of the studies was determined by random assignment. Subjects were studied in the postabsorptive state after a 14-hour fast. A 19-gauge catheter was inserted into a forearm vein for infusion of [6,6-$^2H_2$]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 (Rohm & Haas, Philadelphia, PA) box for sampling of arterialized venous blood.

After obtaining a baseline sample for determination of the background isotopic enrichment and plasma glucose concentration, a primed, continuous (0.22 μmol/kg/min) infusion of [6,6-$^2H_2$]glucose (99%; Isotec, Miamisburg, OH) dissolved in sterile isotonic saline and sterilized by passage of the solution through a membrane 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 and Beck-Nielsen[4]: adjusted prime = normal prime (17.6 μmol/kg) × [actual plasma glucose (mmol/L)/5 (= normal plasma glucose)].

The fasting plasma glucose concentration was measured at the bedside using a Precision Q.I.D. glucometer (Medisense; Abbott Laboratories, Chicago, IL). After 165 minutes of [6,6-$^2H_2$]glucose infusion, 3 blood samples were collected at 5-minute intervals for determination of the plasma glucose concentration and [6,6-$^2H_2$]glucose enrichment. Blood samples for measurement of plasma insulin, counterregulatory hormones, and cytokines (interleukin-6 [IL-6] and tumor necrosis factor [TNF]) were also collected after 175 minutes.

At time 0, after a 3-hour equilibration period of [6,6-$^2H_2$]glucose infusion, 150 mg indomethacin or placebo was administered. Blood samples for measurement of plasma glucose, [6,6-$^2H_2$]glucose enrichment, glucoregulatory hormones, and cytokines were obtained every 15 minutes for the first 2 hours after the intervention and every hour thereafter until the end of the study. Blood samples for free fatty acids (FFAs) were collected at time 0 and 45 minutes and 6 hours after the intervention.

Assays

All measurements were performed in duplicate, and all samples from each individual were analyzed in the same run. The glucose concentration and [6,6-$^2H_2$]glucose enrichment in plasma were measured by gas chromatography/mass spectrometry using selected in monitoring. The method was adapted from Reinauer et al[14] using phenyl-β-D-glucose as an internal standard.

The plasma insulin concentration was measured by commercial radioimmunoassay ([RIA] Pharmacia Diagnostics, Uppsala, Sweden). C-peptide was determined by ^{125}I-RIA (Byk Santec, Dietzenbach, Germany) and plasma cortisol by fluorescence polarization immunoassay on technical device × (Abbott Laboratories, Chicago, IL). Growth hormone was determined by chemiluminescence immunometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA), glucagon by RIA (Linco Research, St. Charles, MO; Glucagon antiserum elicited in guinea pigs against pancreatic-specific glucagon, cross-reactivity with glucagon-like substances of intestinal origin <0.1%), and plasma epinephrine and norepinephrine by high-performance liquid chromatography with fluorescence detection using α-methylnorepinephrine as internal standard.

Cytokine assays. TNF concentrations were measured by an enzyme-amplified sensitivity immunoassay (Medgenix, Amersfoort, The Netherlands) with a detection limit of 5 pg/mL. Plasma IL-6 concentrations 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[15] in their derivative form, since it is known that the fasting state is not a steady state in patients with type 2 diabetes.[4] The effective distribution volume for glucose was assumed to be 165 mL/kg. The results are reported as the mean ± SEM. The data were analyzed by a 2-sided nonparametric 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 less than .05 was considered to represent a statistically significant difference.

RESULTS

Plasma Glucose and EGP

Mean baseline plasma glucose concentrations were not significantly different between the experiments (10.3 ± 1.6 and 11.2 ± 1.7 mmol/L, control *v* indomethacin). In the control experiment, plasma glucose and EGP decreased gradually in all subjects by 22% ($P < .001$) and 17% ($P < .004$), respectively, during the 6-hour observation period. After administration of indomethacin, plasma glucose and EGP increased transiently in all subjects. Plasma glucose increased from 11.2 ± 1.7 mmol/L

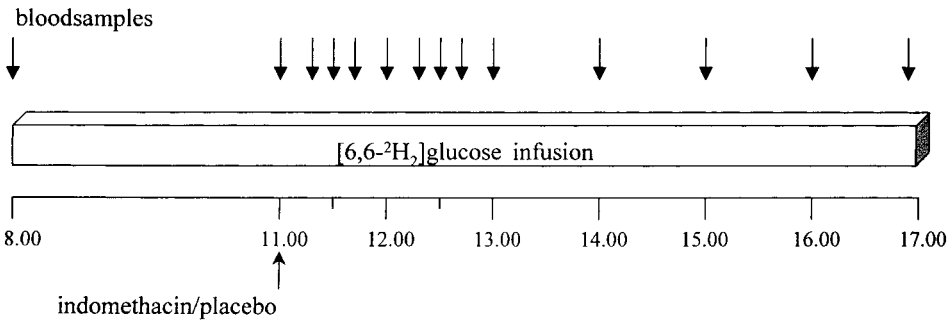

Fig 1. Study design.

to a maximum of 12.8 ± 1.7 mmol/L (or 14%, $P < .05$ *v* control). EGP increased from 12.0 ± 1.7 μmol/kg/min to a maximum of 17.8 ± 1.9 μmol/kg/min (or 48%, $P < .05$ *v* control) (Fig 2).

Hormones and Cytokines

Baseline values for insulin, C-peptide, and counterregulatory hormones were not different between the two studies (Figs 2 and 3). In the control experiment, plasma insulin and C-peptide decreased gradually in all patients from 88 ± 15 to 72 ± 17 pmol/L (or 18%, $P < .001$) and from 952 ± 134 to 720 ± 88 pmol/L (or 22%, $P < .001$). After administration of indomethacin, plasma insulin and C-peptide decreased transiently in all subjects from 78 ± 11 pmol/L to a nadir of 38 ± 5 pmol/L (or 52%) at $t = 1.75$ hours ($P < .05$ *v* control) and from 992 ± 120 pmol/L to a nadir of 497 ± 75 pmol/L at $t = 1.5$ hours ($P < .05$).

Basal 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 statistically significant increase in growth hormone was noted 2 and 3 hours after administration of indomethacin, reaching basal levels again at 4 hours (Fig 4).

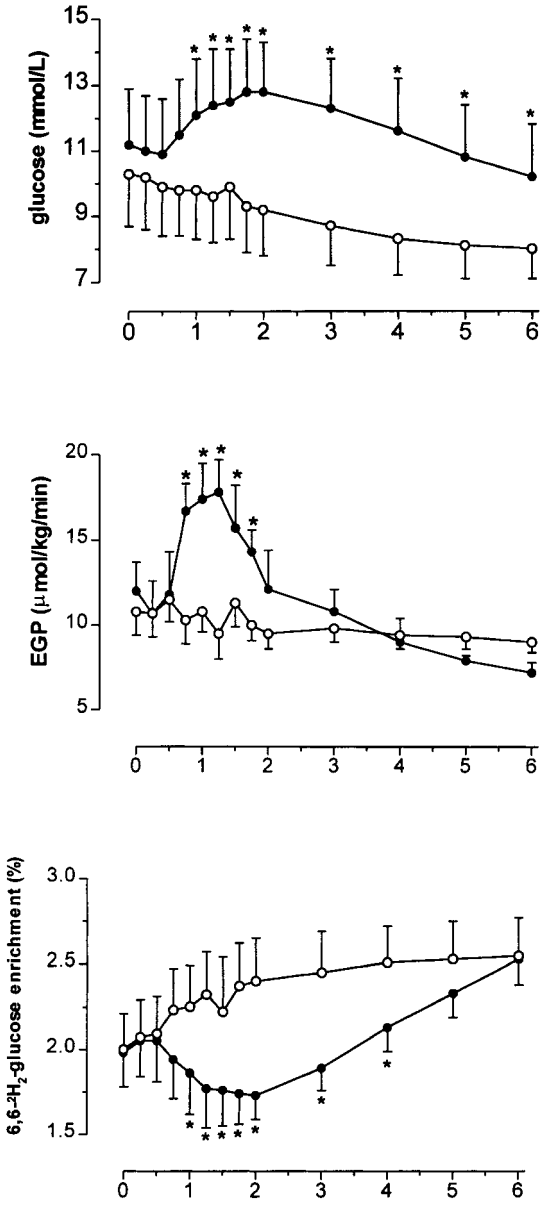

Fig 2. Plasma glucose concentration, EGP, and 6,6-$^2H_2$-glucose enrichment after administration of indomethacin (●) or placebo (○). The x-axis is the time in hours. *Statistically significant difference between the groups ($P < .05$).

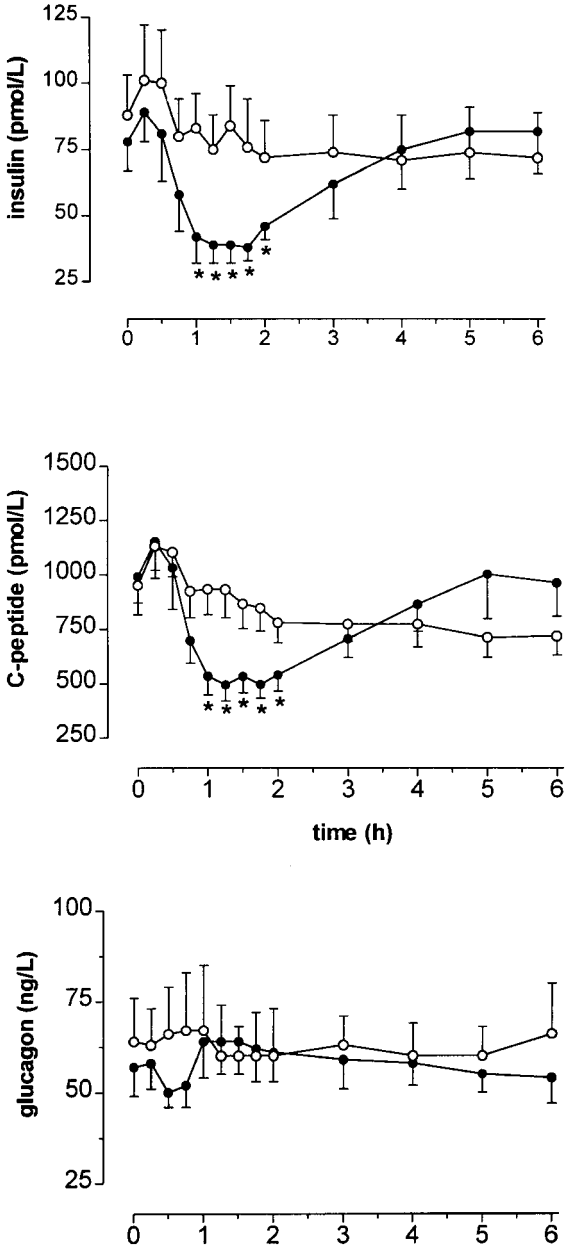

Fig 3. Plasma insulin, C-peptide, and glucagon after administration of indomethacin (●) or placebo (○). The x-axis is the time in hours. *Statistically significant difference between the groups ($P < .05$).

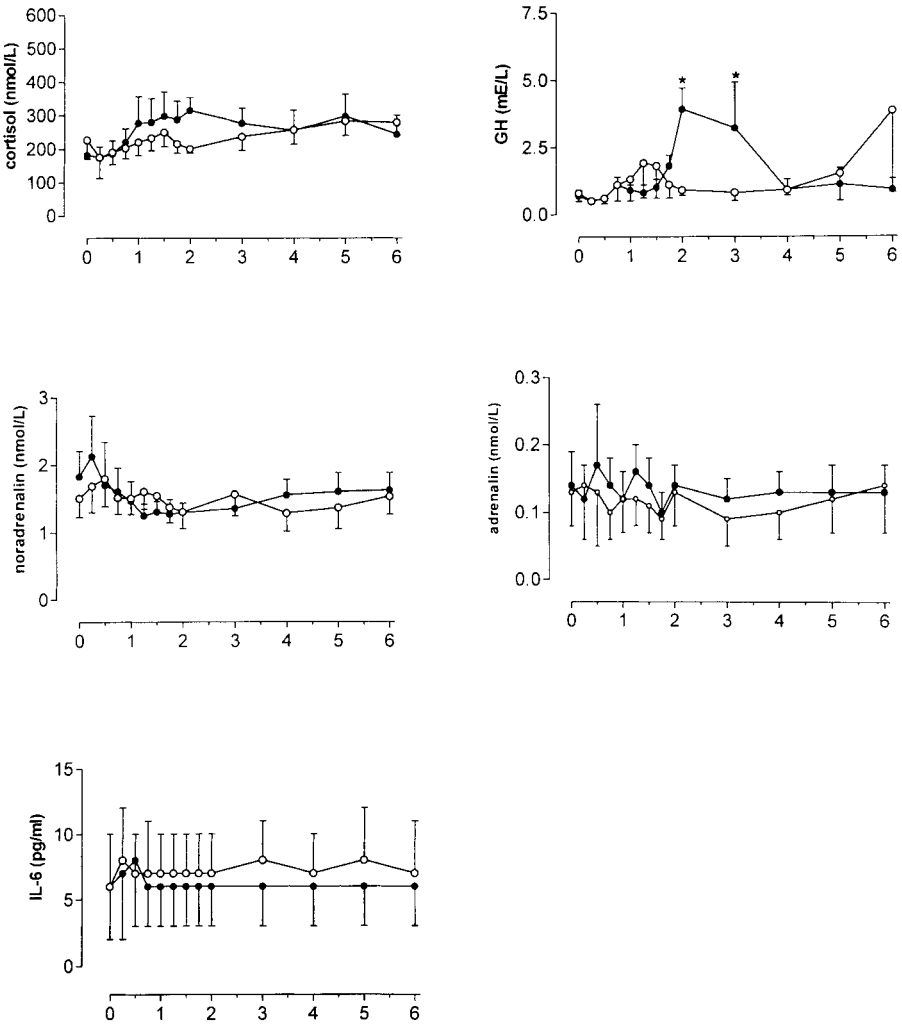

Fig 4. Plasma cortisol, growth hormone (GH), adrenaline, noradrenaline, and IL-6 after administration of indomethacin (●) or placebo (○).

Plasma FFAs were elevated but not statistically different between the two studies at baseline. At the end of the study, plasma FFA levels were lower in the indomethacin experiment ($P < .05$). Basal levels of TNF were below the detection limit of the assay during both experiments and remained unchanged (data not shown). Basal IL-6 levels were not elevated and not statistically different between the two studies. The plasma levels did not change significantly during either of the experiments.

DISCUSSION

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

Indomethacin increased EGP to a similar extent in patients with type 2 diabetes compared with healthy subjects (~6 μmol/kg/min *v* 5 to 7 μmol/kg/min from basal).[12,13] Nonetheless, the increase in plasma glucose was much higher in the diabetics (3.5 mmol/L *v* 1.5 to 2 mmol/L from basal). The combination of the same increase in EGP 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 subjects and patients with type 2 diabetes is the finding that insulin secretion was significantly reduced by indomethacin in type 2 diabetics but not in healthy individuals. A statistically significant increase in growth hormone was measured 2 and 3 hours after administration of indomethacin. Growth hormone itself can stimulate EGP,[16] but it is unlikely that EGP was driven by growth hormone or vice versa. The changes in EGP and insulin concentrations occurred within 45 minutes after administration of indomethacin, whereas plasma growth hormone concentrations started to increase more than 90 minutes after administration of indomethacin. Moreover, if growth hormone was driven by the increase in EGP, an inhibition rather than a stimulation of growth hormone secretion would be expected.[17] At the end of the indomethacin experiments, FFA concentrations were somewhat lower versus the control experiments. This may be due to the rebound in the insulin concentration after initial inhibition.

In our study, the increase in the glucose concentration and glucose production coincided with the decrease in peripheral C-peptide levels and insulin concentrations. Sindelar et al[18] recently reported data on the relationship of the portal vein insulin concentration and basal hepatic glucose production in overnight-fasted conscious dogs. Within 15 minutes after a selective decrease of portal insulin 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. Therefore, in contrast to healthy subjects, in type 2 diabetes, the observed stimulatory effect of indomethacin on EGP is likely 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 4 studies to our knowledge.[12,13,19,20] In 3 of these 4, basal insulin levels remained unaffected, whereas in the fourth, a small but significant decrease from 9.5 to 6.4 μU/mL was observed 1 hour after 50 mg indomethacin versus 8.0 to 6.9 μU/mL after placebo.[19]

The effect of indomethacin on glucose-induced acute insulin secretion is different from its effect under basal circumstances. All studies but one[19] in humans showing an inhibitory effect of indomethacin on insulin secretion (reviewed in Robertson[21]) were performed in experimental settings involving glucose infusion. Therefore, the effect of indomethacin on the peripheral insulin level differs in basal versus glucose-stimulated conditions. In our type 2 diabetic patients, insulin secretion was stimulated as deduced from the 2- to 3-fold increase in basal insulin levels compared with normal values.[12] It can thus be postulated that the effect of indomethacin under conditions in which insulin secretion is stimulated chronically as in the present study resembles the situation of acute glucose-stimulated insulin secretion. Indomethacin has no effect on insulin secretion under basal conditions, as reflected by our experiments in healthy humans.[12]

Although indomethacin is a prostglandin synthesis inhibitor, it is unlikely that the effect of indomethacin on the β cell is due to inhibition of prostaglandin synthesis. Prostaglandin E_2, synthesized by the pancreatic islet, inhibits glucose-induced insulin secretion.[22] 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 IL-1β both in vitro and in vivo.[23] IL-1β stimulates the production of the inducible form of cyclooxygenase (COX-2), the enzyme responsible for generation of prostaglandin E_2 from arachidonic acid. The effect of IL-1 can be either direct by increasing the gene expression of COX-2 mRNA or indirect by production of NO.[24] Thus, stimulation of IL-1 by indomethacin could result in the inhibition of insulin secretion through stimulation of COX-2. A similar IL-1–mediated effect of indomethacin can stimulate growth hormone release[25,26] via stimulation of growth hormone–releasing hormone by IL-1.[27] Growth hormone secretion can thus be stimulated directly by indomethacin independently of EGP.

Another possibility for the inhibition of insulin secretion by indomethacin is its ability to affect the insulin receptor itself, by inhibiting autophosphorylation of the β-subunit of the insulin receptor.[28] Very recent publications indicate that a functional insulin receptor is a prerequisite for normal glucose-stimulated insulin secretion.[29] Insulin stimulates its own release by a positive-feedback loop through binding to its own receptor in the β cell. An impairment of the function of the insulin receptor by indomethacin by inhibiting autophosphorylation of the β-subunit could lead to inhibition of insulin secretion.

The dose of 150 mg indomethacin used in this study is equivalent to the daily therapeutic recommended dose as an antiinflammatory agent. Our data suggest that this dose can influence glucoregulation in type 2 diabetics. In conclusion, in patients with type 2 diabetes mellitus, indomethacin blocks insulin secretion and stimulates EGP.

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