Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 55 (2006) 1255–1262
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Selective antagonism of the hepatic glucocorticoid receptor reduces hepatic glucose production

Dale S. Edgerton[a,*], Peer B. Jacobson[b], Terry J. Opgenorth[b], Bradley Zinker[b], David Beno[b], Tom von Geldern[b], Lars Ohman[c], Melanie Scott[a], Doss Neal[a], Alan D. Cherrington[a]

[a]*Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232, USA*
[b]*Abbott Laboratories, Abbott Park, IL, USA*
[c]*Karo Bio AB, Huddinge, Sweden*

Received 16 March 2006; accepted 29 May 2006

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

This work was presented in part at the 65th Annual Meeting of the American Diabetes Association, New Orleans, LA, June 2003.

* Corresponding author. Molecular Physiology and Biophysics, Vanderbilt University Medical Center, 710 RRB, 2200 Pierce Ave, Nashville, TN 37232-0615. Tel.: +1 615 322 7014; fax: +1 615 322 1462.
E-mail address: dale.edgerton@vanderbilt.edu (D.S. Edgerton).

0026-0495/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2006.05.011

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