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Selective antagonism of the hepatic glucocorticoid receptor reduces hepatic glucose production

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Abstract

A liver-selective glucocorticoid (GC) receptor antagonist (A-348441) was used to determine the effect of reduced hepatic GC signaling on hepatic glucose production. Fasted conscious dogs were studied in the presence (GRA, n = 6) or absence (CON, n = 6) of the intraduodenally administered GC receptor antagonist (100 mg/kg). All dogs were maintained on a pancreatic clamp and in a euglycemic state for 7 hours to ensure that any changes in glucose metabolism were the direct result of the effects of A-348441, which was given at the start of a 5-hour experimental period. In the GRA group, the arterial plasma insulin level was 4.6 ± 0.7 and 4.8 ± 0.6 μU/mL during the basal and the last 30 minutes of the experimental periods, respectively. In the CON group, it was 4.0 ± 0.3 and 4.5 ± 0.5 μU/mL in the 2 periods, respectively. The arterial plasma glucagon level was 49 ± 4 and 46 ± 3 pg/mL in the 2 periods in the GRA group, and 45 ± 3 and 42 ± 3 pg/mL in the CON group. Net hepatic glucose balance progressively decreased in the GRA group from 1.31 ± 0.18 to 0.49 ± 0.30 mg/kg per minute, whereas in the CON group, net hepatic glucose balance was 1.17 ± 0.09 and 1.43 ± 0.18 mg/kg per minute during the basal and last 30 minutes of the experimental periods, respectively. No significant change in net renal or gut glucose balance or nonhepatic glucose uptake was observed in either group. This study demonstrates that the GC receptor plays an important role in the regulation of basal hepatic glucose production and represents a significant potential therapeutic target.

1. Introduction

Dysregulation of hepatic glucose production (HGP) plays a key role in type 2 diabetes mellitus and contributes to fasting hyperglycemia [1,2]. Cortisol acts as a functional antagonist of insulin action, impairing insulin-dependent glucose uptake, increasing lipolysis, and enhancing HGP [3,4]. Phospho*enol*pyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), key enzymes in the hepatic gluconeogenic (GNG) pathway, are up-regulated by cortisol [5-7], and cortisol increases proteolysis [8], thus augmenting the supply of GNG substrates reaching the liver. Glucocorticoid (GC) excess substantially increases the risk of developing obesity, insulin resistance, and diabetes [9,10]. On the other hand, GC deficiency reduces fasting blood glucose levels and improves glucose control in diabetic patients [11].

Glucocorticoid antagonists, such as RU-486, ameliorate diabetes in humans, including in patients with Cushing's syndrome, ectopic corticotropin (ACTH) production, or adrenal carcinoma [9]. Although systemic GC receptor (GR) antagonism is sufficient to improve glucose metabolism, this strategy cannot be used as a generalized long-term therapy for type 2 diabetes mellitus. Initially, systemic exposure to RU-486 results in adrenal insufficiency, with its attendant problems, and long-term exposure to a systemic antagonist leads to activation of the hypothalamic-pituitary-adrenal axis and subsequent hypersecretion of cortisol [12]. Thus, over time, systemic GR antagonist (GRA) therapy tends to become self-limiting due to increased GC production.

Although individuals with type 2 diabetes mellitus do not typically exhibit elevated circulating cortisol levels, GR

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number and tissue-specific conversion of cortisone to cortisol can produce relative GC excess [13,14]. Cortisol is synthesized by the adrenal cortex or converted from inactive cortisone into active cortisol by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1). 11β-HSD-1 is active in the liver, and splanchnic cortisol production may equal or exceed adrenal production, although substantial splanchnic cortisol uptake results in little or no net production [15]. 11β-HSD-1 activity is increased in visceral fat in obese subjects [16-18], and splanchnic cortisol uptake is increased by obesity, potentially because of increased liver cortisol uptake [15]. Therefore, increased GC action in the liver may provide a link between obesity and hepatic insulin resistance, and blocking cortisol signaling in the liver may be an effective means of limiting the excess HGP found in diabetes.

Delivery of a GRA targeted to the liver could minimize the undesirable systemic effects of widespread GR antagonism while still producing the desired decrease in HGP in diabetes. Mice with hepatic-specific disruption of the GR gene exhibit reduced glycemia and decreased expression of PEPCK and G6Pase during prolonged starvation and streptozotocin-induced diabetes [19]. Likewise, hepatic reduction of GR messenger RNA by antisense oligonucleotide treatment in *ob/ob* and *db/db* mice and high fat–fed diabetic rats reduced glucose levels and PEPCK and G6Pase activities, and enhanced insulin-mediated suppression of HGP [20,21]. A-348441 is an orally bioavailable liver-selective GRA, which was produced by linking a GRA (RU-486) to a bile acid so that the activity of both molecules was retained [22]. The resultant conjugate molecule selectively targets hepatocytes and is the first liver-selective GRA with significant antidiabetic activity [23]. A-348441 has been shown to antagonize GC–up-regulated hepatic genes, normalize postprandial glucose in diabetic mice, and reduce hepatic glucose output in insulin-resistant Zucker *fa/fa* rats [23]. The purpose of this study was to determine the effect of antagonism of the hepatic GR on glucose metabolism in the conscious dog. The dog is a useful model for this study because the catheterization of the hepatic portal vein is feasible, which allows for the precise control of the delivery of pancreatic hormones to the liver, and basal HGP is similar in the dog and human, whereas it is 10 to 15 times higher in the rodent [24]. The animals were fasted 60 hours to maximize the chance of observing GNG inhibition because the percent contribution of GNG to glucose production increases as fasting is prolonged and GNG accounts for a larger portion of HGP in diabetes.

2. Materials and methods

2.1. Surgical procedure

Experiments were conducted on twelve 60-hour fasted conscious mongrel dogs of either sex (18-25 kg). Housing and diet have been previously described [25]. The surgical facility met the standards published by the American Association for the Accreditation of Laboratory Animal Care, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. All dogs underwent a laparotomy 2 weeks before the experiment to implant infusion catheters into the duodenum and the jejunal and splenic veins, sampling catheters into the femoral artery and the portal, left common hepatic and left renal veins, and Transonic flow probes (Transonic Systems, Ithaca, NY) around the hepatic artery and portal vein, as described elsewhere [25]. Each dog was used for only 1 experiment. All dogs studied were healthy, as indicated by (1) leukocyte count less than 18 000/mm^3, (2) a hematocrit level greater than 35%, (3) a good appetite, and (4) normal stools.

2.2. Experimental design

Intraportal catheters (splenic and jejunal) were used for the infusion of insulin (Lilly, Indianapolis, IN) and glucagon (Lilly). Angiocaths (Deseret Medical, Becton-Dickinson, Sandy, UT) were inserted percutaneously into leg veins for [3-^{3}H]glucose (DuPont NEN, Boston, MA), indocyanine green (ICG, Sigma, St Louis, MO), and peripheral glucose (20% dextrose, Baxter Healthcare, Deerfield, IL) infusion. Animals were allowed to rest quietly in a Pavlov harness for 30 minutes before the experiments started. The protocol consisted of an equilibration period (−140 to −40 minutes), a basal period (−40 to 0 minutes), and an experimental period (0-270 minutes). At −140 minutes, a priming dose of [3-^{3}H]glucose (.00359 Bq) was given, and constant infusions of [3-^{3}H]glucose (.000035 Bq/min) and ICG (0.07 mg/min) were started. At the same time, a constant infusion of somatostatin (0.8 μg/kg per minute) was started in a peripheral vein to inhibit endogenous pancreatic hormone secretion, and a constant intraportal glucagon (0.5 ng/kg per minute) infusion was started to replace basal secretion of this hormone. The intraportal insulin infusion rate was adjusted, as necessary, to maintain glucose at basal levels, and plasma glucose was measured every 5 minutes. The last change in insulin infusion rate was made at least 20 minutes before the start of the control period. During the experimental period, glucose was infused through a peripheral vein as needed to maintain euglycemia. At the start of the experimental period (0 minutes), A-348441 + vehicle (GRA group, 100 mg/kg, n = 6) or vehicle alone (CON group, n = 6) was delivered by constant infusion via the duodenal catheter for 30 minutes. Duodenal instead of oral delivery was used to ensure complete administration and to provide an even rate of drug absorption from the gastrointestinal tract. A-348441 and vehicle (0.2% hydroxypropyl methylcellulose) were provided by Abbott Laboratories (Abbott Park, IL). The average A-348441 level in the portal vein in the GRA group was 1.06 ± 0.30 μg/mL at the end of the 30-minute dosing period, after which it fell to 0.32 ± 0.13 μg/mL by 270 minutes. Arterial and hepatic vein compound levels were approximately one half and two thirds of the portal vein level, respectively. The compound level in liver tissue taken at the end of the experiment was

Table 1
Arterial plasma insulin, glucagon, epinephrine, ACTH, and cortisol levels and hepatic sinusoidal plasma insulin and glucagon levels during the basal (−40 to 0 minutes) and experimental (0-270 minutes) periods in the CON and GRA groups

	Time (min)									
	−40	0	30	60	90	120	150	180	240	270
Arterial plasma insulin (μU/mL)										
CON	4 ± 0	4 ± 1	4 ± 1	4 ± 0	4 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1	5 ± 1
GRA	4 ± 1	5 ± 1	5 ± 1	5 ± 1	4 ± 1	5 ± 1	5 ± 1	5 ± 1	5 ± 1	5 ± 1
Hepatic sinusoidal insulin (μU/mL)										
CON	13 ± 2	10 ± 2	11 ± 2	11 ± 2	12 ± 2	11 ± 2	10 ± 1	10 ± 1	14 ± 3	10 ± 1
GRA	14 ± 4	13 ± 4	13 ± 4	13 ± 3	12 ± 4	12 ± 4	14 ± 5	14 ± 4	14 ± 4	14 ± 3
Arterial plasma glucagon (pg/mL)										
CON	38 ± 5	32 ± 3	33 ± 3	34 ± 3	38 ± 3	39 ± 3	34 ± 5	37 ± 6	34 ± 6	31 ± 3
GRA	38 ± 7	38 ± 6	38 ± 5	36 ± 5	36 ± 6	37 ± 5	34 ± 7	33 ± 8	34 ± 6	36 ± 4
Hepatic sinusoidal glucagon (pg/mL)										
CON	48 ± 5	43 ± 4	43 ± 3	43 ± 4	50 ± 5	51 ± 4	45 ± 7	45 ± 6	44 ± 6	40 ± 4
GRA	51 ± 7	48 ± 6	49 ± 5	52 ± 4	48 ± 6	50 ± 6	46 ± 6	50 ± 6	45 ± 5	46 ± 4
Arterial plasma epinephrine (pg/mL)										
CON	109 ± 23	91 ± 16	97 ± 40	104 ± 39	103 ± 39	98 ± 28	106 ± 18	128 ± 25	155 ± 51	155 ± 30
GRA	80 ± 13	75 ± 15	72 ± 15	66 ± 18	68 ± 21	78 ± 24	89 ± 35	83 ± 35	70 ± 35	104 ± 45
Arterial plasma norepinephrine (pg/mL)										
CON	168 ± 57	134 ± 23	125 ± 32	160 ± 46	160 ± 46	161 ± 44	152 ± 23	188 ± 42	165 ± 48	193 ± 56
GRA	123 ± 16	110 ± 11	118 ± 12	122 ± 23	116 ± 14	111 ± 12	116 ± 23	107 ± 34	104 ± 13	104 ± 23
Arterial plasma ACTH (pg/mL)										
CON	57 ± 10	50 ± 6	48 ± 5	46 ± 4	44 ± 2	45 ± 2	43 ± 1	47 ± 5	47 ± 3	50 ± 3
GRA	61 ± 7	59 ± 7	57 ± 6	51 ± 5	48 ± 4	50 ± 5	51 ± 6	49 ± 5	48 ± 2	49 ± 4
Arterial plasma cortisol (μg/dL)										
CON	3 ± 1	3 ± 1	3 ± 1	2 ± 0	1 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 1
GRA	4 ± 1	4 ± 1	4 ± 1	3 ± 0	2 ± 0	2 ± 1	3 ± 1	2 ± 1	2 ± 0	2 ± 0

Values are means ± SEM; n = 6 per group.

4.0 ± 1.2 μg/g (~4 μmol/L). A-348441 was not present in the CON group.

2.3. Analytical procedures

Hematocrit; liver glycogen; plasma glucose, [^{3}H]glucose, glucagon, insulin, cortisol, epinephrine, norepinephrine, and nonesterified free fatty acids (FFAs); blood alanine, glycine, serine, threonine, lactate, glutamine, glutamate, glycerol, β-hydroxybutyrate, and acetoacetate concentrations were determined as previously described [25,26]. A-348441 levels were determined in EDTA-treated plasma samples and in liver homogenates extracted with *tert*-butyl methyl ether. Samples were quantitated on a PE Sciex API 2000 liquid chromatography/tandem mass spectrometry system (Concord, Ontario, Canada).

2.4. Calculations

Net hepatic balance (NHB) was calculated by using the arteriovenous (AV) difference method according to the formula NHB = $Load_{out} - Load_{in}$, where $Load_{out}$ = [H] × HF and $Load_{in}$ = [A] × AF + [P] × PF and where [H], [A], and [P] are the substrate concentrations in the hepatic vein, femoral artery, and portal vein blood or plasma, respectively, and HF, AF and PF are the blood flow in the hepatic vein, hepatic artery, and portal vein, respectively, as determined by the ultrasonic flow probes. Hepatic blood flow was also determined from ICG extraction across the liver to verify the ultrasonic measurements. A positive hepatic balance value represents net output by the liver, whereas a negative value represents net hepatic uptake. Net gut and renal glucose balance was also determined, as calculated for the liver, by using portal or renal vein glucose concentrations and flow, respectively. Plasma glucose and [^{3}H]glucose values were multiplied by 0.73 in all vessels to convert them to blood glucose values as validated elsewhere [27]. Tracer-determined unidirectional renal and gut glucose release was measured by subtracting unidirectional renal or gut glucose uptake from net renal or gut glucose balance, respectively. Unidirectional glucose uptake (milligrams per kilogram per minute) was determined for the respective

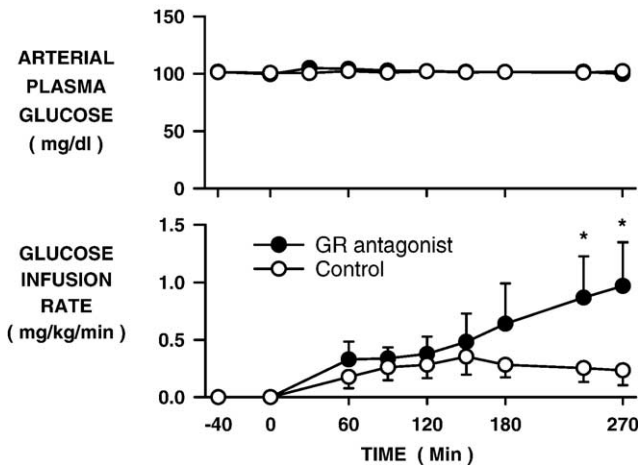

Fig. 1. Arterial plasma glucose levels and GIRs in conscious dogs treated with A-348441 (●) or vehicle (○) during the basal (−40 to 0 minutes) and experimental periods (0-270 minutes) (mean ± SEM, n = 6 per group, *P < .05 vs vehicle group). Glucose infusion rate was previously reported [23].

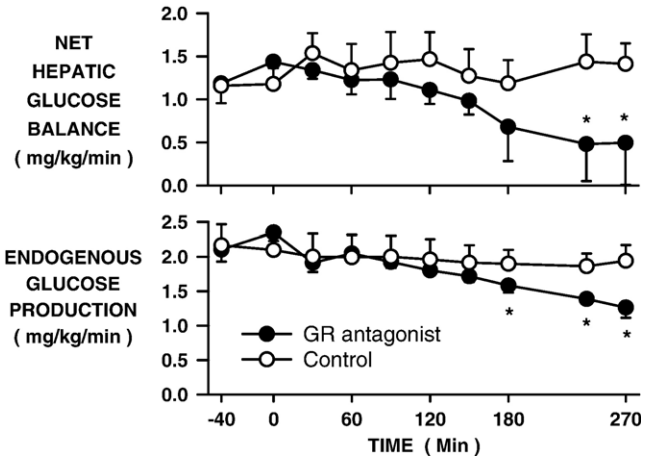

Fig. 2. Net hepatic glucose balance and endogenous glucose production (R_a) in conscious dogs treated with A-348441 (●) or vehicle (○) during the basal (−40 to 0 minutes) and experimental periods (0-270 minutes) (mean ± SEM, n = 6 per group, *P < .05 vs vehicle group). R_a was previously reported [23].

organs by dividing net [^{3}H]glucose balance (disintegrations per minute per kilogram per minute) by arterial [^{3}H]glucose specific activity (disintegrations per minute per milligram). The approximate insulin and glucagon levels in plasma entering the liver sinusoids were calculated by using the formula [A] × %AF + [P] × %PF, where [A] and [P] are arterial and portal vein hormone concentrations, respectively, and %AF and %PF are the respective percent contributions of arterial and portal flow to total hepatic blood flow. Tracer-determined whole-body glucose appearance and utilization were measured by using a primed, constant infusion of [3-^{3}H]glucose. Data calculation was carried out with the 2-compartment model [28] using canine parameters [29]. Endogenous glucose appearance was calculated by subtracting the glucose infusion rate (GIR) from whole-body glucose appearance. Hepatic GNG flux and net hepatic glycogenolysis (conversion of non–carbohydrate precursors and glycogen into glucose-6-phosphate) were determined by using the AV difference method [30]. Gluconeogenic flux to G6P was determined by summing the net hepatic uptake rates of the GNG precursors (alanine, glycine, serine, threonine, glutamine, glutamate, glycerol, lactate, and pyruvate) and dividing by 2 to account for the incorporation of 3 carbon precursors into the 6-carbon glucose molecule. Net hepatic glycogenolysis was determined by subtracting the net hepatic balance of the above-mentioned substrates (in glucose equivalents) and an estimate of glucose oxidation (GO) from net hepatic glucose balance. Based on previous measurements of GO in a variety of experimental conditions, GO was assumed to be 0.2 mg/kg per minute [30]. Net glycogenolysis would be underestimated to the extent that antagonism of the GR may have increased GO; however, the estimate of GNG flux is not affected by assumptions about GO.

2.5. Statistical analysis

The data were analyzed for differences from the basal period and for differences from the control group. Statistical comparisons were carried out by using 2-way repeated-measures analysis of variance (Sigmastat, SPSS, Chicago, IL). One-way analysis of variance comparison tests were used post hoc when significant F ratios were obtained. Significance was established when P < .05 (2-sided test).

3. Results

In both groups the arterial and hepatic sinusoidal plasma insulin and glucagon levels remained basal and unchanged (Table 1). Arterial epinephrine, norepinephrine, ACTH, and cortisol levels were also basal and not different between groups (Table 1).

As a result of glucose infusion during the experimental period, arterial plasma glucose levels were basal and unchanged in both groups (Fig. 1). In the CON group, the average GIR was 0.25 mg/kg per minute, whereas there was a steady increase to approximately 1 mg/kg per minute by the end of the experiment in the GRA group (P < .05; Fig. 1).

Net hepatic glucose balance remained at the basal rate in the CON group (1.43 ± 0.18 mg/kg per minute during the last hour of the experimental period; +22%), but fell from basal to 0.49 ± 0.30 mg/kg per minute in the GRA group

Table 2
Net renal glucose balance, whole-body glucose utilization (R_d), and nonhepatic glucose uptake during the basal (−40 to 0 minutes) and experimental (0-270 minutes) periods in the CON and GRA groups

	Time (min)									
	−40	0	30	60	90	120	150	180	240	270
Net renal glucose balance (mg/kg per minute)										
CON	−0.18 ± 0.16	−0.27 ± 0.09	−0.09 ± 0.10	−0.16 ± 0.10	−0.08 ± 0.07	−0.25 ± 0.13	−0.10 ± 0.07	−0.15 ± 0.08	−0.05 ± 0.10	−0.15 ± 0.13
GRA	−0.13 ± 0.10	−0.13 ± 0.08	−0.02 ± 0.05	−0.15 ± 0.12	−0.01 ± 0.04	−0.11 ± 0.09	0.00 ± 0.04	−0.15 ± 0.11	−0.10 ± 0.04	−0.05 ± 0.02
Whole-body glucose utilization (mg/kg per minute)										
CON	2.14 ± 0.26	2.09 ± 0.20	2.15 ± 0.23	2.20 ± 0.25	2.23 ± 0.25	2.25 ± 0.21	2.24 ± 0.15	2.19 ± 0.09	2.11 ± 0.08	2.08 ± 0.09
GRA	2.36 ± 0.16	2.36 ± 0.17	2.40 ± 0.17	2.38 ± 0.16	2.34 ± 0.16	2.32 ± 0.19	2.30 ± 0.22	2.30 ± 0.24	2.31 ± 0.28	2.32 ± 0.29
Nonhepatic glucose uptake (mg/kg per minute)										
CON	1.16 ± 0.08	1.18 ± 0.18	1.66 ± 0.18	1.51 ± 0.30	1.69 ± 0.32	1.75 ± 0.27	1.63 ± 0.23	1.47 ± 0.21	1.69 ± 0.26	1.65 ± 0.18
GRA	1.19 ± 0.23	1.44 ± 0.31	1.51 ± 0.09	1.55 ± 0.13	1.57 ± 0.19	1.49 ± 0.16	1.47 ± 0.13	1.32 ± 0.17	1.35 ± 0.09	1.47 ± 0.16

Values are means ± SEM; n = 6 per group. R_d was previously reported [21].

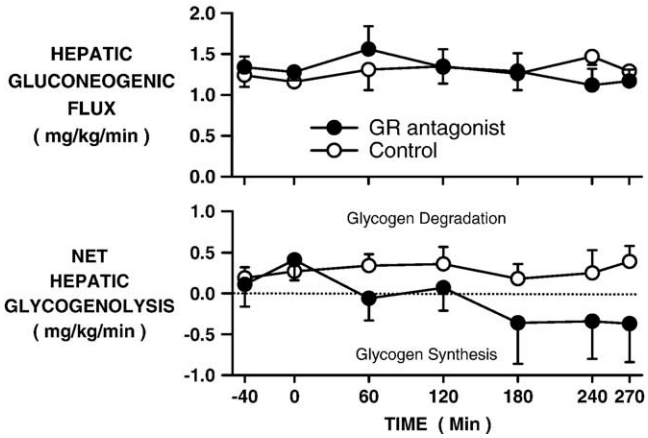

Fig. 3. Net hepatic GNG flux and glycogenolysis in conscious dogs treated with A-348441 (●) or vehicle (○) during the basal (−40 to 0 minutes) and experimental periods (0-270 minutes) (mean ± SEM, n = 6 per group).

(−63%, $P < .05$; Fig. 2). No net or unidirectional gut glucose release occurred in either group. Endogenous glucose production (R_a) did not change over time in the CON group but fell by 40% in the GRA group ($P < .05$; Fig. 2). Antagonism of the hepatic GR had no effect on renal glucose production, whole-body glucose utilization (R_d), or nonhepatic glucose uptake (Table 2). At the end of the experiment, hepatic glycogen content was 23 ± 3 and 27 ± 7 mg/g liver in the CON and GRA groups, respectively.

As determined by the AV difference method, hepatic GNG flux was not different between groups (Fig. 3). Net hepatic glycogenolytic flux was also not statistically different in the 2 groups, although it tended to decrease over time in the GRA group (0.26 ± 0.18 to −0.35 ± 0.30 mg/kg per minute; Fig. 3) but not in the CON group. Lactate, glycerol, and alanine are quantitatively the most important GNG precursors, but other GNG amino acids (serine, threonine, glycine, glutamine, and glutamate) and pyruvate also contribute to the process. There was no difference between groups in arterial blood levels or net hepatic uptake rates of lactate, glycerol, or the summed GNG amino acids (Table 3).

Arterial plasma FFA levels and net hepatic FFAs did not differ between groups (Table 4). Similarly, there was no change in the arterial blood ketone (β-hydroxybutyrate and acetoacetate) levels or net hepatic output (Table 4).

4. Discussion

There is a strong correlation between elevated HGP and fasting hyperglycemia in diabetes. Because cortisol opposes insulin action in the liver, selective antagonism of the hepatic GR might lower HGP. Two independent methods demonstrated that treatment with a GRA reduced glucose production. The effect was significant after 3 hours and appeared limited to the liver.

Hepatic glucose production is the product of gluconeogenesis and glycogenolysis in the liver, and changes in either or both of these flux rates could mediate the metabolic effects of GR antagonism. Although the net balance and tracer methods demonstrated a decrease in basal glucose production, the absolute decrease was relatively small (~50%), preventing a definitive conclusion regarding the effect of GR antagonism on the 2 processes. However, because during the basal state 85% of HGP was derived from gluconeogenesis (Fig. 3), and glucose production decreased by about 50%, the decrease in HGP in the GRA group was at least partially the result of a decrease in GNG (exit of gluconeogenically derived glucose from the liver). However, because no change in GNG flux to G6P

Table 3
Arterial blood level and net hepatic balance for lactate, glycerol, and total GNG amino acids (alanine, serine, threonine, glycine, glutamine, glutamate, and pyruvate) during the basal (−40 to 0 minutes) and experimental (0-270 minutes) periods in the CON and GRA groups

	Time (min)									
	−40	0	30	60	90	120	150	180	240	270
Arterial blood lactate (μmol/L)										
CON	520 ± 95	468 ± 87	458 ± 62	419 ± 56	376 ± 56	388 ± 42	340 ± 38	339 ± 30	361 ± 33	366 ± 29
GRA	412 ± 61	356 ± 72	401 ± 98	393 ± 81	349 ± 64	385 ± 79	412 ± 106	331 ± 57	346 ± 55	341 ± 48
Net hepatic lactate balance (μmol/kg per minute)										
CON	−5.29 ± 1.24	−5.53 ± 1.16	−5.68 ± 1.08	−6.17 ± 1.52	−5.79 ± 1.32	−6.39 ± 1.34	−5.94 ± 1.12	−6.18 ± 0.82	−7.59 ± 1.00	−7.18 ± 0.65
GRA	−7.01 ± 1.13	−6.08 ± 0.99	−7.07 ± 1.36	−7.70 ± 1.55	−6.01 ± 1.24	−6.64 ± 1.17	−6.18 ± 1.09	−6.35 ± 1.40	−5.79 ± 0.62	−5.84 ± 1.27
Arterial blood glycerol (μmol/L)										
CON	83 ± 18	71 ± 10	73 ± 12	72 ± 14	79 ± 14	94 ± 17	87 ± 18	84 ± 19	112 ± 18	99 ± 20
GRA	75 ± 19	64 ± 15	67 ± 17	64 ± 18	58 ± 16	77 ± 21	67 ± 9	58 ± 15	74 ± 11	74 ± 12
Net hepatic glycerol balance (μmol/kg per minute)										
CON	−1.49 ± 0.35	−1.39 ± 0.36	−1.32 ± 0.32	−1.48 ± 0.43	−1.54 ± 0.42	−1.79 ± 0.47	−1.84 ± 0.49	−1.92 ± 0.55	−2.49 ± 0.54	−2.06 ± 0.42
GRA	−1.47 ± 0.43	−1.28 ± 0.35	−1.31 ± 0.34	−1.25 ± 0.31	−1.35 ± 0.39	−1.56 ± 0.50	−1.48 ± 0.20	−1.26 ± 0.37	−1.77 ± 0.39	−1.59 ± 0.27
Arterial blood gluconeogenic amino acids (μmol/L)										
CON	1617 ± 135	1452 ± 115		1447 ± 114		1410 ± 90		1416 ± 93	1426 ± 118	1424 ± 121
GRA	1602 ± 92	1554 ± 88		1549 ± 80		1529 ± 149		1504 ± 134	1534 ± 121	1483 ± 154
Net GNG amino acid balance (μmol/kg per minute)										
CON	−7.04 ± 1.23	−6.01 ± 0.82		−6.96 ± 1.53		−6.82 ± 1.43		−5.86 ± 1.33	−6.21 ± 1.07	−5.07 ± 0.78
GRA	−6.37 ± 0.72	−6.89 ± 0.59		−8.44 ± 0.66		−6.68 ± 0.81		−6.74 ± 0.69	−4.84 ± 0.55	−5.52 ± 0.88

Values are means ± SEM; n = 6 per group.

Table 4
Arterial plasma level and net hepatic uptake for FFAs and arterial blood level and net hepatic output for ketones during the basal (−40 to 0 minutes) and experimental (0-270 minutes) periods in the CON and GRA groups

	Time (min)									
	−40	0	30	60	90	120	150	180	240	270
Arterial plasma FFAs (μmol/L)										
CON	594 ± 133	499 ± 92	617 ± 124	570 ± 128	597 ± 100	581 ± 76	604 ± 71	677 ± 100	780 ± 107	754 ± 89
GRA	587 ± 83	495 ± 75	555 ± 102	486 ± 82	533 ± 91	551 ± 110	534 ± 80	542 ± 110	578 ± 107	622 ± 116
Net hepatic FFA uptake (μmol/kg per minute)										
CON	−1.54 ± 0.61	−1.97 ± 0.64	−1.72 ± 0.92	−2.07 ± 1.00	−2.50 ± 1.07	−1.58 ± 0.90	−1.63 ± 0.59	−1.85 ± 0.83	−2.15 ± 0.70	−1.51 ± 0.88
GRA	−2.28 ± 0.69	−2.21 ± 0.48	−2.02 ± 0.61	−2.32 ± 0.69	−1.81 ± 0.30	−1.93 ± 0.64	−2.46 ± 0.76	−2.68 ± 0.75	−2.39 ± 0.74	−2.08 ± 0.91
Arterial blood ketones (μmol/L)										
CON	203 ± 51	205 ± 60		203 ± 59		211 ± 72		219 ± 66	227 ± 71	238 ± 76
GRA	146 ± 10	116 ± 13		134 ± 18		123 ± 14		144 ± 23	135 ± 25	157 ± 17
Net hepatic ketone output (μmol/kg per minute)										
CON	2.72 ± 0.39	2.26 ± 0.95		2.14 ± 0.79		2.84 ± 0.86		3.28 ± 1.40	4.01 ± 1.47	3.66 ± 1.60
GRA	3.58 ± 1.01	2.72 ± 0.93		2.33 ± 0.59		2.89 ± 0.69		3.05 ± 1.09	3.39 ± 1.21	3.69 ± 1.33

Values are means ± SEM; n = 6 per group.

was observed and net hepatic glycogenolysis tended to decrease, GNG carbon may have been diverted into glycogen instead of being released from the liver as glucose. Such a change could be mediated by a reduction in G6Pase flux [5,7] or by changes in flux through glycogen synthase and/or phosphorylase.

Although cortisol activates glycogen synthase [31], glycogen phosphorylase has also been shown to be activated by the hormone, resulting in net glycogen breakdown [32]. In addition, cortisol may enhance glucagon's effect on glycogenolysis. Previous studies in the dog demonstrated that long-term elevation of cortisol (5 days/5-fold) augmented whole-body glucose production, with increased glucose cycling through glycogen and hepatic GNG as a result of enhanced substrate delivery to the liver [33]. Interpretation of the results in that study is confounded, however, by higher insulin levels in the cortisol group compared with the control group. In another study, long-term cortisol infusion was followed by insulin deficiency [34]. The resulting HGP was greater in the presence of cortisol as a result of increased glycogenolysis, although baseline hepatic glycogen content may have been greater in the treatment group, which could have impacted the glycogenolytic response. Taken together, these data suggest that cortisol can increase HGP through effects on glycogen turnover. Although not significantly different, after GRA in the present study, net hepatic glycogenolysis was 0.67 mg/kg per minute lower compared with the control group.

Because cortisol's effects on enzyme activity are mainly transcriptionally mediated, it is not surprising that the reduction in HGP was not significant until 3 hours after antagonism of the GR. Through the interaction of the GR and PGC-1, GCs can increase the expression of PEPCK and G6Pase and thus raise glucose production [35]. In rats treated with the same dose of A-348441 used in the present study, GC induction of G6Pase messenger RNA was blocked by 60% [23]. Although no significant change in GNG flux to G6P was observed, it is possible that GNG substrate delivery rather than PEPCK activity was rate limiting to flux through the GNG pathway. In that case, even if GRA reduced the expression of PEPCK, the effect of GRA on GNG flux to G6P would have been minimal because the effects of A-348441 are selectively targeted to the liver and no change in hepatic GNG substrate delivery occurred (Table 3). Although glucose production was reduced by about 50%, the effects of antagonizing the GR on glucose production may have been greater at higher doses of A-348441, with long-term treatment, or in individuals with type 2 diabetes mellitus, in whom gluconeogenesis and HGP are inappropriately elevated compared with healthy individuals [36].

Whereas unconjugated RU-486 increases plasma cortisol and ACTH levels [12,23], the levels of these hormones were not affected by A-348441, suggesting hepatic selectivity. In addition, because the GR is expressed in gastrointestinal mucosal cells [37], antagonism of gut GR may have occurred. Although the gut has the capacity to produce glucose [38], gut glucose release was not observed in either group. In addition, the GR is also expressed in the kidney [39], and although renal GNG may account for a small portion of whole-body glucose production [40], net renal glucose balance remained near zero throughout the experiment in both groups. These results suggest that if gut or kidney GR antagonism occurred there was no perceptible effect on glucose production by those organs. Likewise, tracer-determined whole-body glucose utilization and non-hepatic glucose uptake were not affected by the treatment with the antagonist despite the ability of cortisol to decrease glucose utilization in muscle. Cortisol activates lipolysis, but there was no change in arterial plasma FFA or glycerol levels between groups. In addition, arterial epinephrine and norepinephrine levels were not affected by treatment. Thus, these results suggest that in the dog, bile acid conjugation successfully limited the effects of GRA to the liver. Although cholic acid itself has been shown to inhibit the expression of GNG genes by antagonism of the GR

[41,42], the amount of bile acid that accompanies A-348441 would represent a very small percentage of overall plasma cholic acid and thus would not likely contribute to the therapeutic effect.

In summary, this study demonstrates that the GR plays an important role in the regulation of basal HGP and thus represents a significant potential therapeutic target in the treatment of diabetes.

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