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Counterregulatory Responses to Hypoglycemia Differ between Glimepiride and Glyburide in Non Diabetic Individuals

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Abstract

Objective—Reported rates of hypoglycemia in patients with type 2 diabetes mellitus are lower with glimepiride as compared to glyburide. The aim of this study was to determine whether physiologic differences in counterregulatory neuroendocrine and metabolic mechanisms during hypoglycemia provide a basis for the observed clinical differences between glimepiride and glyburide.

Research Design and Methods—Non-diabetic volunteers (age 38 ± 2 yrs, BMI 26 ± 1 kg/m²) were studied in a single-blind fashion during separate 2 day randomized protocols consisting of 2 hr hyperinsulinemic (9 pmol/kg/min) euglycemic (4.9 ± 0.1 mmol) and hypoglycemic (2.9 ± 0.1 mmol/L) clamps. Individuals received biologically equivalent doses of glimepiride (4 mg) or glyburide (10 mg) 1 hr prior to each glucose clamp (n=11) as well as a control group of placebo studies. Glucose kinetics were calculated using D-Glucose-6-6d₂.

Results—Insulin and C-peptide levels were increased ($p < 0.05$) during euglycemia in both sulfonylurea groups as compared to placebo. However, despite equivalent hypoglycemia, insulin and C-peptide levels were higher ($p < 0.05$) only after glyburide. Glucagon responses and endogenous glucose production (EGP) were decreased ($p < 0.05$) during hypoglycemia following glyburide administration as compared to glimepiride. Glyburide reduced ($p < 0.05$) norepinephrine responses during euglycemic clamps. In addition combined epinephrine and norepinephrine responses during hypoglycemia were reduced ($p < 0.05$) following glyburide as compared to placebo. Leptin levels fell by a greater amount ($p < 0.05$) during hypoglycemia with both sulfonylureas as compared to placebo.

Conclusions—In summary, glimepiride and glyburide can both similarly increase insulin and C-peptide levels during hyperinsulinemic euglycemia. However, during moderate hyperinsulinemic hypoglycemia (2.9 mmol/L) glyburide resulted in increased C-peptide and insulin, but blunted glucagon, sympathetic nervous system and EGP responses. We conclude that

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glyburide can acutely reduce key neuroendocrine and metabolic counterregulatory defenses during hypoglycemia in healthy individuals.

Keywords

Glimepiride; glyburide; hypoglycemia; neuroendocrine counterregulation; glucagon

INTRODUCTION

Recent data indicate that 25.6 million individuals age 20 years or older in the United States have diabetes. Of this number ~ 90–95% have type 2 diabetes mellitus (T2DM)(1). In order to reduce long term complications of the disease, individuals with T2DM require life-long treatment and good metabolic control. In addition to lifestyle modification, pharmacologic therapy consisting of oral hypoglycemic drugs, insulin and/or agonists of the glucagon like peptide-1 axis are used alone or in combination for the treatment of hyperglycemia.

A cornerstone of T2DM treatment involves the use of sulfonylureas to stimulate endogenous insulin release to address one of the key pathophysiologic defects causing diabetes. Unfortunately, hypoglycemia is a common complication of sulfonylurea use and can contribute to increased morbidity and mortality in diabetic patients. However, frequency of hypoglycemic episodes differs between sulfonylureas (2, 3). Glyburide (glibenclamide) and glimepiride are both second generation sulfonylurea drugs commonly used in the treatment of T2DM, with the former having a higher prevalence of hypoglycemia as compared to glimepiride (4). The reasons for the difference in hypoglycemic prevalence are incompletely understood, although several potential causes have been suggested. These differences include the finding that glimepiride absorption is delayed by food whereas glyburide is not affected by administration with meals. Additionally, sulfonylureas act on sulfonylurea receptors (SURs) found within ATP-sensitive potassium (K^{ATP}) channels. Previous animal studies have determined that glimepiride and glyburide have distinctive binding affinities and kinetics for pancreatic SUR1 subunits of K^{ATP} channels. This establishes the potential for differential effects of sulfonylureas on beta cell insulin secretion (5).

Previous studies investigating the effects of sulfonylureas on glucagon release during hypoglycemia have been somewhat conflicting with reports of both reduced and/or no effects of sulfonylureas on glucagon during hypoglycemia (6, 7).

To date, there does not appear to be a study comparing the effects of the two most commonly used sulfonylureas on insulin secretion during clamped euglycemia and hypoglycemia. Additionally, data determining the effects of glimepiride and glyburide on glucose kinetics, including the key metabolic response of endogenous glucose production (EGP) are also not available. Therefore the aim of study was to compare the effects of glimepiride and glyburide during both hyperinsulinemic euglycemia and hypoglycemia to determine if physiologic differences in neuroendocrine, metabolic or autonomic nervous system counterregulatory responses might provide mechanistic insight into why differences in rates of hypoglycemia occur in clinical practice when using the two sulfonylureas.

RESEARCH DESIGN AND METHODS

Subjects

Fourteen (7F/7M) non diabetic volunteers (age 38 ± 2 yrs, BMI 26 ± 1 , HbA_{1C} $5.1 \pm 0.1\%$) were studied. Each subject had a normal blood count, plasma electrolytes, liver and renal function and no evidence of either impaired fasting glucose or overt diabetes mellitus. All gave written informed consent. Studies were approved by the Vanderbilt University Human Subjects Institutional Review Board.

Experimental Design

The study protocol consisted of three 2-day studies each separated by at least two months (Fig. 1). Eight individuals participated in all three of the 2-day studies. Three individuals participated in two of the 2-day studies and the remainder underwent a single day study, with $n=11$ in each group. During each 2-day study, participants received either placebo, glimepiride or glyburide, which were administered in a randomized, single blind fashion. Participants were non-smokers and were instructed to avoid any exercise and consume their usual weight maintaining diet for three days before each experiment. Additionally all subjects refrained from consuming alcohol for 24 hrs prior to each study. Subjects were admitted to the General Clinical Research Center (GCRC) the evening prior to the study. After a standardized evening meal and an overnight 10 hr fast, two intravenous cannulas were inserted under 1% lidocaine local anesthesia. One cannula was placed in a retrograde fashion into a vein on the back of the hand of the non-dominant arm. This hand was placed in a heated box ($55\text{--}60^{\circ}\text{C}$) during the study so that arterialized blood could be obtained (8). The other cannula was placed in the arm for infusions.

Euglycemia Experiments

A euglycemia experiment was performed on day 1 of each 2-day study. At time zero, after an overnight fast, a primed (6 mg/kg) continuous infusion (0.06 mg/kg/min) of sterile, pharmacy prepared 6'-dideuterated glucose (Sigma Aldrich, St. Louis, MO) was administered via a precalibrated infusion pump (Harvard Apparatus, South Natick, MA). An equilibration period of 150 min was allowed to elapse followed by a 120 min hyperinsulinemic-euglycemic experimental period. Subjects received either placebo, glimepiride (4mg) or glyburide (10mg) one hour prior to the euglycemic clamps. Glimepiride (Amaryl) and glyburide (DiaBeta) tablets were obtained from Aventis. At time 150 min, a primed constant (9 pmol/kg/min) infusion of insulin (Human Regular Insulin, Eli Lilly, Indianapolis, IN) was started and continued until time 270 minutes. During the clamp period, plasma glucose was measured every five min and a 20% dextrose infusion was adjusted so that plasma glucose levels were held constant at 4.9 ± 0.1 mmol/L (9). Potassium chloride (20mmol/L) was infused during the clamp to reduce insulin-induced hypokalemia. After the experiment subjects were given lunch and a standardized evening meal and remained on the GCRC.

Hypoglycemia Experiments

A hypoglycemia experiment was performed on day 2 of each 2-day study. These experiments followed the same format as the above euglycemic experiments, with a 150 min starting period and subjects receiving the same intervention as day 1 at 1 hr prior to the hypoglycemic clamp. Exceptions were that when the primed constant (9.0 pmol/kg/min) infusion of insulin was started, the rate of fall of glucose was controlled (~ 0.08 mmol/min) and a glucose nadir (2.9 mmol/L) was achieved using a modification of the glucose clamp technique (10). During the clamp period, plasma glucose was measured every five min and a 20% dextrose infusion was adjusted so that plasma glucose levels were held constant at 2.9 ± 0.1 mmol/L (10). Neuroendocrine counterregulatory hormone release begins at a hypoglycemic value of 3.9 mmol/L (11). Thus a hypoglycemic level of 2.9 mmol/L is able to produce a robust and easily measurable counterregulatory response. After the experiment subjects were given a meal and plasma glucose levels were stabilized to euglycemia.

Tracer Methodology/Calculations

D-Glucose-6,6-D2 was purchased from Sigma-Aldrich, St. Louis, MO. The stable isotope was prepared in a sterile solution and infused as a primed (6 mg/kg) continuous infusion of 0.06 mg/kg/min. A 2.5 hr equilibration period was allowed before the 2 hr glucose clamp period began. The derivatization of glucose to quantitate isotopic enrichment was achieved by acetate derivatives and analyzed by EI-Gas Chromatography/Mass Spectrometry as described by Hachey et al (12). Rates of glucose appearance (R_a), endogenous glucose production (EGP), and glucose utilization were calculated according to the Steele equation (13). EGP was calculated by determining the total R_a (this comprises both EGP and any exogenous glucose infused to maintain the desired hypoglycemia) and subtracting it from the amount of exogenous glucose infused. To maintain a constant specific activity, isotope delivery was increased commensurate with increases in exogenous glucose infusion. CVs of the basal and final 30 minute periods of atom percent excess (APE) are reported in Supplement 2. During these studies, only glucose flux results from the steady-state basal and the final 30-min periods of the euglycemic and hypoglycemic clamps are reported.

Analytical Methods

Plasma glucose concentrations were measured in triplicate using the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Blood for hormones and intermediary metabolites were drawn twice during the control period and every 15 min during the experimental period. Cardiovascular parameters (pulse, systolic, diastolic, and mean arterial pressure) were measured noninvasively by a Dinamap (Critikon, Tampa, FL) every 10 min throughout each study starting at 80 min. Glucagon was measured according to a modification of the method of Aguilar-Parada et al (14) with an interassay coefficient of variation (CV) of 12%. Insulin was measured as previously described (15) with an interassay CV of 9%. Catecholamines were determined by HPLC (16) with an interassay CV of 12% for epinephrine and 8% for norepinephrine. Two modifications to the procedure for catecholamine determination were made: 1) a five-point rather than a one-point standard calibration curve was used, and 2) the initial and final samples of plasma with known amounts of epinephrine and norepinephrine were spiked so that accurate identification of the

relevant respective catecholamine peaks could be made. Cortisol was assayed using the Clinical Assays Gamma Coat Radioimmunoassay (RIA) kit with an interassay CV of 6%. Growth hormone was determined by RIA (17) with a CV of 8.6%. Pancreatic polypeptide was measured by RIA using the method of Hagopian et al. (18) with an interassay CV of 8%.

Hypoglycemic symptoms were quantified using a previously validated semiquantitative questionnaire (19). Each individual was asked to rate his/her experience of symptoms twice during the control period and every 15 min during experimental periods. Symptoms measured included sweating, tremor/shaking, feeling hot, thirsty/dry mouth, agitation/irritability, palpitations, feeling tired/fatigued, confusion, dizziness, difficulty of thinking, blurriness of vision and sleepiness. The ratings of the first six symptoms were summed to obtain an autonomic symptom score, whereas the total of the ratings from the last six symptoms provided a neuroglycopenic symptom score.

Statistical Analysis

Data are expressed as mean $\pm$ SE and were analyzed using standard, parametric, one- and two-way analysis of variance (ANOVA) and with repeated measures where appropriate (Graph Pad Software, Inc., San Diego, CA). Data was also analyzed using paired and unpaired two-tailed t-tests. In all cases p value of <0.05 was accepted as statistically significant. Baseline values were considered to be an average of the two time points drawn before starting the clamp procedure. The present study investigates the in-vivo integrated clinical physiologic responses to hypoglycemia. These include epinephrine, norepinephrine, pancreatic polypeptide (autonomic nervous system); insulin, c-peptide, glucagon, growth hormone, cortisol (neuroendocrine counterregulatory hormones); glucose kinetics and intermediary metabolites (metabolic); hypoglycemic symptoms and cardiovascular responses. The leptin responses are non-primary and should be considered exploratory in nature.

RESULTS

Glucose

Plasma glucose was maintained equivalently (4.9 ± 0.1 mmol/L) during the euglycemic clamps. Plasma glucose reached a steady state and equivalent hypoglycemia (2.9 ± 0.1 mmol/L) by 180 min and was maintained during all hypoglycemic clamp procedures (Fig.1).

Insulin and C-peptide

Insulin levels during euglycemia were increased ($p < 0.05$) following glimepiride (592 ± 54 pmol/L) and glyburide (581 ± 54 pmol/L) as compared to placebo (435 ± 26 pmol/L) (Table 1). Insulin levels were also increased ($p < 0.05$) during hypoglycemia following glyburide (492 ± 35 pmol/L) as compared to glimepiride (405 ± 18 pmol/L) and placebo (398 ± 31 pmol/L) (Fig. 2). Similarly C-peptide levels were increased during euglycemia ($p < 0.05$) with both sulfonylurea drugs (glimepiride 0.9 ± 0.2 nmol/L and glyburide 1.5 ± 0.3 nmol/L) as compared to placebo (0.4 ± 0.1 nmol/L) (Table 1). During hypoglycemia

C-peptide levels were higher ($p<0.05$) following glyburide (0.5 ± 0.2 nmol/L) as compared to placebo (0.1 ± 0.2 nmol/L) (Fig. 2).

Neuroendocrine Counterregulatory Hormones

Glucagon responses were similar during euglycemia in all groups, but were significantly reduced ($p<0.05$) during hypoglycemia after glyburide administration (90 ± 9 ng/L) as compared to placebo (132 ± 14 ng/L) and glimepiride (135 ± 21 ng/L) (Fig. 2).

Norepinephrine responses increased ($p<0.05$) during hyperinsulinemic euglycemia following placebo or glimepiride, but not glyburide. There were no statistical differences in epinephrine or norepinephrine responses during hypoglycemia amongst groups. However, when epinephrine and norepinephrine responses were grouped together as an “index” of sympathetic nervous system activity, there were significant ($p<0.05$) reductions in combined catecholamine responses following glyburide as compared to glimepiride and placebo.

Cortisol and growth hormone levels were unaffected by glimepiride or glyburide during either euglycemia or hypoglycemia (Table 1).

Pancreatic Polypeptide

Pancreatic polypeptide (PP) responses were reduced ($p<0.05$) during euglycemia following placebo and glyburide but not glimepiride and were increased ($p<0.05$) during hypoglycemia in all groups as compared to euglycemia. PP responses were also higher ($p<0.05$) during hypoglycemia following glimepiride as compared to placebo (Table 2, Fig. 4).

Leptin

Leptin responses were similar in all groups during euglycemia. During hypoglycemia there were greater reductions in leptin following either glimepiride or glyburide as compared to euglycemia (Table 2, Fig. 4).

Glucose Kinetics

Glucose kinetics were not changed during the euglycemic protocols. However, EGP was reduced and GIR rates were increased ($p<0.05$) during hypoglycemia following glyburide administration (Fig. 3). Specific activity in APEs of D-Glucose-6,6-D2 reached steady state at the start (CVs 1–4%) and end of clamp (CVs 2–4%) in all protocols (Suppl. 2).

Intermediary Metabolites

There were no differences amongst the groups for lactate and glycerol during euglycemia and hypoglycemia (Table 1).

Hypoglycemic Symptoms

Autonomic and neuroglycopenic symptom scores increased similarly 11 ± 2 during hypoglycemia ($p<0.05$) in all groups.

Cardiovascular Parameters

There were no significant differences in systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and heart rate during any of the euglycemic studies.

During hypoglycemia diastolic blood pressure was decreased by 9 ± 2 mmHg ($p < 0.05$) after glimepiride and by a greater amount 12 ± 13 mmHg ($p < 0.05$) after glyburide administration as compared to euglycemia (0 ± 1 mmHg). Additionally there was a greater reduction in diastolic blood pressure during hypoglycemia following glyburide as compared to placebo (12 ± 13 mmHg vs. 3 ± 4 mmHg, respectively ($p < 0.05$)).

DISCUSSION

This present study has investigated the acute in-vivo physiologic responses between glyburide and glimepiride during clamped hyperinsulinemic euglycemia and moderate hypoglycemia in healthy individuals. Our results clearly demonstrate that: 1) Both glyburide and glimepiride (a newer second generation sulfonylurea) increased endogenous insulin and C-peptide secretion under hyperinsulinemic euglycemic conditions; 2) Both sulfonylureas acutely suppress leptin levels during clamped hypoglycemia; 3) Multiple differences in physiologic counterregulatory responses exist during clamped hypoglycemia between the two sulfonylureas. These differences notably include increased insulin and C-peptide levels following glyburide, reduced glucagon and sympathetic nervous system responses, limited endogenous glucose production following glyburide and reduced EGP following glyburide as compared to glimepiride.

Sulfonylureas remain a widely used and popular option for use in T2DM. Drawbacks include the increased risk of hypoglycemia, weight gain and possibly increased β -cell dysfunction. The advent of late second generation (sometimes called third generation) sulfonylureas such as glimepiride have reduced the risk and incidence of hypoglycemia and weight gain when used in clinical practice (2, 4, 20). However, the in-vivo physiologic mechanisms for these findings are not fully understood.

In this present study, we therefore determined the acute effects of a single biologically and clinically comparable half maximal dose of two commonly used sulfonylurea drugs glyburide and glimepiride under controlled glucose clamp conditions so that in-vivo physiologic responses during both euglycemic and hypoglycemic conditions could be determined. Both drugs are absorbed at similar rates (~ 1 hr) and reach peak plasma levels between 2–4 hr (21, 22). Healthy individuals were studied to determine a normal, healthy insulin response as well as normal counterregulatory responses to hyperinsulinemic euglycemic and hypoglycemic clamps.

Our findings can be grouped into two main categories. Firstly, novel determinations that are common to the spectrum of second generation sulfonylureas and secondly, specific differences between glyburide and glimepiride.

Both sulfonylureas increased insulin and C-peptide levels during hyperinsulinemic euglycemic clamps. C-peptide levels fell by $\sim 37\%$ during placebo euglycemic clamps,

which is usual for the prevailing level and duration of hyperinsulinemia used in our study. This indicates that during euglycemia, the sulfonylureas could acutely overcome the suppressive effect of exogenous hyperinsulinemia and substantially stimulate endogenous insulin release. This finding helps to provide context as to how sulfonylureas can maintain glucose lowering effects even under basal (postabsorptive) conditions in metabolically well controlled T2DM individuals. Our findings also demonstrate the potential risk of hypoglycemia when using these drugs as endogenous insulin secretion is not suppressed even by hyperinsulinemic euglycemia.

However, glyburide alone further exacerbates the potential for hypoglycemia by increasing insulin and C-peptide levels during hypoglycemia. Szoke et al (6) and Landstedt-Hallin et al (23) reported similar findings during hypoglycemic clamp studies in healthy individuals and T2DM respectively. In the present study, plasma glucagon was similarly suppressed during all euglycemic clamp studies. However, glyburide resulted in a significant blunting of glucagon release during hypoglycemia as compared to glimepiride or placebo. Previous studies in healthy individuals (7) and T2DM (23) have also reported blunting of glucagon responses during hypoglycemia, but were not observed in healthy individuals by Szoke et al (6). Although it should be noted that the hypoglycemic value (~2.5mmol/L) used in the Szoke et al (6) study was lower than the present and other reports which may have provided a greater overriding drive for glucagon release. Nevertheless, the finding of increased insulin secretion but reduced glucagon release is consistent with the current intra-islet hypothesis of (24) regulation of glucagon responses during hypoglycemia. The intra-islet hypothesis posits that a reduction in intra-islet insulin levels is an important physiologic mechanism for glucagon release during hypoglycemia in humans (24). Additionally, Evans et al (25) have demonstrated that local hypothalamic injection of glyburide into the ventromedial hypothalamus (VMH) during hypoglycemia in conscious rats also results in suppressed glucagon responses. Glyburide also appeared to reduce sympathetic nervous system responses during the hyperinsulinemic clamps. Glyburide alone blunted the increase in norepinephrine levels during hyperinsulinemic euglycemia. Additionally, when we combined epinephrine and norepinephrine responses during hypoglycemia to give a novel and more comprehensive index of sympathetic nervous system activity (adrenomedullary and sympathetic neural) there was a clear blunting of the catecholamine responses following glyburide as compared to placebo. Our finding is also supported by Evans et al whom have reported that direct infusion of glyburide into the VMH can suppress epinephrine responses during hypoglycemia in conscious rats (25). Only one other study (7) has reported epinephrine and norepinephrine levels during hypoglycemia following glyburide. These investigations reported numerical (but not significant) reductions in epinephrine but no differences in norepinephrine.

This present study also appears to be the first to incorporate glucose tracers to determine glucose kinetics during euglycemic and hypoglycemic clamps following glimepiride and glyburide administration. Glucose kinetics were similar during all euglycemic clamps. However, there was a marked reduction in EGP during hypoglycemia following glyburide as compared to glimepiride or placebo. These findings are consistent with glyburide effects to suppress glucagon and sympathetic nervous system activity (epinephrine and norepinephrine) while also increasing insulin levels during hypoglycemia. The similarity of

circulating levels of lactate and glycerol amongst the three groups during hypoglycemia also indicates that changes in pancreatic islet hormones and sympathetic nervous system activity were having primary hepatic rather than peripheral metabolic effects.

Both glimepiride and glyburide suppressed leptin levels by a greater amount during hypoglycemia as compared to placebo. Studies in rat pre-adipocytes (26) have reported direct effect of sulfonylureas (glyburide) on leptin expression. Thus the acute reduction in leptin levels during hypoglycemia, but not euglycemia, is interesting and may indicate a “counterregulatory feeding signal”. Secondly, glimepiride increased pancreatic polypeptide responses during both euglycemia and hypoglycemia compared to placebo or glyburide. Pancreatic polypeptide (PP) is a partial marker for parasympathetic nervous system activity that is released from specific PP cells in pancreatic islets. Thus the increased PP responses may indicate direct parasympathetic nervous system stimulation or more likely differential pancreatic islet PP cell activation by glimepiride.

Sulfonylureas act on (SURs) which are components of K_{ATP} channels. K_{ATP} channels are widely expressed on various tissues including pancreas, neurons, skeletal smooth muscle and cardiac muscle (5, 27). Pancreatic K_{ATP} channels can be further subdivided according to the specific form of inwardly rectifying potassium channel (K_{ir} 6.0, 6.1. and 6.2) and SUR (SUR1 and SUR2) subunits of which they are comprised (5, 27–30). Previous animal studies have determined that SUR1 and K_{ir} 6.2 subunit combinations are expressed differentially on PP, alpha, delta and beta pancreatic islet cells (5).

The above establishes a role for differential physiologic effects by sulfonylureas on specific islet cells. Additionally, sulfonylureas also differ in receptor binding affinity, potency and elimination (31). Furthermore, Kramer et al (32, 33) have reported that individual sulfonylureas can interact with differing components of K_{ATP} channels. The same group has also demonstrated that glimepiride had a 3–4 fold lower binding affinity as compared to glyburide (32, 33). Although this study cannot identify the precise molecular mechanisms responsible for the differing physiologic effects of glimepiride and glyburide, the above studies certainly point to a plausible physiologic basis for our findings. The present study was conducted in non-diabetic individuals with normally functioning islet cells (beta and alpha). This provides an understanding of the normal physiologic responses occurring after the acute administration of the two sulfonylureas. Further studies could investigate the acute and perhaps the chronic effects of glimepiride and glyburide on integrated counterregulatory responses in T2DM humans.

In summary, this study has investigated the acute physiologic in-vivo differences of glimepiride and glyburide in healthy individuals. During hypoglycemia glyburide increased insulin levels, but resulted in decreased glucagon, decreased combined epinephrine and norepinephrine and reduced endogenous glucose production counterregulatory responses. Leptin levels were more greatly suppressed by both sulfonylureas as compared to placebo during hypoglycemia whereas insulin was equally stimulated during euglycemia by glyburide and glimepiride. In conclusion, this study has determined that glyburide can reduce the principal neuroendocrine and metabolic counterregulatory defenses against falling plasma glucose in healthy individuals. Our results provide further mechanistic

physiologic explanation for the observed increased incidence of hypoglycemia following glyburide as compared to glimepiride use in clinical practice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

T2DM	Type 2 Diabetes Mellitus
SURs	Sulfonylurea receptors
ATP	Adenosine triphosphate
K+	Potassium
BMI	body mass index
HbA1c	glycated hemoglobin
hr.	hour
mg/kg/min	milligram/kilogram/minute
APE	atom percent excess
CV	coefficient of variation
HPLC	high-performance liquid chromatography
EGP	endogenous glucose production
GIR	glucose infusion rate
mmHg	millimeters of mercury
MAP	mean arterial pressure
VMH	ventromedial hypothalamus
Hypo	hypoglycemia
Eugly	euglycemia

Figure 1. Experimental protocol and plasma glucose levels during hyperinsulinemic euglycemic (4.9 ± 0.1 mmol/L) and hypoglycemic (2.9 ± 0.1 mmol/L) clamps in healthy overnight fasted individuals.

Figure 2. Insulin, C-peptide and glucagon levels during hyperinsulinemic euglycemic ($4.9 \pm 0.1 \text{ mmol/L}$) and hypoglycemic ($2.9 \pm 0.1 \text{ mmol/L}$) clamps following glyburide 10mg or glimepiride 4mg in healthy overnight fasted individuals.

Figure 3. Rates of endogenous glucose production (EGP), glucose disappearance (RD) and glucose infusion rate (GIR) during hyperinsulinemic euglycemic (4.9 ± 0.1 mmol/L) and hypoglycemic (2.9 ± 0.1 mmol/L) clamps following glyburide 10mg or glimepiride 4mg in healthy overnight fasted individuals.

Figure 4. Change from baseline of pancreatic polypeptide and leptin levels during hyperinsulinemic euglycemic (4.9 ± 0.1 mmol/L) and hypoglycemic (2.9 ± 0.1 mmol/L) clamps following glyburide 10mg or glimepiride 4mg in healthy overnight fasted individuals.

Table 1

Neuroendocrine and Metabolic responses during clamped hyperinsulinemic euglycemia and hypoglycemia (2.9 ± 0.1 mmol/L)

	Placebo				Glimepiride				Glyburide			
	Euglycemia		Hypoglycemia		Euglycemia		Hypoglycemia		Euglycemia		Hypoglycemia	
	Basal	Final	Basal	Final	Basal	Final	Basal	Final	Basal	Final	Basal	Final
Insulin pmol/L	42±6	435±26*	65±10	398±31*	61±10	592±54*†	72±8	405±18*	39±5	581±54*‡	101±25	492±35*‡
C-peptide nmol/L	0.63±0.21	0.4±0.1	0.7±0.2	0.1±0.02*	0.5±0.1	0.9±0.2*†	0.8±0.2	0.3±0.1*	0.7±0.1	1.5±0.3*‡	1.1±0.3	0.5±0.2*
Glucagon ng/L	51.3±5.7	42.6±5*	53.5±6.5	132±14*	46±5	39±5*	54±6	135±21*	50±5	39±4*	56±5	91±9*‡
Epinephrine pg/mL	40±7	36±5	32±4	855±78*	32±4	36±4	32±5	780±103*	42±5	45±5	41±4	672±98*
Norepinephrine pg/mL	187±32	267±34*	229±20	380±27*	230±22	284±86*	226±32	374±31*	224±29	255±27	219±34	339±32*
Cortisol nmol/L	343±54	331±38	338±50	956±21*	338±55	357±37	395±61	967±72*	426±62	405±77	339±56	323±104
GH µg/L	0.8±2.3	0.8±0.2	0.7±0.2	17±3*	0.9±0.4	1.4±0.9	0.6±0.3	24±8*	0.9±0.3	1.6±0.7	0.5±0.1	20±4*
Glycerol µmol/L	68±10	42±8*	64±8	54±6	68±13	42±12*	74±14	56±8*	74±12	37±7*	64±12	43±7*
Lactate mmol/L	0.9±0.1	1.2±0.2*	1±0.1	1.5±0.2*	0.7±0.1	1.14±0.1*	0.9±0.1	1.6±0.1*	0.8±0.1	1.2±0.1*	0.9±0.1	1.4±0.2*

* p<0.05 compared to baseline

† p<0.05 compared to euglycemia placebo final

‡ p<0.05 compared to hypoglycemia placebo and Glimepiride final
Values are means ±SE

Table 2
Pancreatic Polypeptide and Leptin responses during clamped hyperinsulinemic euglycemia and hypoglycemia (2.9±0.1mmol/L)

	Placebo		Glimepiride		Glyburide	
Euglycemia						
	Basal	Final	Basal	Final	Basal	Final
Pancreatic Polypeptide (ng/L)	95±18	74±20	71±11	73±17	88±14	73±12
Leptin (ng/ml)	12.2±3.9	12.5±4	11±3.2	11.2±3.2	10.8±3.4	10.7±3.3
Hypoglycemia						
	Basal	Final	Basal	Final	Basal	Final
Pancreatic Polypeptide (ng/L)	111±22	808±162 *	126±47	1445±251 *†	98±11	1071±154 *
Leptin (ng/ml)	14.4±4	13.1±6	15.6±3.8	13±3.1 *	12.2±3.9	9.4±3 *

^{*} p<0.05 compared to baseline
[†] p<0.05 compared to hypoglycemia placebo
Values are means ± SE