Glyburide Increases the Secretion, Tissue Uptake, and Action of Insulin in Conscious Normal Dogs

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The action of glyburide on glucose homeostasis involves pancreatic and extrapancreatic mechanisms. The relative importance of each of these processes in the hypoglycemic response to sustained administration of glyburide is unknown. In addition, the effect of this drug on the hepatic extraction of insulin is controversial. This investigation uses direct techniques in conscious normal dogs to examine the impact of glyburide therapy (2.5 mg twice daily for 4 weeks) on glucose homeostasis. Preparatory surgery included placement of Doppler flow probes on hepatic vessels and insertion of catheters in carotid artery, portal vein, hepatic vein, and renal vein. After recovery from surgery, animals underwent an intravenous glucose tolerance test ([IGTT] 0.3 g · kg^{-1} intravenous glucose bolus) and an insulin infusion clamp test ([IICT] 2 mU · kg^{-1} · min^{-1} intravenous insulin during 150 minutes) followed by glyburide therapy. After 4 weeks, the IGTT and IICT were repeated. Glyburide increased the insulin secretory response during the late phase of the IGTT and augmented glucose clearance during the IICT. Hepatic extraction of insulin was also stimulated by glyburide. We conclude that the hypoglycemic action of long-term glyburide administration involves stimulation of both insulin secretion by the pancreas and glucose disposal by peripheral tissues. In addition, glyburide augments the extraction of insulin by the liver, and such an effect might prevent the development of sustained high levels of insulin in blood perfusing peripheral tissues.

THE MECHANISMS of action of oral sulfonylureas are complex, incompletely understood, and to some extent differ among them.[1,2] Since these drugs are capable of enhancing insulin secretion and are ineffective in insulin-dependent diabetes, their antihyperglycemic action has been considered to result mostly from stimulatory effects on insulin release by the β cells of the pancreas. Increased insulin secretion appears to be the dominant action with short-term administration of 2 weeks or less.[3] By contrast, extrapancreatic actions that involve the liver, skeletal muscle, and adipose tissue may have a major role in the long-term antihyperglycemic effects of oral sulfonylureas.[1,4,5] An increased systemic availability of insulin resulting from a reduction in the hepatic extraction of this hormone has also been proposed to play a role in the effects of sulfonylureas on glucose homeostasis.[3,6-9] The enhanced pancreatic secretion and reduced hepatic clearance of insulin by sulfonylureas should increase plasma insulin levels. Yet plasma insulin levels are not high during long-term therapy with these drugs. This paradox might be due to diminished insulin secretion in response to the lower glucose concentration in the extracellular fluid. In addition, an increased tissue response to insulin might further reduce the demand for insulin secretion. Direct measurement of these parameters might clarify these issues.

The diminished hepatic clearance of insulin with sulfonylurea therapy reported by other investigators is based on data acquired with indirect techniques. We hypothesize that the glucose-lowering effects of sulfonylureas acting on hepatic and extrahepatic tissues would be associated with increased, not decreased, tissue uptake of insulin. This view would be consistent with our previous demonstration of the opposite phenomenon, ie, a decreased tissue uptake of insulin in a state of tissue resistance to this hormone, which develops in acidemic states.[10]

Using direct techniques in conscious dogs,[10-12] the current study examines the effects of long-term administration of glyburide on glucose homeostasis mediated by changes in plasma levels, metabolic effects, and/or tissue uptake of insulin. Our results demonstrate that glyburide exerts major pancreatic and extrapancreatic actions, with the latter including an increased hepatic uptake of insulin and an augmented peripheral-tissue response to insulin.

MATERIALS AND METHODS

Studies were performed on 30 adult mongrel dogs of either sex ranging in weight from 19 to 31 kg. Preparatory surgery was performed after an overnight fast under general anesthesia with intravenous pentobarbital sodium (30 mg/kg body weight) as previously described.[10] After a midline incision, pulse range-gated ultrasonic Doppler flow probes were placed around the portal vein and hepatic artery, and catheters were positioned in a carotid artery and the portal vein, common hepatic vein, and renal vein (left or right). These catheters exited at the back of the neck and were occluded with short stainless wire plugs. Catheters were flushed daily with 2 mL heparinized saline (50 U/mL) to ensure patency. Baseline studies were performed after at least a 2-week period of recovery from surgery. Only animals who appeared in healthy condition, with good appetites and normal stools, were used. Upon completion of these studies in the control period (details to follow), the animals received an oral dose of 2.5 mg glyburide twice daily for a 4-week period. We chose a relatively high dosage of this oral sulfonylurea to better expose its effects on glucose homeostasis. Studies similar to those performed in the control state were performed after glyburide therapy.

Experimental Protocols

All studies were performed in conscious dogs trained to remain quiet in "ad hoc" slings. The animals were fasted overnight; dogs in

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the glyburide-treated period did not receive the drug the morning of the experimental protocol. Urine was collected via a urethral catheter. Physiologic saline solution was infused to replace the volume of blood loss due to sampling. Three control blood samples drawn 10 minutes apart (−20, −10, and 0 minutes) for assay of glucose, insulin, and glucagon were obtained simultaneously from all catheterized vessels. All animals underwent two experimental protocols: an intravenous glucose tolerance test (IGTT) and an insulin infusion clamp test (IICT).

IGTT

A 0.3-g/kg body weight glucose solution (50%) was infused as a bolus dose at time 0 minutes in a peripheral vein. Blood samples were drawn thereafter from all vessels at 15-minute intervals for 180 minutes. Urine was collected throughout the protocol.

IICT

An interval of at least 3 days separated this test from the IGTT. Insulin was infused in the portal vein at 2 $mU \cdot kg^{-1} \cdot min^{-1}$ from 0 to 150 minutes. Hypoglycemia was prevented by infusion of dextrose (5%) into the external jugular vein at rates sufficient to maintain basal glucose concentration. Basal glucose levels in arterial blood during the clamp studies were 88 ± 5 and 61 ± 7 mg/100 mL ($P < .01$) in control and glyburide-treated dogs, respectively. Adjustments of glucose infusion rates were based on plasma glucose determinations made every 5 minutes. Blood samples from all vessels were obtained every 15 minutes for 180 minutes.

Analytical Methods

Blood samples for determination of plasma glucose, insulin, and glucagon were collected in chilled tubes containing 500 U Trasylol (FBA Pharmaceutical, New York, NY) and 1.2 mg EDTA per milliliter of blood. Glucose levels were measured by a glucose autoanalyzer (Beckman Instruments, Fullerton, CA) using a glucose oxidase method. Plasma immunoreactive insulin was assayed with dextran-coated charcoal,[13] and glucagon with Unger's 30K antibody.[14] Blood flow was measured with an ultrasonic range-gated pulsed Doppler flow meter.[12] Blood flow measurements were corrected for plasma flow based on hematocrits.

Calculations

Hepatic vein plasma flow was taken as the sum of plasma flows in the portal vein and hepatic artery. Fractional hepatic extraction of insulin was calculated according to the formula, [(hormone presented to the liver − hormone leaving the liver)/hormone presented to the liver] × 100, where the amount of hormone presented to the liver was the sum of the contribution from the portal vein and hepatic artery (concentration × flow), and the amount leaving the liver was the product of hepatic vein concentration times hepatic vein plasma flow. Net hepatic glucose balance was determined by the equation, (glucose leaving the liver − glucose presented to the liver)/body weight. A positive balance indicates hepatic glucose output, and a negative balance, hepatic glucose uptake.

Insulin secretion was estimated as the difference in plasma insulin level between samples drawn from the portal vein and carotid artery, multiplied by the portal plasma flow. Insulin action in the control period and during glyburide treatment was examined by measuring insulin-stimulated glucose uptake using glucose clearance.[15] Glucose clearance was calculated as the ratio of glucose consumption (ie, glucose infusion rate plus hepatic glucose production) during insulin infusion to the simultaneously measured arterial glucose level.

Statistical analyses were performed using ANOVA for paired or unpaired groups of data as appropriate. A nonlinear regression function was modeled with the data points for each glucose clearance evaluated in control and glyburide-treated animals. Individual nonlinear regressions were subsequently combined to obtain the glucose clearance value for control and glyburide groups. Statistical significance was set at P less than .05.

RESULTS

General Remarks

The dogs remained easy to manage, and none of the protocols appeared to have any ill effects. The term "basal" is used in connection with pooled data from samples drawn at −20, −10, and 0 minutes. The terms "early" and "late" for the IGTT and IICT protocols are used in connection with pooled data from samples drawn between 0 and 75 minutes for the former and between 75 and 150 minutes for the latter.[10]

Plasma Flow Values

Table 1 summarizes results for the IGTT and IICT protocols. Glyburide-treated dogs had a slight but statistically significant lower hepatic flow in the basal state as compared with controls in the IGTT protocol. In addition, plasma flow in the hepatic vein decreased mildly but significantly in the late phase as compared with baseline in control and glyburide-treated dogs in the IGTT and IICT protocols. The percent contribution of hepatic artery blood flow to total hepatic blood flow during both study protocols was not altered by administration of glyburide.

Table 1. Plasma Flows for the IGTT and IICT Protocols in the Portal Vein, Hepatic Artery, and Hepatic Vein in Control and Glyburide-Treated Dogs

	Portal Vein	Hepatic Artery	Hepatic Vein
IGTT protocol			
Basal			
Control	16 ± 1.3	6 ± 0.7	22 ± 1.7
Glyburide	14 ± 1.0*	5 ± 0.6*	19 ± 1.1*
Early phase			
Control	16 ± 1.3	6 ± 0.6	22 ± 1.6
Glyburide	15 ± 1.0	6 ± 0.7	21 ± 1.2
Late phase			
Control	14 ± 1.4	5 ± 0.6	19 ± 1.7
Glyburide	12 ± 0.8	5 ± 0.8	17 ± 1.1
IICT protocol			
Basal			
Control	15 ± 1.3	6 ± 0.7	21 ± 1.7
Glyburide	15 ± 1.5	5 ± 0.7	20 ± 2.0
Early phase			
Control	14 ± 1.2	6 ± 0.7	20 ± 1.7
Glyburide	13 ± 1.3	5 ± 0.6	18 ± 1.7
Late phase			
Control	12 ± 1.3§	6 ± 0.9	18 ± 1.8
Glyburide	11 ± 1.2	5 ± 0.7	16 ± 1.7

*$P < .05$ v control during same period.
†$P < .05$ v control for basal and early data.
‡$P < .05$ v glyburide, early data.
§$P < .05$ v same group, basal level.

Plasma Glucose Levels

Figure 1 depicts plasma glucose levels during the IGTT protocol in control and glyburide-treated dogs. Basal levels were 93 ± 5 and 67 ± 7 mg/100 mL ($P < .01$) in the carotid artery, 100 ± 4 and 77 ± 8 mg/100 mL ($P < .01$) in the hepatic vein, 92 ± 4 and 67 ± 7 mg/100 mL in the portal vein ($P < .01$), and 99 ± 5 and 77 ± 9 mg/100 mL in the renal vein ($P < .01$) in control and glyburide-treated dogs, respectively. Plasma glucose levels in the basal state were highest in the hepatic vein and renal vein. At 15 minutes after glucose infusion, plasma glucose levels were 162 ± 5 and 140 ± 10 mg/100 mL ($P < .01$) in the carotid artery in control and glyburide groups, respectively. After 15 minutes, a progressive decrease in plasma glucose levels was observed in all vessels for the two study groups. At all times in all vessels, the control group had higher plasma glucose levels than the glyburide group. Differences were statistically significant at 30 minutes and 90 through 180 minutes in all vessels.

Plasma Level and Secretory Rate of Insulin

The insulin secretory response was evaluated in control and glyburide-treated dogs during the IGTT. Plasma insulin levels in samples from the portal vein, carotid artery, hepatic vein, and renal vein in control and glyburide-treated groups are depicted in Fig 2. At all points of observation, the portal vein and renal vein had the highest and lowest plasma insulin levels, respectively, in both study groups. Basal plasma insulin levels were not significantly different between the two study groups (54 ± 8 and 52 ± 5 μU/mL in portal vein, 24 ± 3 and 25 ± 4 μU/mL in hepatic vein, 17 ± 3 and 21 ± 4 μU/mL in carotid artery, and 10 ± 2 and 12 ± 2 μU/mL in renal vein in control and glyburide groups, respectively). At 15 minutes after glucose infusion, the maximal response was observed: insulin levels were 214 ± 19 and 139 ± 16 μU/mL ($P < .01$) in the portal vein in control and glyburide groups, respectively. Conversely, the glyburide group had significantly higher insulin levels than the control group at 90 through 180 minutes.

Figure 3 depicts the effects of the IGTT on insulin secretion in control and glyburide groups. The basal rate of insulin secretion was 620 ± 124 and 438 ± 70 μU/kg/min ($P < .05$) in control and glyburide groups, respectively. As expected, a brisk increase in the insulin secretory rate was observed immediately after glucose infusion in both groups, with the highest value detected at 15 minutes. The glyburide group had a lower insulin secretory rate than the controls (718 ± 125 and 1,177 ± 203 μU/kg/min, $P < .01$) in the early phase, but a higher rate in the late phase (555 ± 124 and 346 ± 83 μU/kg/min, $P < .01$). Thus, a delayed augmentation of the insulin secretory response to a glucose load corresponding to the second phase of the IGTT was found in the glyburide group.

Hepatic Extraction of Insulin

Glyburide-treated animals had consistently higher hepatic extraction of insulin than the control group and did not show a significant change in response to both the IGTT and IICT compared with basal levels. Figure 4 depicts hepatic extraction of insulin in the basal state, in the IICT, and in the IGTT for the control and glyburide groups. Hepatic insulin extraction was 44% ± 2% and 50% ± 2% ($P < .05$) at baseline, 40% ± 2% and 45% ± 1% ($P < .05$) during the IICT, and 46% ± 2% and 50% ± 2% ($P < .08$)

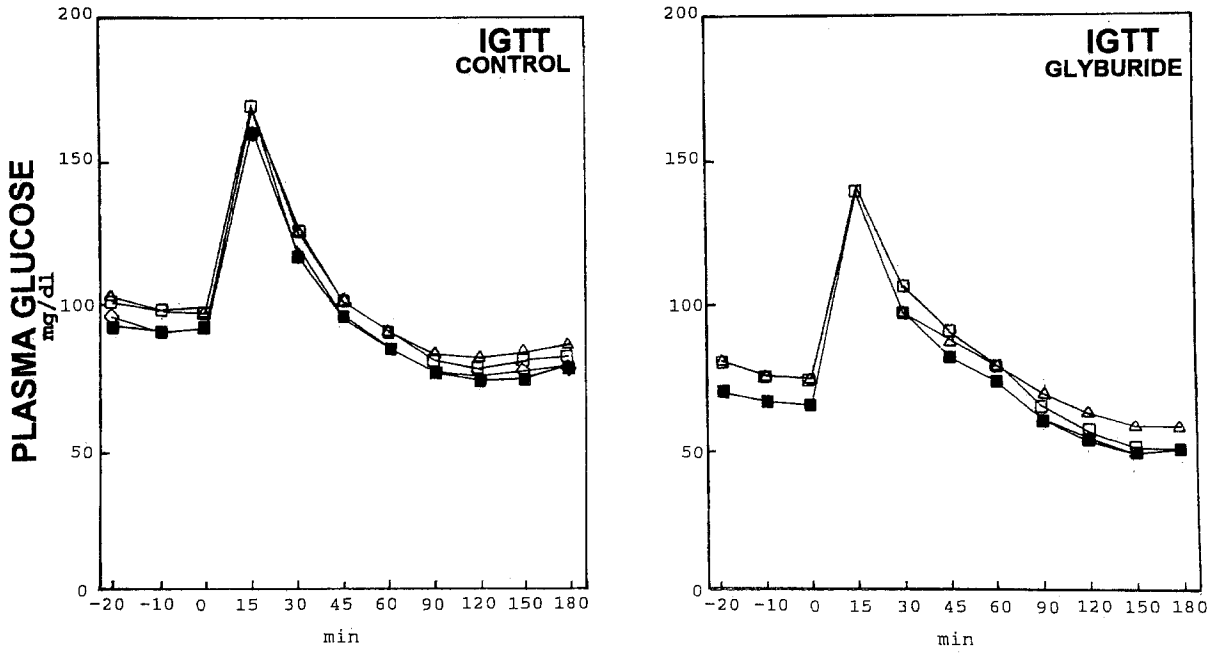

Fig 1. Plasma glucose levels in the basal state and after the IGTT in control and glyburide-treated dogs. Each symbol represents the mean value of all studies at the indicated time of sampling for each of the following vessels: (◇) carotid artery, (■) portal vein, (△) hepatic vein, and (□) renal vein.

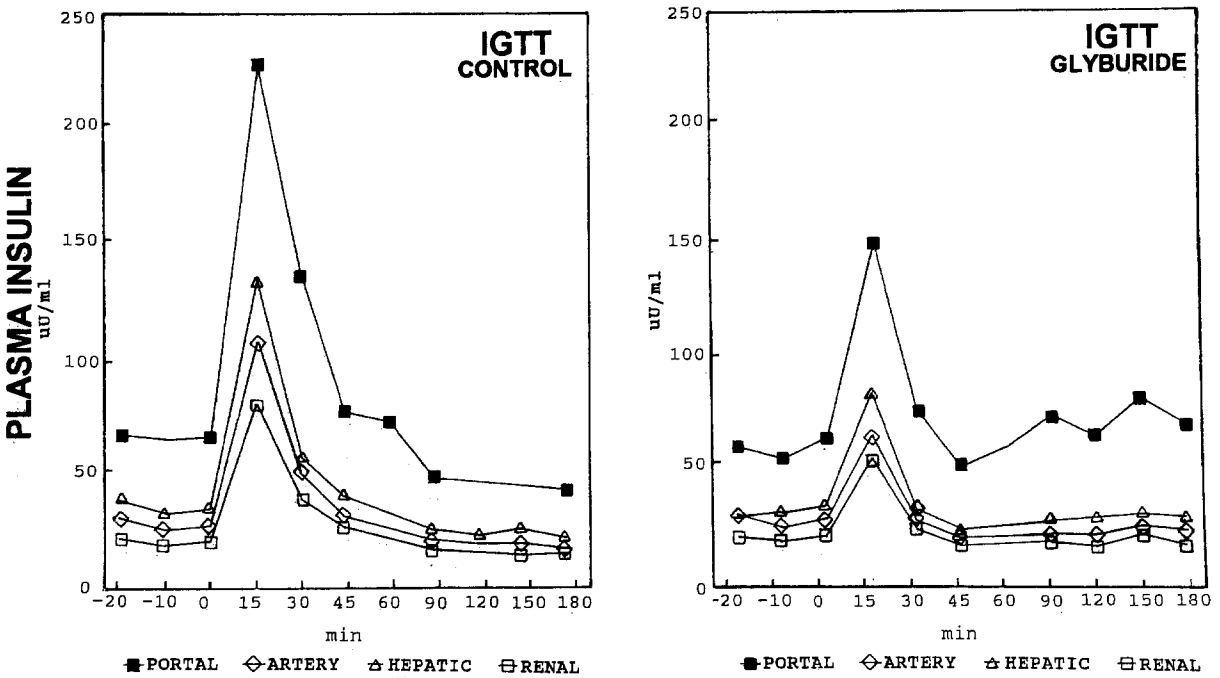

Fig 2. Plasma insulin levels in the basal state and after the IGTT in control and glyburide-treated dogs. Each symbol represents the mean value of all studies at the indicated time of sampling for each of the following vessels: (◇) carotid artery, (■) portal vein, (△) hepatic vein, and (□) renal vein.

during the IGTT in control and glyburide groups, respectively. Plasma insulin levels in control and glyburide groups during the insulin clamp were as follows. The early plasma insulin levels were 279 ± 9 and 224 ± 8 μU/mL ($P < .01$) in the portal vein, 127 ± 8 and 95 ± 7 μU/mL ($P < .01$) in the hepatic vein, 142 ± 9 and 81 ± 8 μU/mL ($P < .01$) in the carotid artery, and 80 ± 7 and 64 ± 6 μU/mL ($P < .01$) in the renal vein in control and glyburide groups, respectively. The late plasma insulin levels were 493 ± 9 and 322 ± 8 μU/mL ($P < .01$) in the portal vein, 229 ± 8 and

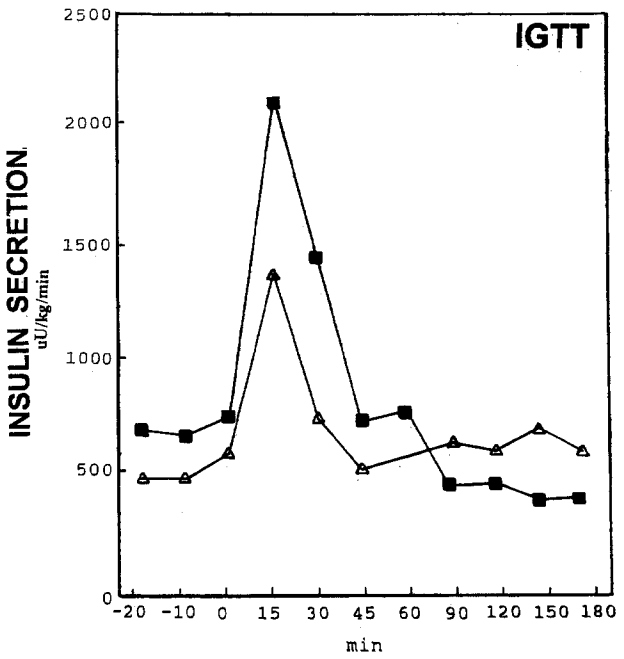

Fig 3. Insulin secretory rate in the basal state and after the IGTT in (■) control and (□) glyburide-treated dogs. Each symbol represents the mean value of all studies at the time indicated.

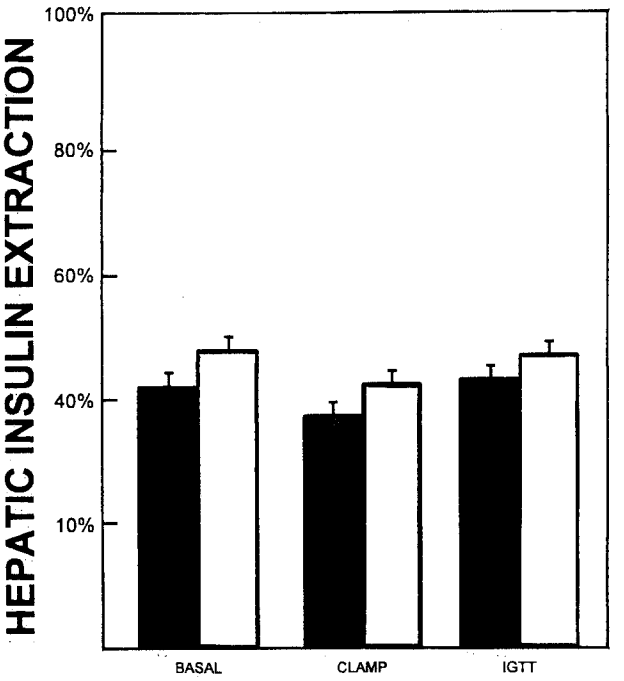

Fig 4. Hepatic extraction of insulin in the basal state and during the IGTT and IICT (clamp) for the (■) control and (□) glyburide group. Each column represents the mean ± SE of all points of observation in the basal state or in each of two experimental protocols.

136 ± 7 μU/mL ($P < .01$) in the hepatic vein, 219 ± 9 and 104 ± 8 μU/mL ($P < .01$) in the carotid artery, and 131 ± 7 and 81 ± 6 μU/mL ($P < .01$) in the renal vein in control and glyburide groups, respectively. Postinfusion results were 266 ± 6 and 116 ± 5 μU/mL ($P < .01$) in the portal vein, 86 ± 5 and 53 ± 5 μU/mL ($P < .05$) in the hepatic vein, 106 ± 6 and 47 ± 5 μU/mL ($P < .01$) in the carotid artery, and 50 ± 5 and 32 ± 4 μU/mL ($P < .07$) in the renal vein in control and glyburide groups, respectively. Plasma insulin levels were significantly lower at all points of observation during the IICT protocol in glyburide-treated dogs versus controls.

Plasma Glucagon Levels

Basal plasma glucagon levels were 246 ± 25 and 288 ± 44 pg/mL (nonsignificant [NS]) in the portal vein in control and glyburide groups, respectively. Control dogs receiving the IGTT had a significant increase in glucagon levels at 180 minutes compared with the corresponding basal values, and such a hormonal response occurred in association with the hypoglycemia of the late post–glucose infusion period. The insulin clamp significantly decreased plasma glucagon in all vessels in the two study groups. The early results were 161 ± 14 and 206 ± 21 pg/mL ($P < .05$) in the portal vein, 126 ± 17 and 137 ± 13 pg/mL (NS) in the hepatic vein, 102 ± 10 and 137 ± 14 pg/mL ($P < .05$) in the carotid artery, and 82 ± 7 and 136 ± 13 pg/mL ($P < .05$) in the renal vein in control and glyburide groups, respectively. The late results were 88 ± 6 and 135 ± 13 pg/mL ($P < .01$) in the portal vein, 74 ± 5 and 89 ± 5 pg/mL ($P < .05$) in the hepatic vein, 65 ± 3 and 82 ± 5 pg/mL ($P < .01$) in the carotid artery, and 65 ± 3 and 79 ± 4 pg/mL ($P < .01$) in the renal vein in control and glyburide groups, respectively. Plasma glucagon values at 180 minutes were 89 ± 12 and 125 ± 17 pg/mL (NS) in the portal vein, 71 ± 6 and 80 ± 6 pg/mL (NS) in the hepatic vein, 65 ± 4 and 81 ± 6 pg/mL ($P < .05$) in the carotid artery, and 63 ± 4 and 81 ± 7 pg/mL ($P < .05$) in the renal vein in control and glyburide groups, respectively. Plasma glucagon levels were higher at most points of observation in the glyburide group than in the control group, and the differences reached statistical significance at many points.

Hepatic Glucose Production

Figure 5 depicts the effects of the insulin infusion protocol on hepatic glucose production in the two study groups. A major significant suppression of hepatic glucose output was observed with the IICT in both groups. In the control group, levels were 9.7 ± 1.1 (basal), 3.6 ± 0.6 (early), 2.6 ± 0.4 (late), and 2.4 ± 0.6 (postinfusion) μmol/kg/min. Hepatic glucose output in the glyburide group was 9.3 ± 1.0 (basal), 2.5 ± 0.4 (early), 1.6 ± 0.3 (late), and 1.7 ± 0.4 (postinfusion) μmol/kg/min. Hepatic glucose production was lower in the glyburide group in comparison to the control group, and the difference reached significance for the early data. Hepatic glucose production in the control group was 2.6 ± 0.4 μmol/kg/min (late); the corresponding value for the glyburide group was 1.6 ± 0.3 μmol/kg/min ($P < .05$).

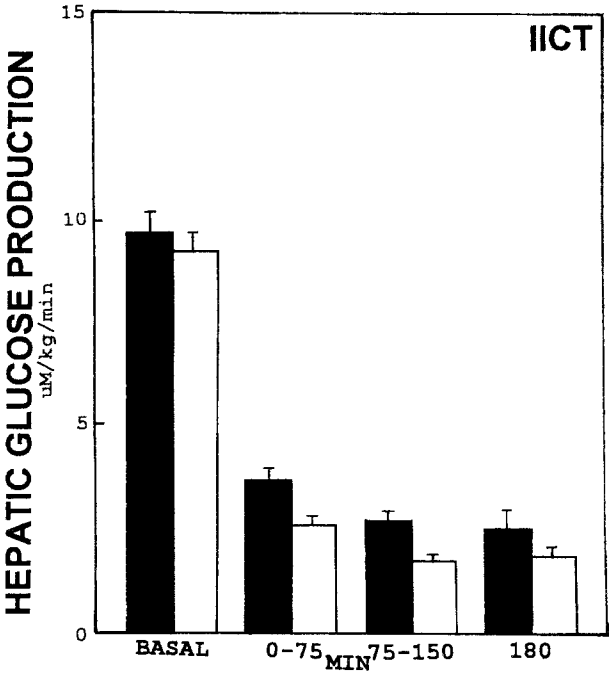

Fig 5. Net hepatic glucose production in the basal state and in response to the IICT in (■) control and (□) glyburide-treated dogs. Each column represents the mean ± SE of all studies at the time indicated.

Glucose Clearance

Insulin-induced stimulation of peripheral glucose consumption as assessed by glucose clearance during the IICT is depicted in Fig 6. A progressive time-dependent increase in glucose clearance was observed in the two study groups over the 150-minute period of insulin infusion. The combined nonlinear regression function that describes glucose clearance for the glyburide group was significantly higher

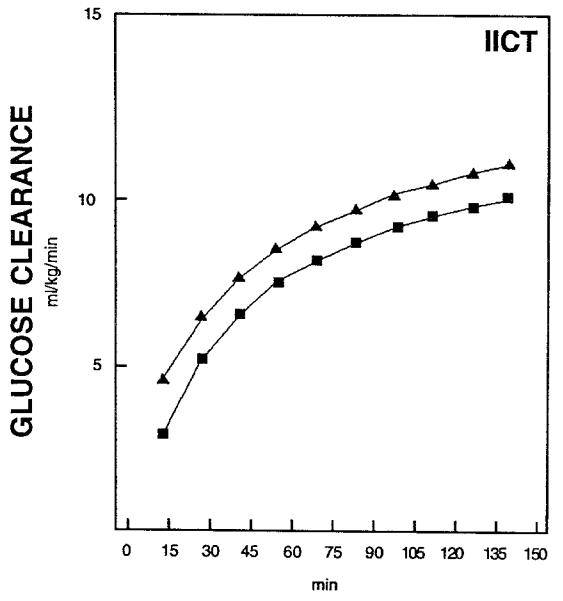

Fig 6. Glucose clearance in response to the IICT in (■) control and (▲) glyburide-treated dogs.

($P < .05$) than that for the control group. Values observed at 75 minutes from the start of insulin clamp were 7.8 ± 0.7 and 8.9 ± 0.8 mL/kg/min ($P < .05$) in control and glyburide groups, respectively. Thus, glyburide increased the tissue response to insulin in the normal dog.

DISCUSSION

The present study demonstrates that administration of glyburide for 4 weeks produces extrapancreatic effects in tissue uptake of both glucose and insulin. The data support the notion advanced by other investigators[16-18] that sulfonylureas alter hepatic extraction of insulin, increase insulin action on peripheral tissues, and stimulate insulin secretion during the late phase of glucose infusion.

Our results establish that glyburide increases the hepatic extraction of insulin (Fig 4). This finding, demonstrated in conscious dogs evaluated with direct techniques, was first reported in a preliminary study from our laboratory[19]; shortly thereafter, other investigators demonstrated that hepatic insulin extraction increases with glyburide administration in an in vitro preparation.[2] Although early studies have shown that sulfonylureas increase insulin binding to specific receptors of isolated hepatocyte membranes,[20-22] more recent studies performed with hepatocytes in tissue cultures did not find such effects.[23-27] In addition, others have proposed that sulfonylureas reduce hepatic clearance of insulin.[3,6-9] Consequently, the effects of sulfonylureas on hepatic insulin uptake have been controversial. Recent studies that evaluated single-pass uptake of insulin by the perfused liver[2] demonstrated a stimulatory effect of glyburide as compared with control conditions. The present investigation establishes that this oral sulfonylurea augments hepatic extraction of insulin in conscious animals. In theory, the increased extraction of insulin may be a primary drug effect on the liver, or may be secondary to a drug-induced augmentation of insulin secretion. Since plasma levels of insulin during the insulin clamp were lower at all points of observation in the sulfonylurea-treated group, we propose that the greater liver uptake of insulin in the glyburide group was not the result of a higher rate of delivery of this hormone. Consequently, we envision that a primary direct effect of glyburide on extrapancreatic tissues such as the liver and possibly the skeletal muscle is likely present, since the enhanced uptake of insulin demonstrated in the liver was associated with an increased response to this hormone. A primary decrease in hepatic insulin extraction that was independent of changes in insulin secretion and accompanied by a diminished sensitivity to this hormone was previously identified and reported by our laboratory in acidemic states.[10] We propose that glyburide exerts an overall response of a comparable nature but with an effect opposite to that occurring in acidemia with respect to the physiologic action and hepatic extraction of insulin.

The glyburide-induced increased hepatic extraction of insulin shown in this study will tend to decrease insulin levels in peripheral blood. However, the modest increase in hepatic insulin extraction in the glyburide-treated animals is not large enough to explain the substantially lower plasma insulin values observed during the clamp in this group. A lower basal insulin secretion, increased peripheral insulin receptors, and upregulation of receptors secondary to lower basal insulin levels might have contributed to the lower insulin levels during the clamp in the glyburide group. The evidence presented here contradicts studies supporting the notion that sulfonylurea treatment of type II diabetes promotes chronic hyperinsulinemia due to its effect on the liver,[3,6-9] and as a consequence is counterproductive because it might lead to more severe insulin resistance and possibly other side effects. However, it must be recognized that this study examines the effects of glyburide in normal dogs; thus, caution should be exercised in the extrapolation of our results to the diabetic state.

The existence of both pancreatic and extrapancreatic effects, other than the previously discussed increased hepatic extraction of insulin, was also documented in this study with long-term administration of glyburide. The lower plasma glucose levels observed in dogs treated long-term with glyburide as compared with control animals during fasting and following glucose loading might have secondarily altered insulin secretion and glucose clearance. Consequently, caution must be exercised in interpreting these data with respect to the direct effects of the sulfonylurea. In the basal state, glyburide-treated animals had a lower plasma glucose concentration, but the absolute levels, as well as the secretion rate, of insulin were not elevated; these findings might reflect, in part, the effect of the prevailing lower plasma glucose concentration, which tends to decrease insulin secretion. In addition, the rate of basal glucose clearance was increased while glucose levels were lower in the glyburide-treated group. This may suggest that insulin action and/or insulin-independent glucose utilization were increased. Chronic hyperinsulinemia can either increase or decrease insulin action, depending on the dose of insulin used.[28-30] When low-dose insulin is used and hypoglycemia does not occur, insulin resistance develops—conversely, when high-dose insulin is used and hypoglycemia develops, insulin action improves. Thus, the presence of hypoglycemia may mask some of the physiological effects of hyperinsulinemia, and this may be related to the activation of the adrenergic nervous system. Our data indicate that glyburide does enhance basal glucose clearance and improve insulin-stimulated glucose uptake, and these changes may be in part due to excess insulin and decreased plasma glucose levels rather than to direct effects of glyburide on insulin action.

The lower fasting glucose levels in glyburide-treated dogs versus controls resulted in a weaker stimulus for insulin secretion, which might account for the lower basal insulin secretion in the former group. Examination of the insulin secretory rate in response to a standard intravenous glucose load also showed lower plasma insulin levels in portal blood in the glyburide group, which would tend to support that long-term oral intake of this sulfonylurea fails to elicit insulinotropic effects. Yet the stimulus for insulin secretion in response to glucose infusion was of lower intensity in the glyburide-treated group based on the lower plasma glucose level observed immediately after the bolus dose. This resulted in lower plasma insulin levels and pancreatic insulin output than in control animals in the early period. However, a delayed insulin secretory response that per-

sisted longer (late period, Fig 3) was found in glyburide-treated animals as compared with the control group. Administration of the oral sulfonylurea was also associated with other actions on extrapancreatic tissues.

The insulin-induced increment in glucose disposal observed with the insulin clamp (IICT), as evaluated with glucose clearance, was significantly larger in the glyburide-treated group in comparison to the control group (Fig 6). Consequently, the salutary effects on glucose homeostasis of long-term administration of glyburide include an increased glucose clearance in peripheral tissues.

Long-term administration of glyburide altered the levels of glucagon, since portal blood values in the fasting state and after glucose infusion were higher in glyburide-treated dogs compared with control animals. Yet, significant differences in the estimated rate of glucagon secretion were not detected between the two groups with any protocol. The higher glucagon levels observed during the basal state in the glyburide group might be explained as a counterregulatory response to the lower glucose level, but the reason for the elevated glucagon levels after glucose infusion is unclear. Despite the higher plasma glucagon levels in the glyburide group, hepatic glucose output was generally lower in comparison to control levels. This observation suggests a decreased response of the liver to glucagon-induced glucose release. The data are consistent with an effect of sulfonylureas that decreases the counterregulatory response by reducing glucagon-induced hepatic glycogenolysis.

Insulin infusion, as expected, significantly inhibited net hepatic glucose output in control and glyburide groups, and this effect was not different in the two groups of studies. Mean values for hepatic glucose production were lower in the glyburide group at all points of observation (Fig 5) yet did not attain statistical significance. Although glyburide inhibits the high hepatic glucose production that accompanies the diabetic state, this effect is less evident when this agent is administered to normal animals.

The mild reduction in splanchnic blood flow observed in glyburide-treated dogs is not unexpected, considering that these compounds are specific inhibitors of adenosine triphosphate (ATP)-dependent K^+ channels (K^+_{ATP}). Activation of K^+_{ATP} of vascular smooth muscle in response to administration of minoxidil and diazoxide, two K^+ channel activators that are among the most potent antihypertensive agents currently available, produces arteriolar dilation. Conversely, inactivation of K^+_{ATP} channels in the arteriolar wall by sulfonylureas[31] might account for the vascular constriction and reduction of blood flow in various tissues, including the abdominal viscera, reported in the current investigation.

In summary, this study demonstrates that long-term glyburide administration to the conscious dog has pancreatic and extrapancreatic effects on glucose homeostasis. The extrapancreatic effects include a glyburide-induced augmentation of glucose clearance, a derived index of glucose consumption in peripheral tissues, as well as stimulation of hepatic extraction of insulin.

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REFERENCES

1. Gerich JE: Oral hypoglycemic agents. N Engl J Med 321:1231-1245, 1989

2. Chen H, Hamel FG, Siford G, Duckworth WC: Alteration of rat hepatic insulin metabolism by glyburide and glipizide. J Pharmacol Exp Ther 264:1293-1298, 1993

3. Almer LO, Johansson E, Melander A, et al: Influence of sulphonylureas on the secretion, disposal and effect of insulin. Eur J Clin Pharmacol 22:27-32, 1982

4. Gavin JR III: Glyburide: New insights into its effects on the beta cell and beyond—Introduction. Am J Med 89:1S-2S, 1990 (suppl 2A)

5. Porte D Jr, Kahn SE: Mechanisms for hyperglycemia in type II diabetes mellitus: Therapeutic implications for sulfonylurea treatment—An update. Am J Med 90:8S-14S, 1991 (suppl 6A)

6. Scheen AJ, Lefebvre PJ, Luyckx AS: Glipizide increases plasma insulin but not C-peptide level after a standardized breakfast in type 2 diabetic patients. Eur J Clin Pharmacol 26:471-474, 1984

7. Beck-Nielsen H, Hother-Nielsen O, Andersen PH, et al: In vivo action of glibenclamide. Diabetologia 29:515A, 1986 (abstr)

8. Groop L, Luzi L, Melander A, et al: Different effects of glyburide and glipizide on insulin secretion and hepatic glucose production in normal and NIDDM subjects. Diabetes 36:1320-1328, 1987

9. Melander A, Lebovitz HE, Faber OK: Sulfonylureas. Why, which, and how? Diabetes Care 13:18-25, 1990 (suppl 3)

10. Adrogué HJ, Chap Z, Okuda Y, et al: Acidosis-induced glucose intolerance is not prevented by adrenergic blockade. Am J Physiol 225:E812-E823, 1988

11. Adrogué HJ: Nephrology Forum: Glucose homeostasis and the kidney. Kidney Int 42:1266-1282, 1992

12. Adrogué HJ, Chap Z, Ishida T, et al: Role of the endocrine pancreas in the kalemic response to acute metabolic acidosis in conscious dogs. J Clin Invest 75:798-808, 1985

13. Herbert V, Lau KS, Gottlieb CW, et al: Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25:1375-1379, 1965

14. Faloona GR, Unger RH: Glucagon, in Jaffe BM, Berman HR (eds): Methods of Hormone Radioimmunoassay. New York, NY, Academic, 1974, pp 317-330

15. Gottesman I, Mandarino L, Gerich J: Use of glucose uptake and glucose clearance for the evaluation of insulin action in vivo. Diabetes 33:184-191, 1984

16. Kolterman OG: The impact of sulfonylureas on hepatic glucose metabolism in type II diabetics. Diabetes Metab Rev 3:399-414, 1987

17. Beck-Nielsen H, Hother-Nielsen O, Pedersen O: Mechanism of action of sulphonylureas with special reference to the extrapancreatic effect: An overview. Diabetic Med 5:613-620, 1988

18. Del Prato S, Vigili de Kreutzenberg S, Riccio A, et al: Hepatic sensitivity to insulin: Effects of sulfonylurea drugs. Am J Med 90:29S-36S, 1991 (suppl 6A)

19. Adrogué HJ, Pena J, Comstock JP: Enhanced hepatic extraction of insulin (HExI) and glucagon (HExGn) by chronic glyburide therapy in normal dogs. Clin Res 39:445A, 1991 (abstr)

20. Feinglos MN, Lebovitz HE: Sulphonylureas increase the number of insulin receptors. Nature 276:184-185, 1978

21. Bachmann W, Bottger I, Haslbeck M, et al: Extrapancreatic

action of sulphonylureas: Effect of gliquidone on insulin and glucagon binding to rat liver plasma membranes. Eur J Clin Invest 9:411-415, 1979

22. Greenstein BD: Improved insulin receptor assay: Effects of an antidiabetic sulfonylurea on liver membrane insulin receptors from obese hyperglycemic mice. Br J Pharmacol 66:317-322, 1979

23. Dolais-Kitabgi J, Alengrin F, Freychet P: Sulphonylureas in vitro do not alter insulin binding or insulin effect on amino acid transport in rat hepatocytes. Diabetologia 24:441-444, 1983

24. Nowak SM, McCaleb ML, Lockwood DH: Extrapancreatic action of sulfonylurea: Hypoglycemic effects are not dependent on altered insulin binding or inhibition of transglutaminase. Metabolism 32:398-402, 1983

25. Salhanick AI, Konowitz P, Amatruda JM: Potentiation of insulin action by a sulfonylurea in primary cultures of hepatocytes from normal and diabetic rats. Diabetes 32:206-212, 1983

26. Fleig W, Noether-Fleig G, Fussgaenger R, et al: Modulation by a sulfonylurea of insulin-dependent glycogenesis, but not of insulin binding, in cultured hepatocytes. Evidence for a post-receptor mechanism of action. Diabetes 33:285-290, 1984

27. Davidson MB, Sladen G: Effect of glyburide on glycogen metabolism in cultured rat hepatocytes. Metabolism 36:925-930, 1987

28. Wardzala LJ, Hirshman M, Pofcher E, et al: Regulation of glucose utilization in adipose cells and muscle after long-term experimental hyperinsulinemia in rats. J Clin Invest 76:460-469, 1985

29. Rizza RA, Mandarino LJ, Genest J, et al: Production of insulin resistance by hyperinsulinaemia in man. Diabetologia 28:70-75, 1985

30. McGuinness OP, Myers SR, Neal D, et al: Chronic hyperinsulinemia decreases insulin action but not insulin sensitivity. Metabolism 39:931-937, 1990

31. Longmore J, Weston AH: The role of K^+ channels in the modulation of vascular smooth muscle tone, in Cook NS (ed): Potassium Channels. New York, NY, Wiley, 1990, pp 259-299