Dapagliflozin, a Selective SGLT2 Inhibitor, Improves Glucose Homeostasis in Normal and Diabetic Rats

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OBJECTIVE—The inhibition of gut and renal sodium-glucose cotransporters (SGLTs) has been proposed as a novel therapeutic approach to the treatment of diabetes. We have identified dapagliflozin as a potent and selective inhibitor of the renal sodium-glucose cotransporter SGLT2 in vitro and characterized its in vitro and in vivo pharmacology.

RESEARCH DESIGN AND METHODS—Cell-based assays measuring glucose analog uptake were used to assess dapagliflozin's ability to inhibit sodium-dependent and facilitative glucose transport activity. Acute and multi-dose studies in normal and diabetic rats were performed to assess the ability of dapagliflozin to improve fed and fasting plasma glucose levels. A hyperinsulinemic-euglycemic clamp study was performed to assess the ability of dapagliflozin to improve glucose utilization after multi-dose treatment.

RESULTS—Dapagliflozin potently and selectively inhibited human SGLT2 versus human SGLT1, the major cotransporter of glucose in the gut, and did not significantly inhibit facilitative glucose transport in human adipocytes. In vivo, dapagliflozin acutely induced renal glucose excretion in normal and diabetic rats, improved glucose tolerance in normal rats, and reduced hyperglycemia in Zucker diabetic fatty (ZDF) rats after single oral doses ranging from 0.1 to 1.0 mg/kg. Once-daily dapagliflozin treatment over 2 weeks significantly lowered fasting and fed glucose levels at doses ranging from 0.1 to 1.0 mg/kg and resulted in a significant increase in glucose utilization rate accompanied by a significant reduction in glucose production.

CONCLUSIONS—These data suggest that dapagliflozin has the potential to be an efficacious treatment for type 2 diabetes. ***Diabetes*** **57:1723–1729, 2008**

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EC_{50}, half-maximal response; FPG, fasting plasma glucose; SGLT, sodium-glucose cotransporter.

The inhibition of renal glucose reabsorption is a novel approach to the treatment of diabetes. In normal individuals, glucose present in the plasma is filtered by the kidneys, but virtually all of it is reabsorbed, such that <1% of glucose is excreted in urine (1,2). Inhibition of this reabsorption process is predicted to reduce the renal threshold for glucose, allowing the excretion of excess glucose in the urine and thus lowering plasma glucose levels. Because this mechanism of action does not require insulin secretion or insulin action to effect glucose lowering, it could be efficacious in a wide variety of diabetic patients. Furthermore, since hyperglycemia per se has been shown to reduce insulin sensitivity and impair β-cell function in animal models, the correction of hyperglycemia is predicted to improve these important physiological defects in type 2 diabetes (3–10).

Significant nonclinical proof of concept exists for this approach to antihyperglycemic therapy. Phlorizin, a relatively nonselective but potent inhibitor of the sodium-glucose cotransporters (SGLTs), which mediate renal reabsorption and intestinal absorption of glucose (11), has been demonstrated to be glucosuric and antihyperglycemic in diabetic animal models (3–10). Studies with T-1095, a renal SGLT inhibitor, have also shown similar antidiabetic effects (12–16).

Inhibition of the SGLTs is complicated by differences in two important isoforms: SGLT1 and SGLT2. SGLT1 is highly expressed in the gastrointestinal tract and is the major transporter of dietary glucose and galactose. Inactivating mutations in SGLT1 have been associated with glucose-galactose malabsorption syndrome in humans (Online Mendelian Inheritance in Man [OMIM] 606824), which causes life-threatening dehydration on a glucose- or galactose-containing diet (17). SGLT1 is also expressed in the liver, lung, and kidney, while SGLT2, for which glucose is the primary substrate, appears to be selectively expressed in the kidney (18,19). SGLT2 expression in the kidney is localized to the S1 segment of the proximal tubule, where >90% of renal glucose reabsorption occurs, whereas SGLT1 is present in the more distal S3 segment of the proximal tubule (20). In rats, mRNA hybrid depletion studies suggested that SGLT2 is the major transporter responsible for renal reabsorption of glucose (20). In humans, mutations in SGLT2 are associated with familial renal glucosuria (OMIM 233100) (21,22), a benign syndrome in which glucose excretion occurs in the absence of hyperglycemia. Thus, SGLT2 appears to be the major transporter responsible for renal glucose transport, mediating glucose reuptake from the glomerular filtrate.

We propose that selective inhibition of SGLT2 vs. SGLT1 would promote renal glucose excretion and reduce hyperglycemia without the potential for gastrointestinal side

TABLE 1
In vitro inhibition of human and rat SGLT2 and SGLT1 by dapagliflozin and phlorizin

	Human SGLT2	Human SGLT1	Rat SGLT2	Rat SGLT1
Dapagliflozin	1.12 ± 0.065 (n = 18)	1,391 ± 7 (n = 16)	3.0 ± 0.5 (n = 5)	620 ± 70 (n = 6)
Phlorizin	35.6 ± 4.2 (n = 11)	330 ± 50 (n = 10)	75 ± 8 (n = 4)	302 ± 30 (n = 4)

Data are mean EC_{50} ± SE (nmol/l).

effects predicted with inhibition of SGLT1. In this report, we describe the in vitro and in vivo pharmacology of the SGLT2-selective inhibitor dapagliflozin, currently under clinical investigation for use as an antidiabetic agent. The predicted reduction in ambient hyperglycemia with dapagliflozin would be expected not only to reduce A1C levels in diabetic individuals, but also to produce improvements in hepatic and peripheral insulin sensitivity and β-cell function, as has been shown nonclinically for phlorizin (3–10) and T-1095 (14,16).

RESEARCH DESIGN AND METHODS

Reagents and chemicals. Dapagliflozin was synthesized by Bristol-Myers Squibb Discovery Chemistry (23). Primers, vectors, restriction enzymes, and tissue culture media were purchased from Invitrogen (Carlsbad, CA). All other chemicals unless otherwise specified were purchased from Sigma (St. Louis, MO).

Cloning and cell line construction for human and rat SGLT1 and SGLT2. Human SGLT1 (hSGLT1) and human SGLT2 (hSGLT2) full-length cDNA sequences were cloned by PCR using Marathon Ready human kidney cDNA (Clontech, Mountain View, CA), with primers designed from published sequences (GenBank accession numbers NM_003041 and NM_000343). The hSGLT1 and hSGLT2 sequences were cloned into pIRESneo for mammalian expression and were stably transfected into Chinese hamster ovary (CHO) cells. SGLT-expressing clones were selected based on resistance to G418 antibiotic (Geneticin; Invitrogen) and activity in the ^{14}C-α-methyl-D-glucopyranoside (^{14}C-AMG) uptake assay. Rat SGLT1 (rSGLT1) and rat SGLT2 (rSGLT2) sequences were cloned from rat kidney and intestine cDNA into the pTRE2 vector containing a tetracycline-inducible promoter and stably transfected into the CHO-AA8 Tet-Off cell line (Clontech, Mountain View, CA). Clones were selected based on resistance to G418 and hygromycin (Calbiochem, San Diego, CA) and activity in the ^{14}C-α-methyl-D-glucopyranoside uptake assay, described below.

SGLT1 and SGLT2 assays. Cells expressing hSGLT1, hSGLT2, rSGLT1, or rSGLT2 were maintained using standard cell culture techniques. Assays for sodium-dependent glucose transport in 96-well plates were initiated by adding 100 μl/well of protein-free assay buffer containing sodium (HEPES/Tris pH 7.4, 137 mmol/l NaCl, 5.4 mmol/l KCl, 2.8 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$), 10 μmol/l ^{14}C-α-methyl-D-glucopyranoside and inhibitor, or DMSO vehicle, and plates were incubated for 2 h at 37°C. Sodium-dependent ^{14}C-α-methyl-D-glucopyranoside uptake was calculated by subtracting the counts per minute observed under sodium-free uptake conditions from the counts observed under sodium-containing conditions. Inhibitors were assayed at eight concentrations in triplicate in the presence of sodium, and the percent inhibition was calculated by comparing counts per minute in inhibitor-containing wells with counts per minute in wells containing only DMSO vehicle. Phlorizin was evaluated in parallel in every assay. A dose-response curve was fitted to an empirical four-parameter model using XL Fit (IDBS, Guilford, U.K.) to determine the inhibitor concentration at half-maximal response (EC_{50}).

Adipocyte glucose uptake assays. Before the assay, predifferentiated human adipocytes (Zen-Bio, Research Triangle Park, NC) were washed once in Dulbecco's modified Eagle's medium, low glucose, without fetal bovine serum, and incubated for 2 h at 37°C. The cells were then washed twice in Krebs-Ringer bicarbonate HEPES buffer, without glucose. The assay buffer (100 μl/well) consisted of Krebs-Ringer bicarbonate HEPES buffer containing either no insulin or 100 nmol/l insulin, 10 μmol/l 2-^{14}C-deoxy-D-glucose, inhibitor or cytochalasin B, and a DMSO control (n = 6 per set). Cells were incubated at 37°C for 20 min, washed three times in PBS, and lysed in 50 μl/well of 0.1 N NaOH. MicroScint-40 was added, and cells were counted in a TopCount scintillation counter. Percent inhibition was calculated by comparing counts per minute in inhibitor-containing wells to counts per minute in wells without inhibitor.

Animals. Diagrammatic representation of the protocols used in these experiments are available in an online appendix at http://dx.doi.org/10.2337/db07-1472. All animals were maintained at Bristol-Myers Squibb facilities in accordance with the guidelines established by the Department of Veterinary Sciences, and all protocols were approved by the Bristol-Myers Squibb Animal Care and Use Committee. The animals were allowed ad libitum access to food and water unless otherwise stated, and rooms were maintained at 22°C and 50% humidity on a 12-h light/dark cycle. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) were maintained on Harlan Teklad 2018 diet and weighed 250 g at the time of the experiment. Male Zucker diabetic fatty (ZDF) rats (Genetic Models, Indianapolis, IN) were maintained on Purina 5008 chow, and for acute studies, rats were 19 weeks of age and had a mean weight of 422 g. For the first chronic study, male ZDF rats were 17 weeks of age with a mean weight of 399 g; in the second chronic study, male ZDF rats were 15 weeks old with a mean body weight of 397 g. For all ZDF rat studies, rats were randomized into groups where body weight and plasma glucose levels were not statistically different between groups. Blood samples were collected and centrifuged (Eppendorf) at 4°C, 2,500 rpm, for 10 min. Plasma (5 μl) was removed and mixed with 25 μl saline for glucose analysis using the Cobas Mira Analyzer (GMI, Ramsey, MN). All urine volumes were measured and recorded. For urine glucose analysis, 5 μl urine was removed and mixed with 250 μl saline and analyzed on the Cobas Mira Analyzer.

Acute normal and diabetic rat studies. For all animal studies, the vehicle used for drug administration was 5% 1-methyl-2-pyrrolidinone, 20% polyethylene glycol, and 20 mmol/l sodium diphosphate. For glucose tolerance testing, 15 Sprague-Dawley rats were fasted overnight (18 h), weighed, bled via tail tip (30–40 μl), and randomized into five groups (n = 3). Rats were dosed orally with single doses of vehicle or drug (1 ml/kg; 0.01–10 mg/kg drug) and subsequently dosed orally with 50% aqueous glucose solution (2 g/kg). Rats were then bled at 15, 30, and 60 min and 24 h post-dose. Insulin was not measured in these studies.

For glucosuria assessment, overnight-fasted Sprague-Dawley rats were placed into metabolism cages (Lab Products, Seaford, DE) for baseline urine collection over 24 h. Rats were weighed, randomized into five groups (n = 3), dosed orally with single doses of vehicle or drug (1 ml/kg; 0.01–10 mg/kg drug), and subsequently dosed orally with 50% aqueous glucose solution (2 g/kg). Immediately after dosing, rats were returned to metabolism cages for 24-h urine collection and re-fed at 1 h after the glucose challenge. The delta area under the curve for plasma glucose from baseline glucose value was calculated using GraphPad Prism. The urine glucose and urine volume data were normalized per 200 g body weight.

For assessment of acute glucosuria and plasma glucose effects in ZDF rats, the animals were weighed, bled via the tail tip (40–50 μl) in the fed state, and randomized into four groups (n = 6). Rats were dosed with vehicle or drug (1 ml/kg; 0.01–1.0 mg/kg drug) and placed into metabolism cages (without prior acclimation). Blood samples were collected immediately before dosing and at 2, 4, 6, and 24 h post-dose. Urine collections were obtained at 2, 4, 6, and 24 h post-dose. The animals were allowed to re-feed after the 6-h time point. Plasma samples at each time point were analyzed for the presence of glucose and dapagliflozin. Urine glucose and urine volume data were normalized per 400 g body weight. Insulin was not measured in these studies.

Chronic diabetic rat studies. Two studies were performed in ZDF rats to evaluate the effects of multi-dose treatment with dapagliflozin on prandial and fasting plasma glucose (FPG) and the metabolic profile of the rats after 2 weeks of treatment. In study 1, rats were fasted overnight, weighed, bled via the tail tip (40–50 μl), and randomized into four groups (n = 6 per group). Rats were dosed orally with vehicle or drug (1 ml/kg; 0.01–1.0 mg/kg drug) once daily for 14 days. Fasting body weight and plasma samples were obtained on days 8 (18-h fast) and 15 (24-h fast), and fed plasma samples were taken on day 14, 24 h after the previous dose. In study 2, ZDF rats were randomized into two groups (n = 6 per group) and dosed orally with vehicle or drug (1 ml/kg; 0.5 mg/kg drug) once daily for 15 days. Blood samples (40 μl) were obtained from 18-h–fasted rats from all groups by tail bleed on days 1, 8, and 15 of the study to determine plasma glucose levels. Neither fasting nor fed insulin was measured in these studies. A hyperinsulinemic-euglycemic clamp study was conducted with vehicle- and dapagliflozin-treated rats on day 17 and 48 h after the last dose of vehicle or dapagliflozin. Neither food nor water intake were monitored in these studies.

TABLE 2
In vitro inhibition of human adipocyte GLUT activity by dapagliflozin, phlorizin, phloretin, and cytochalasin B (20 μmol/l)

	No BSA added		4% BSA added	
	% Inhibition, basal	% Inhibition, + insulin	% Inhibition, basal	% Inhibition, + insulin
Dapagliflozin	9 ± 1 (3)	8 ± 3 (3)	0 (1)	0 (1)
Phlorizin	10 (1)	12 (1)	11 (1)	5 (1)
Phloretin	78 (1)	79 (1)	77 (1)	77 (1)
Cytochalasin B	88 ± 2 (4)	89 ± 0.3 (4)	86 ± 1 (3)	88 ± 1 (3)

Data in parentheses are *n*. BSA, bovine serum albumin.

Hyperinsulinemic-euglycemic clamp study. The procedures for the clamp study were adapted from the literature (3,14,24,25). Details of the clamp procedure are given in the online appendix.

Statistical analysis. All results are expressed as means ± SE. The statistical analysis of drug effects versus vehicle effects in most cases was performed using a one-way ANOVA followed by the Dunnett's post hoc test. For the hyperinsulinemic-euglycemic clamp data, the comparison of drug effects versus vehicle effects was performed using the Student's *t* test.

RESULTS

In vitro activity. Dapagliflozin exhibits a mean EC_{50} against hSGLT2 of 1.12 nmol/l, compared with the EC_{50} for phlorizin of 35.6 nmol/l (Table 1). Against hSGLT1, dapagliflozin and phlorizin displayed mean EC_{50} values of 1,391 and 330 nmol/l, respectively, indicating that dapagliflozin is highly selective (~1,200-fold) for hSGLT2 vs. hSGLT1. Dapagliflozin is ~32-fold more potent than phlorizin against hSGLT2 and ~4-fold less potent than phlorizin against hSGLT1. Dapagliflozin is also a potent selective inhibitor of rSGLT2, displaying a mean EC_{50} value of 3.0 nmol/l, with ~200-fold selectivity versus rSGLT1. Dapagliflozin is highly selective versus GLUT transporters as assayed in human adipocytes, displaying 8–9% inhibition in protein-free buffer at 20 μmol/l and virtually no inhibition in the presence of 4% bovine serum albumin (Table 2). Protein was added to this assay to simulate the in vivo condition of plasma protein binding. Phlorizin minimally inhibits adipocyte GLUT activity; however, the aglycone of phlorizin, phloretin, inhibits GLUT activity ~77% regardless of whether bovine serum albumin is present in the assay.

Acute in vivo activity. In normal rats, dapagliflozin administration caused significant dose-dependent glucosuria (Fig. 1) and increase in urine volume, with 1 mg/kg producing a 400-fold increase in urine glucose and a threefold increase in urine volume versus vehicle over 24 h post-dose. During an oral glucose tolerance test in normal rats, dapagliflozin administration was associated with a reduction in glucose area under the curve over 1 h post-dose at 1 and 10 mg/kg doses (Fig. 2), demonstrating that this glucosuric agent was able to reduce glucose excursions after an acute glucose challenge in normal rats. In ZDF rats administered single oral doses of dapagliflozin, a dose-dependent increase in urine glucose and urine volume excretion was apparent at 6 h post-dose (Fig. 3*A*) simultaneous with plasma glucose lowering in the same rats (Fig. 4) at doses of 0.01–1.0 mg/kg. The clear dose dependency of the urine glucose excretion effect declined when examined over a 24-h period (Fig. 3*B*), with treated rats in all dose groups demonstrating a twofold enhancement of urine glucose levels compared with vehicle-treated rats. Efficacy in lowering plasma glucose was still observed at 24 h post-dose after a period of refeeding in these rats at a dose of 1 mg/kg. No evidence of hypoglycemia was observed during the course of these studies. At 1 mg/kg, plasma exposure to dapagliflozin at 1 h post-dose was 1.2 μmol/l and estimated to be 5.2 $\mu mol \cdot l^{-1} \cdot h^{-1}$ over the first 6 h of the experiment.

Chronic in vivo efficacy. In the first of two chronic studies, dapagliflozin dose-dependently lowered fasting glucose levels in 18-h–fasted ZDF rats by day 8 of treatment, measured 24 h after the previous dose (Fig. 5). This effect was also evident on day 15 of treatment, where rats were fasted for a 24-h period, and in fed animals, measured on day 14 of the study. These data demonstrate that efficacy in lowering FPG was maintained over a 2-week once-daily treatment regimen. No body weight changes compared with vehicle-treated rats were noted, and no

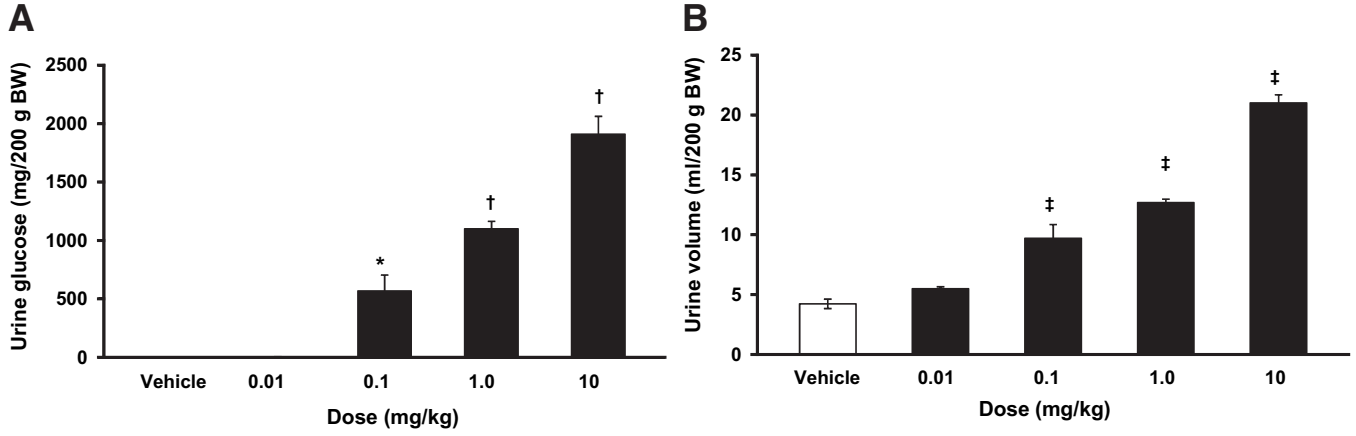

FIG. 1. Single oral doses of dapagliflozin increase urinary glucose excretion (*A*) and urine volume (*B*) in normal Sprague-Dawley rats over 24 h. □, Vehicle; ■, dapagliflozin. Vehicle-treated rats excreted 2.5 mg/200 g body weight (BW), and rats treated with 0.01 mg/kg dapagliflozin excreted 3.7 mg/200 g body weight in *A*. **P* < 0.01, †*P* < 0.0001, ‡*P* < 0.0005, each vs. vehicle.

abnormal behavior was observed; the rats appeared to be well. No marker of renal or liver toxicity was measured.

In the second chronic study, when measured 24 h after the final dose on day 15, ZDF rats treated with 0.5 mg/kg dapagliflozin displayed a 53% decrease in 18-h FPG level compared with vehicle-treated rats (Table 3). On the third day after the final dose, a hyperinsulinemic-euglycemic clamp study was initiated to assess the metabolic effects of dapagliflozin versus vehicle treatment. In the basal stage, urine glucose loss rate was significantly higher in the vehicle-treated rats compared with the dapagliflozin-treated rats (Table 3), perhaps reflective of a trend for higher plasma glucose levels in vehicle-treated rats. The fact that the clamp procedure was initiated 48 h after the last dose of a compound that exhibits a 4- to 5-h half-life in rats (W. Humphreys, W.N.W., unpublished data) suggests that plasma drug levels were negligible during the clamp procedure, although they were not measured. Thus, we expect that urine glucose excretion acutely induced by dapagliflozin was not a significant contributor to the metabolic effects observed during the clamp. Urine volumes were also significantly reduced in dapagliflozin-treated rats during this procedure (S.H., L.X., J.M.W., W.G. Humphreys, W.N.W., J.R.T., unpublished data). Although there appeared to be reduced urinary glucose loss during the insulin infusion stage of the clamp in dapagliflozin-treated rats compared with vehicle-treated rats, this difference was not statistically significant.

Glucose infusion rate in dapagliflozin-treated rats was increased significantly during the insulin infusion stage of the clamp compared with that observed in vehicle-treated rats, suggesting an improvement in whole-body glucose utilization (Table 3). Glucose production during the insulin infusion stage was also reduced significantly in dapagliflozin-treated rats compared with vehicle-treated rats (Table 3). In addition, radio-labeled glucose uptake into liver during the clamp was significantly increased in dapagliflozin-treated rats, whereas glucose uptake into skeletal muscle or white adipose tissue was not significantly changed. These data suggest that 2-week treatment with dapagliflozin ameliorated elevated glucose production in ZDF rats and enhanced liver insulin sensitivity.

TABLE 3
Dapagliflozin treatment of ZDF rats over 15 days results in reduced FPG, increased glucose infusion rate and glucose utilization rate, decreased endogenous glucose production, and increased glucose uptake into liver in the insulin infusion stage of a hyperinsulinemic-euglycemic clamp versus those in vehicle-treated rats

	FPG (day 15)	Basal stage			Insulin infusion stage				Glucose uptake into peripheral tissues during insulin infusion stage		
	Plasma glucose (mg/dl)	Plasma glucose (mg/dl)	Urinary glucose loss rate (mg · kg^{-1} · min^{-1})	Glucose utilization rate(mg · kg^{-1} · min^{-1})	Plasma glucose (mg/dl)	Urinary glucose loss rate (mg · kg^{-1} · min^{-1})	Glucose infusion rate (mg · kg^{-1} · min^{-1})	Glucose utilization rate (mg · kg^{-1} · min^{-1})	Hepatic glucose production (mg ·kg^{-1} · min^{-1})	R_g' (liver) (ng · kg^{-1} · min^{-1})	R_g' (skeletal muscle) (ng · kg^{-1} · min^{-1})	R_g' (white adipose tissue) (ng · kg^{-1} · min^{-1})
Vehicle	295.2 ± 19.5	402 ± 30	0.35 ± 0.15	3.3 ± 0.3	122.8 ± 0.4	0.1 ± 0.05	2.6 ± 0.4	5.3 ± 0.15	3.0 ± 0.32	4.7 ± 0.46	4.2 ± 0.58	0.4 ± 0.06
Dapagliflozin	138.2 ± 7.4‡	307 ± 19	0.014 ± 0.01*	3.9 ± 0.27	121.7 ± 0.6	0.02 ± 0.01	6.0 ± 0.6†	6.6 ± 0.32‡	0.7 ± 0.4‡	7.2 ± 0.32‡	4.3 ± 0.52	0.6 ± 0.1

*$P < 0.05$, †$P < 0.01$, ‡$P < 0.005$, each vs. vehicle. R_g', rate of glucose uptake into tissue.

DISCUSSION

The approach of enhancing urine glucose excretion to correct hyperglycemia has been in the experimental diabetes literature for many years, with phlorizin used as the primary tool. Such an approach to the treatment of hyperglycemia is attractive because it does not rely on insulin secretion or insulin action and could thus be effective in a wide variety of patients. It is also a mechanism predicted to have a low risk of hypoglycemia, because of the lack of impact on counterregulatory mechanisms of glucose homeostasis and the selective inhibition of SGLT2 versus other glucose transporters in the kidney and gut. In addition, it has the potential to be weight-neutral or to promote weight loss, due to the loss of glucose in the urine. Thus, such an approach to diabetes therapy could deliver significant patient benefit. As a potential clinical agent for the treatment of diabetes, however, phlorizin itself is unsuitable given its low oral bioavailability and susceptibility to degradation in vivo (26). Moreover, phlorizin is a relatively nonselective inhibitor of SGLT1 and SGLT2 and, upon degradation, produces the aglycone phloretin, an inhibitor of facilitative glucose transporters

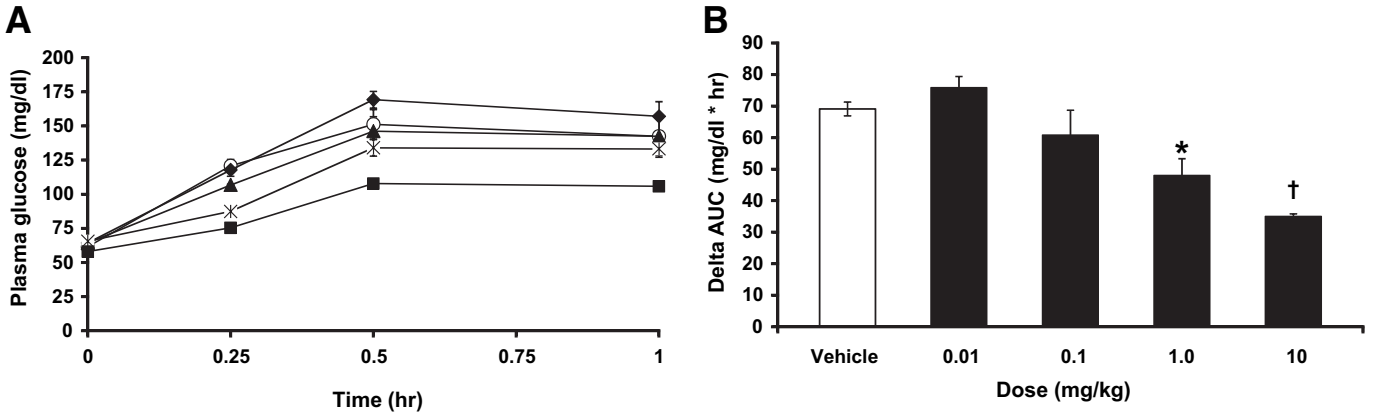

FIG. 2. Dapagliflozin reduces plasma glucose excursions in normal rats. *A*: Plasma glucose excursion curves over 1 h post–glucose challenge. ○, Vehicle; ♦, 0.01 mg/kg; ▲, 0.1 mg/kg; ✳, 1 mg/kg; ■, 10 mg/kg. *B*: The delta area under the curve (AUC) from baseline plasma glucose values for vehicle- and dapagliflozin-treated rats. *$P < 0.05$, †$P < 0.005$, each vs. vehicle.

(27,28). We sought to discover stable potent compounds that were able to selectively inhibit the major transporter of glucose in the renal proximal tubule (SGLT2), without affecting the major transporter of glucose in the small intestine (SGLT1) or major members of the GLUT family. Dapagliflozin was identified as among the most potent and selective compounds from this effort and clearly demonstrated not only enhanced potency versus phlorizin, but greater selectivity for SGLT2 versus SGLT1. Notably, the selectivity seen for hSGLT2 relative to hSGLT1 (~1,200-fold) is not fully maintained in the rat (~200-fold). At the doses used in the rat experiments, we believe that the majority of the pharmacology observed is due to renal SGLT2 inhibition, since at maximal plasma concentration of drug, free plasma concentrations at an oral dose of 1 mg/kg are estimated to be <40 nmol/l (W. Humphreys, W.N.W., unpublished data). At doses higher than 1 mg/kg, we cannot rule out the potential impact of SGLT1 inhibition, in the gut as well as the kidney, on the observed pharmacology. However, no gastrointestinal side effects indicative of significant intestinal SGLT1 inhibition (diarrhea or soft stools) were noted in the rats during the course of these acute and chronic experiments at any dose.

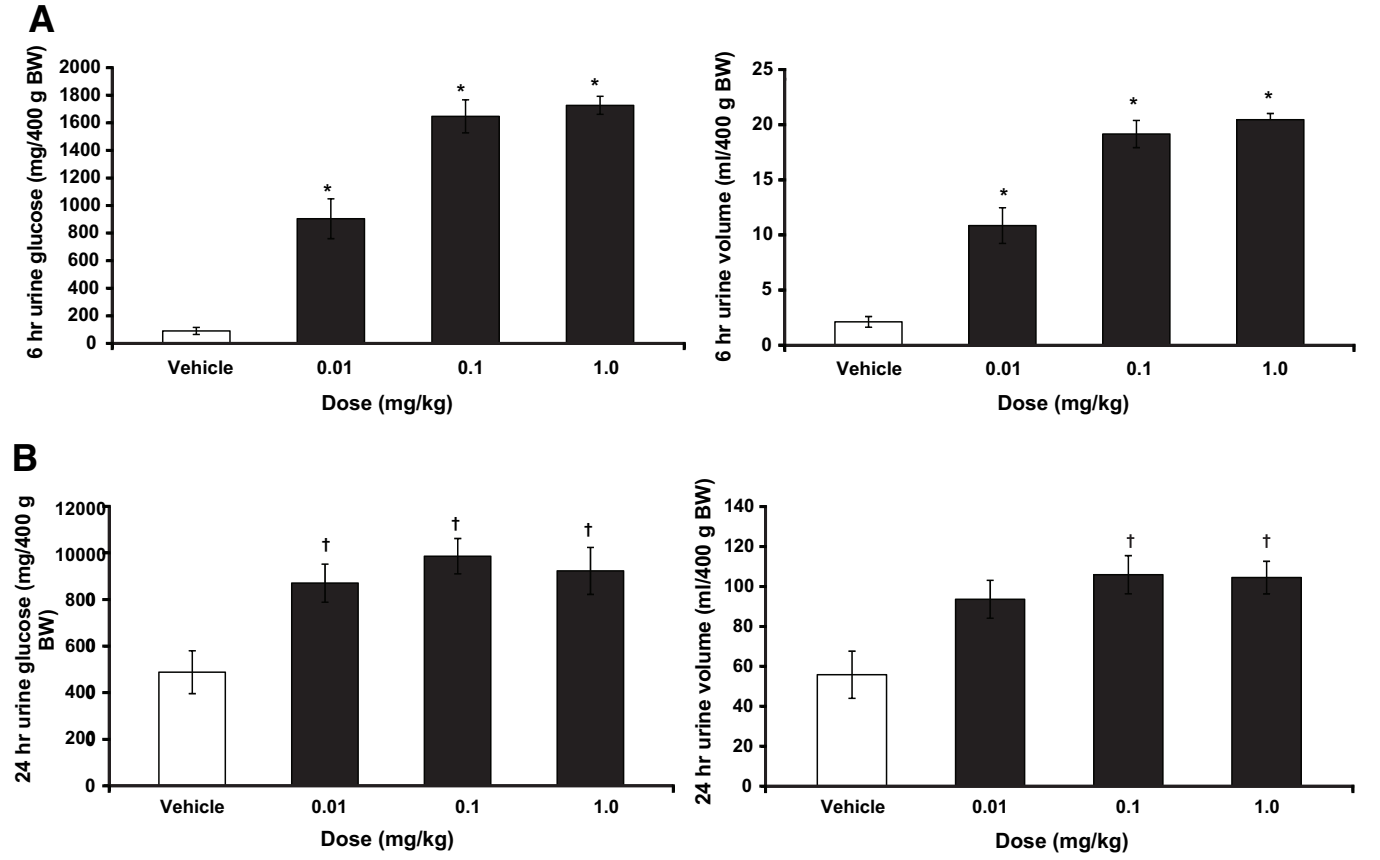

FIG. 3. Single oral doses of dapagliflozin stimulate an increase in urinary glucose excretion (left) and increased urine volume (right) in ZDF rats over 6 h post-dose (*A*) and 24 h post-dose (*B*). □, Vehicle treatment; ■, dapagliflozin treatment. *$P < 0.0001$, †$P < 0.05$, each vs. vehicle. BW, body weight.

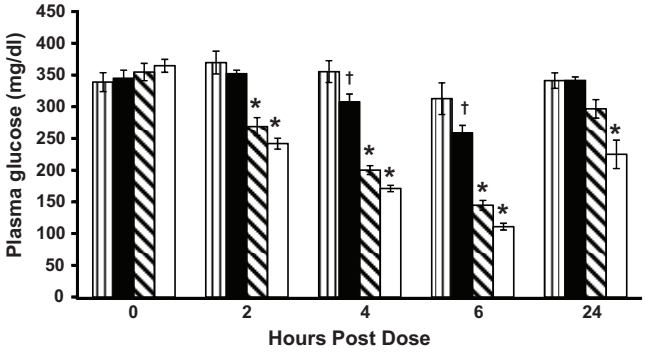

FIG. 4. Dapagliflozin lowers plasma glucose levels in ZDF rats after single oral doses. Rats were in the fed state at the initiation of the experiment. Access to food was restricted over the first 6 h post-dose, but access to food was restored for the remaining 18 h post-dose. ▥, Vehicle; ■, 0.01 mg/kg; ▧, 0.1 mg/kg; □, 1 mg/kg. *$P < 0.0001$, †$P < 0.05$, each vs. vehicle.

At 0.1 mg/kg, dapagliflozin stimulated significant excretion of glucose in the urine in both normal nondiabetic rats, as well as in rats that were already glucosuric because of diabetes. Notably, at 0.01 mg/kg, dapagliflozin stimulated a significant increase in urine glucose excretion in ZDF rats within 6 h post-dose, whereas in normal rats, glucose excretion was not significantly increased at this dose over 24 h. The data are suggestive of a greater impact of dapagliflozin in the diabetic kidney to reduce the renal glucose threshold. The potency of dapagliflozin in stimulating glucosuric responses in normal rats appears to be greater than that observed with sergliflozin (29), T-1095, or phlorizin administered subcutaneously (12). In an oral glucose tolerance test, dapagliflozin doses of 1 and 10 mg/kg in normal rats reduced the plasma glucose excursion significantly compared with vehicle-treated rats, indicating that dapagliflozin administration can have a beneficial impact on glucose tolerance, even in normal animals. Because insulin levels were not monitored in this experiment, no conclusions can be drawn with regard to the effect of dapagliflozin on the insulin response during the oral glucose tolerance test.

Over the first 6 h after ZDF rats were treated with dapagliflozin, significant dose-dependent plasma glucose lowering was observed, simultaneous with enhancement of glucose excretion in the urine (Fig. 4). The data demonstrate an acute ability of dapagliflozin upon oral administration to lower plasma glucose in a highly diabetic insulin-resistant model. Insulin levels were not measured in these studies, since by 15–17 weeks of age, ZDF rats show significant loss of insulin secretory function; previous studies have shown little acute effect on insulin levels in this model with compounds acting by this mechanism (S.H., J.R.T., L.X., W.G. Humphreys, W.N.W., J.R.T., unpublished data). Nonetheless, significant correction of ambient hyperglycemia was achieved acutely by dapagliflozin in these experiments, with no evidence of hypoglycemia observed.

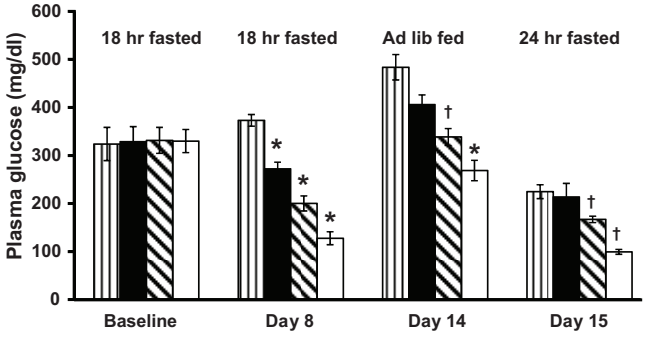

FIG. 5. Dapagliflozin lowers fasting and fed plasma glucose over 15 days of once-daily oral treatment in ZDF rats. ▥, Vehicle; ■, 0.01 mg/kg; ▧, 0.1 mg/kg; □, 1 mg/kg. *$P < 0.0001$, †$P < 0.05$, each vs. vehicle.

Based on our observations that dapagliflozin administration resulted in acute glucosuria accompanied by plasma glucose lowering in fed diabetic rats, we expected that hyperglycemic fasting glucose levels would be lowered in ZDF rats as well. While we anticipated that elevated FPG could be acutely lowered by this mechanism, it was measured only in the 2-week studies and only on days 8 and 15 of treatment. The data confirm that dapagliflozin reduced FPG levels in ZDF rats over the treatment period. Fed plasma glucose levels were measured only on day 14 in the first chronic study, and dose-dependent reductions were observed; however, the acute experiments in ZDF rats demonstrate that this reduction can occur within hours after a single oral dose. Despite the fact that these rats excreted large amounts of glucose over the course of the 2-week study (S.H., J.R.T., L.X., W.G. Humphreys, W.N.W., J.R.T., unpublished data), no significant change in body weight was noted in these experiments. Although food intake was not specifically monitored in these experiments, it is well known that ZDF rats are hyperphagic, thereby perhaps accounting for the lack of body weight effect of dapagliflozin. In other studies, dapagliflozin administration to diet-induced obese rats has been shown to result in body weight loss over 28 days (30).

Reduced hyperglycemia in ZDF rats would be expected to improve insulin sensitivity. The results of the hyperinsulinemic-euglycemic clamp study demonstrated that within 3 days of completing 2 weeks of dapagliflozin treatment, ZDF rats displayed improved glucose utilization accompanied by reduced glucose production and enhanced glucose influx into liver tissue. This suggests that reduction of hyperglycemia per se by dapagliflozin can have potentially significant benefit to the liver in the context of diabetes, perhaps due in part to the effect of glucose itself on glucose-6-phosphatase gene expression, as has been shown in rats (31). Interestingly, no significant enhancement of glucose uptake into skeletal muscle or adipose tissue was observed during the insulin infusion stage of the clamp, suggesting that 2-week treatment with dapagliflozin does not significantly alter insulin sensitivity in these tissues in this model. Overall, the metabolic improvements observed with dapagliflozin are similar to those observed with other compounds that act by this mechanism (3,14). Further investigations to better understand the effects of dapagliflozin on pathways related to insulin sensitivity (in liver, muscle, and adipose tissue) and on insulin secretion are needed. In addition, further exploration of the effects of dapagliflozin on the renal glucose threshold in rats—similar to studies already performed with sergliflozin (29)—are warranted. We also speculate that this mechanism of action could provide renal benefit in the context of diabetes beyond hyperglycemia control, by helping to correct the hyperfiltration apparent in early diabetes, a risk factor for the subsequent development of nephropathy (32). This hypothesis has yet to be tested with dapagliflozin.

In summary, the preclinical pharmacology of dapagliflozin suggests that this agent, by inducing renal glucose

excretion, not only can correct ambient hyperglycemia, but also can produce improvements in the metabolic status of the ZDF rat. Dapagliflozin appears to be more potent than previously characterized SGLT2 inhibitors and has a sustained duration of action in the ZDF rat model. Combined with the observation of no gastrointestinal side effects or hypoglycemia, the data suggest that dapagliflozin represents an attractive therapeutic candidate for the treatment of diabetes.

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