

The Sulfonylurea Glyburide Induces Impairment of Glucagon and Growth Hormone Responses During Mild Insulin-Induced Hypoglycemia

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OBJECTIVE — The sulfonylurea (SU) glyburide may cause severe and prolonged episodes of hypoglycemia. We aimed at investigating the impact of glyburide on glucose counterregulatory hormones during stepwise hypoglycemic clamp studies.

RESEARCH DESIGN AND METHODS — We performed stepwise hypoglycemic clamp studies in 16 healthy volunteers (7 women and 9 men aged 44 ± 10 years). We investigated counterregulatory hormonal and symptom responses at arterialized venous plasma glucose levels (PG) of 3.8, 3.2, and 2.6 mmol/l, comparing 10 mg glyburide orally and placebo in a double-blind, randomized crossover fashion.

RESULTS — The increase in plasma glucagon with time from PG = 3.8 onward was smaller for glyburide than for placebo ($P = 0.014$). Plasma glucagon area under the curve (AUC_{60-180}) was lower after glyburide than after placebo ($1,774 \pm 715$ vs. $2,161 \pm 856$ pmol \cdot l⁻¹ \cdot min, $P = 0.014$). From PG = 3.8 onward, plasma growth hormone (GH) levels with placebo were nearly two times (1.9 [95% CI 1.2–2.9]) as high as with glyburide ($P = 0.011$). AUC_{60-180} for GH was lower after glyburide than after placebo (geometric mean [range] 665 [356–1,275] and 1,058 [392–1,818] mU \cdot l⁻¹ \cdot min, respectively; $P = 0.04$). No significant differences were observed for plasma cortisol, epinephrine and norepinephrine, or incremental symptom scores.

CONCLUSIONS — The SU glyburide induces multiple defects in glucose counterregulatory hormonal responses, notably decreases in both glucagon and GH release.

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Prolonged and severe hypoglycemic episodes may constitute a notorious problem in patients with type 2 diabetes treated with the sulfonylurea (SU) compound glyburide (1). Although this is generally attributed to its long duration of action (2), it is currently uncertain whether and in what respect glyburide (or

other SU) may impair glucose counterregulation.

SU derivatives block ATP-sensitive K⁺ (K_{ATP}) channels via binding to the so-called SU receptor (SUR), which is a subunit of the channel. In endocrine cells, K_{ATP} channels regulate the secretion of hormones such as insulin, prolactin, and

growth hormone (GH) (3). By closing K_{ATP} channels, SUs induce insulin secretion in the pancreatic β -cell. In the brain, these ion channels play an important role in connecting changes of extracellular glucose levels to changes of neurotransmitter release (3).

Normal glucose counterregulation involves the dissipation of insulin secretion and the release of an array of hormones, including glucagon, epinephrine, norepinephrine, cortisol, and GH (4,5). However, to our knowledge, a systematic approach to study the impact of SU on all counterregulatory hormones and at various plasma glucose levels has never been undertaken. To investigate glucose counterregulation, we performed stepwise hypoglycemic clamp studies in 16 healthy volunteers.

Plasma glucagon, epinephrine, norepinephrine, cortisol, and GH levels, as well as symptom responses to clamped hypoglycemia at glucose levels of 3.8, 3.2, and 2.6 mmol/l, were compared after oral administration of 10 mg glyburide or placebo in a double-blind, randomized crossover fashion.

RESEARCH DESIGN AND METHODS

Subjects

Sixteen healthy volunteers (seven women and nine men; 44 ± 10 years of age, mean BMI 25.3 ± 3.5 kg/m²) participated in this study. They were recruited by newspaper advertisements. Eligibility assessments included a full medical history, physical examination, electrocardiography, and laboratory tests (full blood count, creatinine, liver enzymes, thyrotropin and plasma free thyroxine, basal cortisol, fasting blood glucose, HbA_{1c}, lipid profile, and urine pregnancy test, if indicated). Written informed consent was obtained from all participants after the nature of the study had been explained. The study had been approved by the Ethical

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Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; GH, growth hormone; PG, plasma glucose level; K_{ATP}, ATP-sensitive K⁺ channel; RIA, radioimmunoassay; SU, sulfonylurea; SUR, SU receptor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Committee of University Medical Center, Utrecht, the Netherlands.

Study procedures

After an overnight fast, subjects were admitted on two separate occasions, at least 14 days apart, after having abstained from drinking alcohol for at least 48 h. A continuous insulin infusion (Human Actrapid; Novo Nordisk, Gentofte, Denmark), $2 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 180 min, was administered with a variable infusion of dextrose 20% (with 10 mmol KCl added to each 500 ml). Arterialized venous blood samples were collected for plasma glucose determination (Glucose Analyzer; Yellow Spring Instruments, Yellow Spring, OH) (6). During the first 60 min, euglycemia ($\sim 5.1 \text{ mmol/l}$) was maintained, and at 30 min, 10 mg glyburide or placebo was administered orally. Then, stepwise hypoglycemic clamping was performed: between 60 and 80 min, the plasma glucose level (PG) was allowed to decrease gradually to 3.8 mmol/l over 20 min and subsequently was kept constant at 3.8 mmol/l for 20 min between 80 and 100 min. The following was performed twice: PG was decreased to 3.2 mmol/l between 100 and 120 min and kept constant at that level between 120 and 140 min; PG was then decreased to 2.6 mmol/l between 140 and 160 min and kept constant at that level from 160 to 180 min.

Blood samples for the determination of plasma insulin, C-peptide, glucagon, epinephrine, norepinephrine, cortisol, and GH were stored at -20°C for later determination.

Analytical methods

Glucagon was measured in plasma with aprotinin after ethanol extraction, using an in-house competitive radioimmunoassay (RIA) using a polyclonal anti-glucagon antibody (4305-8; Rigshospitalet, Copenhagen), ^{125}I -glucagon (IM 160; Amersham, U.K.) as a tracer and human glucagon (1-29) (H6790; Bachem, Marina del Rey, CA) as a standard. Plasma epinephrine and norepinephrine levels were determined with reverse-phase high-performance liquid chromatography (Decade; Antec, Zoeterwoude, the Netherlands). Plasma insulin was measured with RIA, using a polyclonal anti-insulin antibody (Caris 46; Amersham) and ^{125}I -insulin (IM 166; Amersham).

C-peptide levels were measured with RIA (MD 315; Euro-Diagnostica, Malmö, Sweden). Human GH was measured us-

ing an immunometric technique on an Immulite Analyzer (Diagnostic Products, Los Angeles, CA) with 1 ng/ml equivalent to 2.6 mIU/l (World Health Organization International Ref. Prep 80/505). Cortisol was measured by means of competitive chemiluminescence (ACS-Centaur; Bayer, Tarrytown, NY). Symptom scores for seven autonomic symptoms (pounding heart, feeling tense/nervous, dry mouth, sweaty, cold hands, numb lips, trembling), four neuroglycopenic symptoms (slurred speech, confusion, blurred vision, difficulty concentrating), and three dummy symptoms (difficult breathing, painful legs, seeing yellow haloes) were scored at baseline and every 20 min thereafter, using a semiquantitative scale rating severity from 0 (absent) to 6 (very severe) points (7). Incremental symptom scores were calculated as the difference between symptom scores on $t = 180$ (PG = 2.6 mmol/l) and $t = 60$ (end of euglycemic clamping).

Statistical methods

Results are means \pm SD, unless stated otherwise. The area under the curve (AUC) was calculated using the trapezoidal rule (8). Statistical analyses were performed using SPSS statistical software (version 8.0; SPSS, Chicago, IL).

Glyburide and placebo data were compared pairwise (within patients) using repeated-measures analysis of variance (ANOVA) for longitudinal data sets (i.e., average hormonal profiles). Hormone levels and symptom scores on $t = 180$ and at baseline ($t = 60$ min) on the same study day and comparisons between glyburide and placebo for hormone levels at the end of the clamps and for AUCs (within patients) were compared with paired Student's *t* tests. Comparisons of symptom scores within and between clamps were made using Wilcoxon's signed-rank test, because data were not normally distributed. For the same reason, GH and norepinephrine data were logarithmically transformed before performing a repeated-measures ANOVA. Consequently, paired differences are expressed as ratios.

RESULTS— Glucose average arterialized venous plasma glucose levels during the last 20 min of euglycemic clamping were 4.9 ± 0.4 and $5.0 \pm 0.5 \text{ mmol/l}$. During the three hypoglycemic plateaus aiming for 3.8 , 3.2 , and 2.6 mmol/l ,

plasma glucose levels were 3.9 ± 0.3 and 3.8 ± 0.2 , 3.2 ± 0.2 and 3.2 ± 0.2 , and 2.7 ± 0.2 and $2.6 \pm 0.2 \text{ mmol/l}$ for placebo and glyburide, respectively (all NS) (Fig. 1). Glucose infusion rates did not differ between the two experiments, and cumulative amounts of glucose infused were similar for placebo and glyburide at 60, 100, 140, and 180 min. (all $P > 0.35$).

Insulin and C-peptide

Steady-state peripheral plasma insulin concentrations were comparable for placebo and glyburide clamps ($P = 0.22$) (Fig. 1); plasma insulin levels were $140 \pm 47 \text{ mU/l}$ for placebo and $141 \pm 49 \text{ mU/l}$ for glyburide at $t = 180$ min. On days when glyburide was administered (glyburide days), plasma C-peptide levels were significantly higher from $t = 30$ min onward than on days when placebo was administered (placebo days) ($P < 0.0005$) (Fig. 1). Consequently, C-peptide AUC₃₀₋₁₈₀ was higher for glyburide than for placebo (135 ± 51 vs. $96 \pm 30 \text{ nmol} \cdot \text{l}^{-1} \cdot \text{min}$; mean difference [95% CI] 39 [20–60] $\text{nmol} \cdot \text{l}^{-1} \cdot \text{min}$; $P < 0.0005$), reflecting β -cell stimulation by glyburide, despite progressive hypoglycemia over time.

Glucagon

Both on the placebo day and the glyburide day, there was a significant increase of plasma glucagon levels from $t = 100$ min (glucose = 3.8 mmol/l) onward ($P < 0.0005$) (Table 1, Fig. 2). However, the linear increase with time was significantly smaller for glyburide than for placebo ($P = 0.014$). The glucagon AUC₆₀₋₁₈₀ was lower after glyburide than after placebo ($1,774 \pm 715$ vs. $2,161 \pm 856 \text{ pmol} \cdot \text{l}^{-1} \cdot \text{min}$; mean difference [95% CI] 387 [89–685] $\text{pmol} \cdot \text{l}^{-1} \cdot \text{min}$; $P = 0.014$).

GH

On both on plasma and glyburide days, plasma GH levels increased linearly with time from $t = 100$ min onward ($P = 0.0005$) (Table 1, Fig. 2). Between 100 and 180 min, mean GH levels with placebo were, on average, 1.9 times (95% CI 1.2–2.9) as high as GH levels with glyburide ($P = 0.011$). This resulted in a lower AUC₆₀₋₁₈₀ for GH after administration of glyburide as compared with placebo: geometric mean (range) 665 (356–1,275) and $1,058$ (392–1,818) $\text{mU} \cdot \text{l}^{-1} \cdot \text{min}$, respectively; mean (95% CI) ratio 1.57 (1.02–2.41) ($P = 0.04$).

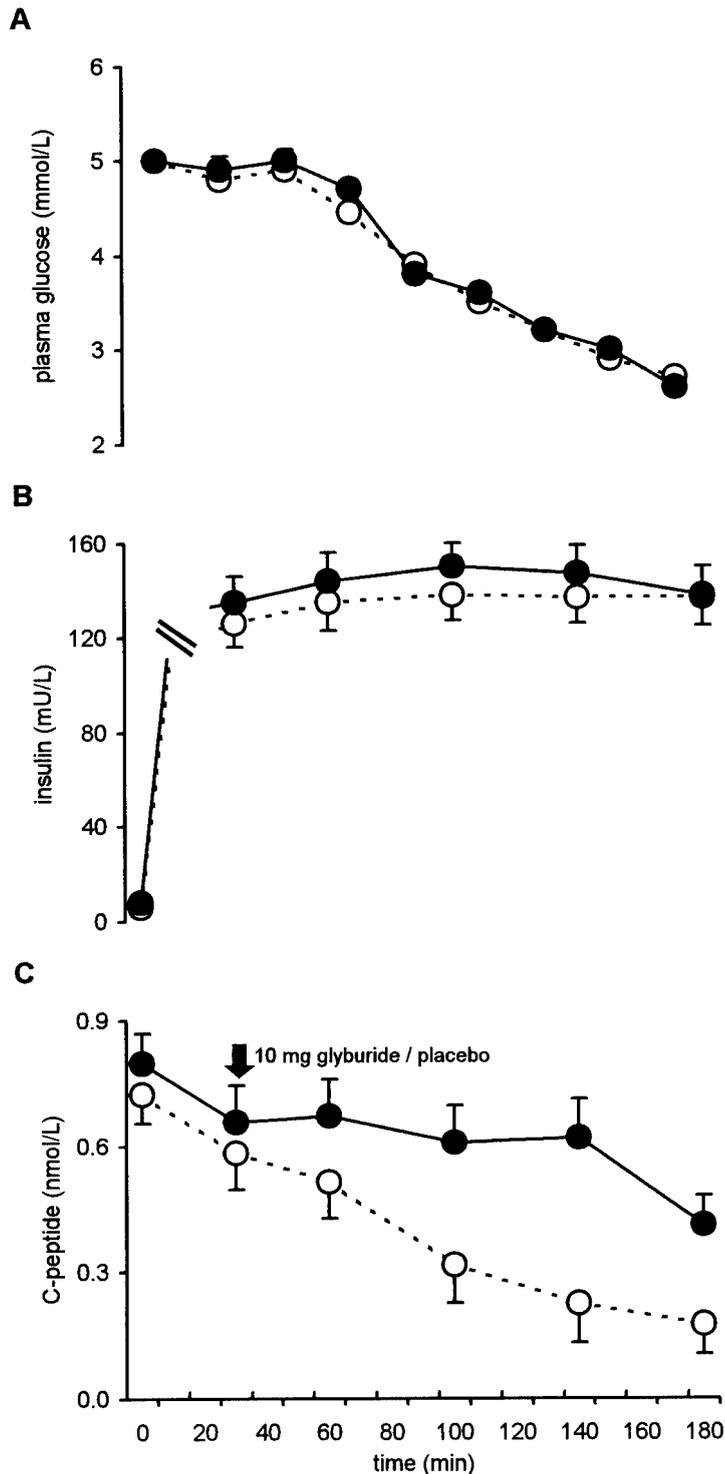


Figure 1—Mean \pm SEM plasma glucose levels (A), circulating insulin levels (B), and C-peptide levels (C) during stepwise hypoglycemic clamps with placebo (○) or 10 mg glyburide orally (●) at $t = 30$ min.

Cortisol

Plasma cortisol levels increased significantly during both experiments (Table 1). Cortisol profiles were similar for placebo and glyburide, and cortisol levels at the

end of the clamp were not significantly different for placebo and glyburide ($P = 0.069$). Cortisol AUC_{60-180} was similar on placebo and glyburide days: 37 ± 19 and $36 \pm 14 \text{ } \mu\text{mol} \cdot \text{l}^{-1} \cdot \text{min}$, respectively.

Catecholamines

Plasma epinephrine significantly increased from baseline ($t = 60$ min) to the end of the clamp on the placebo day (Table 1). Overall, plasma epinephrine profiles did not differ between placebo and glyburide series. Consequently, epinephrine AUC_{60-180} was similar on placebo and glyburide days (130 ± 64 vs. $112 \pm 47 \text{ } \text{nmol} \cdot \text{l}^{-1} \cdot \text{min}$).

Plasma norepinephrine profiles did not differ between placebo and glyburide series (Table 1). Consequently, norepinephrine AUC_{60-180} was similar on placebo and glyburide days (201 ± 64 vs. $230 \pm 100 \text{ } \text{nmol} \cdot \text{l}^{-1} \cdot \text{min}$).

Symptom scores

A significant increase in total symptom scores between baseline ($t = 60$ min) and the end of the clamp ($t = 180$ min) was observed during both placebo and glyburide experiments (placebo: median increment 7 points (range -1 to 34, $P = 0.0005$); glyburide: 11 points (range 2–20, $P = 0.001$); incremental symptom scores did not differ significantly between placebo and glyburide).

CONCLUSIONS— These studies addressed the effect of the SU derivative glyburide on glucose counterregulatory hormone levels. Plasma counterregulatory hormone levels were assessed at three different hypoglycemic levels with hypoglycemic glucose clamps. The present studies indicate that administration of the SU compound glyburide impairs the release of both glucagon and GH in response to insulin-induced hypoglycemia. These studies confirm and extend previous findings of interference of SU with glucagon secretion during nonstepwise hypoglycemia (4,5).

We observed a significant increase in glucagon compared with baseline after clamped hypoglycemia of $\sim 3.8 \text{ mmol/l}$ for 20 min on the placebo day, in accordance with previous findings (9,10). Glyburide interfered with glucagon release during progressive hypoglycemia from plasma glucose levels of $\sim 3.8 \text{ mmol/l}$ onward. When plasma glucose levels were decreased farther, this inhibitory effect increased, as illustrated by a lower slope of the glucagon versus time profile with glyburide compared with placebo.

Glucagon release has been identified to play a primary role in the defense against hypoglycemia (11). It has been

Table 1—Glucose counterregulatory hormone levels and incremental symptom scores before the start (t = 60 min) and at the end (t = 180 min) of stepwise hypoglycemic glucose clamps in 16 healthy subjects, comparing glyburide (10 mg orally) and placebo

	Placebo	Glyburide
Glucagon (pmol/l)		
Start	9 ± 1	8 ± 1
End	38 ± 1*	29 ± 3*
GH (mU/l)		
Start	0.3 (0.2–2)	0.8 (0.1–2)
End	30 (21–41)	23 (14–37)
Cortisol (umol/l)		
Start	0.25 ± 0.04	0.24 ± 0.05
End	0.64 ± 0.05	0.56 ± 0.05
Epinephrine (nmol/l)		
Start	0.1 ± 0.02	0.3 ± 0.02
End	3.1 ± 0.26	3.4 ± 0.35
Norepinephrine (nmol/l)		
Start	1.4 (1.2–1.7)	1.4 (1.0–2.0)
End	2.3 (1.9–2.7)	2.6 (2.1–3.3)
Symptom score (points)		
Start	1 (0–8)	2 (0–5)
End	8 (1–35)	15 (3–22)

Hormonal data are means ± SEM, geometric mean (95% CI) (for GH and norepinephrine), or median (range) (for data for symptom scores). **P* < 0.04

suggested that, apart from regulation via the ambient glucose level and the autonomic nervous system (12), glucagon release by the α -cell is under control of intra-islet mechanisms (13). Indeed, glucagon release can be inhibited by insulin in isolated rat pancreata (14). Rat pancreatic α -cells have been demonstrated to express K_{ATP} channels with SU sensitivity similar to the β -cell (15), which would imply a stimulatory effect of SU on glucagon secretion.

Glucagon release is controlled by an elaborate variety of signals that interplay with each other and may have different influences depending on the (experimental) circumstances. There is evidence that insulin, flowing from the β -cells toward the neighboring α -cells, inhibits glucagon release tonically (14). In the present studies, C-peptide levels were markedly higher after administration of glyburide than after administration of placebo, reflecting sustained endogenous insulin secretion as a result of β -cell stimulation by the SU. It is therefore plausible that the SU inhibits hypoglycemia-induced glucagon release via increased (β -cell) insulin release. Whether other mechanisms are (also) at play is unclear. A direct effect of the SU on the α -cell is less likely, because K_{ATP} channel blockers per se are expected

to stimulate (rather than inhibit) glucagon release (16). The autonomic system has a major effect on the normally occurring glucagon release during hypoglycemia (12,17,18). SU receptors have been shown to be present in the brain and in nerve cells (18,19). However, in our studies, no differences in epinephrine or norepinephrine responses were detected between glyburide and placebo. This may provide indirect evidence that interference with the activation of the autonomic nervous system was not a major reason for the suppression of the glucagon response by glyburide.

To date, no studies have addressed the question of whether other counterregulatory hormonal responses are influenced by SU administration during hypoglycemia. As mentioned, we did not observe differences in plasma catecholamines.

During the placebo experiments, we observed a GH response to insulin-induced hypoglycemia, with a median GH level of 28 (range 10–99) mU/l at the end of the clamp. Circulating insulin levels in the same range as during our studies have been reported to not alter hypoglycemia-induced GH release (20). To our knowledge, the present studies are the first to indicate that GH release is im-

paired by glyburide at moderate hypoglycemia, as reflected by lower average GH levels and lower AUC from baseline to the end of the clamp.

GH release is under a complex regulation by the hypothalamus, with stimulatory effects of GH-releasing hormone and ghrelin and inhibitory effects of somatostatin (21,22). The GH secretory response to insulin-induced hypoglycemia is believed to be mainly mediated via inhibition of the release and/or action of somatostatin (23,24). K_{ATP} channels with similar properties as those in pancreatic β -cells (i.e., closure by glucose and SU) have been observed in rat adenohypophysis cells (25). Interestingly, the SU glipizide is capable of stimulating GH release in these cells (26). In contrast, our in vivo studies show that glyburide inhibits the GH release induced by mild levels of hypoglycemia. Therefore, it could be that in vivo in humans, other factors are at play. One possibility would be that the SU stimulates somatostatin release, which in turn would inhibit GH secretion.

During the placebo experiments, cortisol levels started to increase when plasma glucose levels decreased below 3.2 mmol/l, as reported previously (10). It has been shown that brief exposure to hyperinsulinemia does not alter the cortisol response to hypoglycemia (20). Although there was a tendency toward lower cortisol levels at the end of the glyburide experiments as compared with placebo (*P* = 0.069), we did not observe a significant overall effect on the cortisol response in the presence of glyburide. Failure to detect a significant difference does not exclude this completely, because cortisol starts to increase later than other hormones, i.e., as plasma glucose levels decrease below ~3.2 mmol/l (9,10). However, the magnitude of such a possible difference seems to be limited (~20%).

The impairment by SU of the glucagon response to hypoglycemia described herein occurred at plasma glucose levels of ~3.8 mmol/l, whereas this inhibitory effect increased over time as plasma glucose levels were decreased further to ~3.2 and ~2.6 mmol/l. Clearly, SU induces a prolonged stimulation of insulin secretion, which necessitates full-scale glucose counterregulatory hormonal responses. Because glucagon has been identified to play a primary role in the defense against hypoglycemia (11), impairment of the glucagon response to hypoglycemia

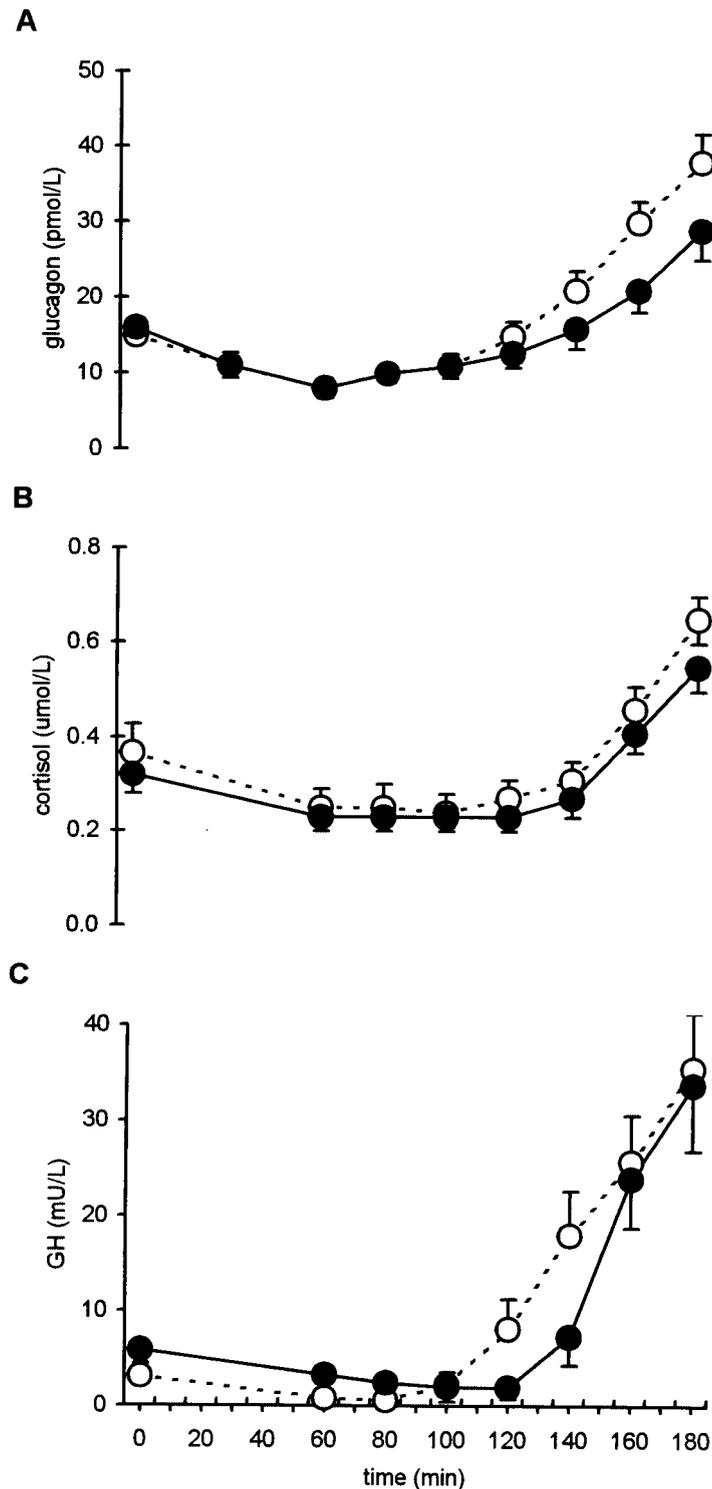


Figure 2—Mean \pm SEM plasma glucagon (A), cortisol (B), and GH (C) levels during stepwise hypoglycemic clamps with placebo (○) or 10 mg glyburide orally (●) at $t = 30$ min.

by SU is potentially dangerous for patients being treated with these drugs. This may possibly be even more important during fasting or exercise or when an SU is combined with insulin. Our observa-

tion that the GH release in response to hypoglycemia is impaired, in addition to the glucagon release in the presence of glyburide, may help explain (at least partly) the protracted nature of severe hy-

poglycemic episodes that has been observed in patients using this drug (27). Although GH is normally not critical for the recovery from hypoglycemia, the glucose counterregulatory effects of GH have been demonstrated to play a role in the recovery from prolonged hypoglycemia (28), which may have even more impact when the glucagon response is diminished. Our data suggest that GH responses at more profound levels of hypoglycemia (PG = 2.6 mmol/l) may not be affected by glyburide. We propose that more profound hypoglycemia possibly overrides the inhibitory effect of SU on the GH response.

In summary, we have demonstrated that the glucagon response to progressive levels of insulin-induced hypoglycemia is increasingly impaired in the presence of glyburide. Epinephrine and norepinephrine responses are not affected, suggesting the absence of major effects of the SU on the autonomic nervous system. These data support the notion that glucagon release in hypoglycemia is substantially influenced by intra-islet mechanisms, possibly by increased intra-islet insulin levels. Furthermore, these studies indicate, for the first time, that SU administration diminishes hypoglycemia-induced GH release, possibly by disinhibition of hypothalamic somatostatin release.

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