Original article

MOLECULAR METABOLISM

A gut—brain neural circuit controlled by intestinal gluconeogenesis is crucial in metabolic health

Maud Soty [1,2,3], Armelle Penhoat [1,2,3], Marta Amigo-Correig [1,2,3], Jennifer Vinera [1,2,3], Anne Sardella [1,2,3], Fanny Vullin-Bouilloux [1,2,3], Carine Zitoun [1,2,3], Isabelle Houberdon [1,2,3], Gilles Mithieux [1,2,3,*]

ABSTRACT

Objectives: Certain nutrients positively regulate energy homeostasis via intestinal gluconeogenesis (IGN). The objective of this study was to evaluate the impact of a deficient IGN in glucose control independently of nutritional environment.
Methods: We used mice deficient in the intestine glucose-6 phosphatase catalytic unit, the key enzyme of IGN (I-*G6pc*$^{-/-}$ mice). We evaluated a number of parameters involved in energy homeostasis, including insulin sensitivity (hyperinsulinemic euglycaemic clamp), the pancreatic function (insulin secretion *in vivo* and in isolated islets) and the hypothalamic homeostatic function (leptin sensitivity).
Results: Intestinal-*G6pc*$^{-/-}$ mice exhibit slight fasting hyperglycaemia and hyperinsulinemia, glucose intolerance, insulin resistance and a deteriorated pancreatic function, despite normal diet with no change in body weight. These defects evoking type 2 diabetes (T2D) derive from the basal activation of the sympathetic nervous system (SNS). They are corrected by treatment with an inhibitor of α-2 adrenergic receptors. Deregulation in a key target of IGN, the homeostatic hypothalamic function (highlighted here through leptin resistance) is a mechanistic link. Hence the leptin resistance and metabolic disorders in I-*G6pc*$^{-/-}$ mice are corrected by rescuing IGN by portal glucose infusion. Finally, I-*G6pc*$^{-/-}$ mice develop the hyperglycaemia characteristic of T2D more rapidly under high fat/high sucrose diet.
Conclusions: Intestinal gluconeogenesis is a mandatory function for the healthy neural control of glucose homeostasis.
© 2014 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords Intestinal gluconeogenesis; Insulin sensitivity; Insulin secretion; Autonomous nervous system; Hypothalamus; Type 2 diabetes

1. INTRODUCTION

The recent worldwide increase in the prevalence of obesity and associated pathologies like type 2 diabetes (T2D) is a public health issue of major importance [36]. Faced with this epidemic, it becomes crucial to develop knowledge to ensure better prevention and treatment of obesity and T2D. T2D is a metabolic disorder characterized by chronic hyperglycemia in a context of insulin resistance (IR) and impaired insulin secretion [15,19]. Insulin resistance is linked to the inefficiency of insulin to stimulate glucose uptake by adipose tissue and skeletal muscles, and to suppress endogenous glucose production (EGP) from glucose-producing organs. On the other hand, T2D patients exhibit basal hyperinsulinemia, at least partially compensating IR, associated with anomalies in glucose-stimulated insulin secretion (GSIS) [9,15]. Lastly, a general hallmark of IR and T2D is linked to increased levels of plasma epinephrine [17,28]. These increased levels reflect the chronic activation of the sympathetic nervous system (SNS). This might play an important role in the onset of T2D, since an increased plasma epinephrine level may impair GSIS [2] and an augmentation of EGP, by stimulating both gluconeogenesis [1,5] and glycogenolysis [3].

In this context, intestinal gluconeogenesis (IGN) is a function shown to regulate energy homeostasis in fed post-absorptive states (for a review see [29]). The induction of IGN results in the release of glucose in the portal vein. Its detection by a portal glucose sensor, recently identified as the sodium glucose co-transporter 3, and the transmission of this signal to the brain by the peripheral neural system, initiate a neural gut—brain axis with benefits for energy homeostasis [37,40]. It has been suggested that this takes place in particular nutritional situations, such as when eating a protein-enriched diet [21,30,38], a fiber-enriched diet [41], and after gastric bypass surgery [27]. It is noteworthy that the increase in IGN is associated with a marked improvement in insulin sensitivity of EGP from the liver [27,30]. In particular, the role of IGN in glucose control under stimulation conditions has been proven by experiments in which the periportal neural afferents were inactivated, since improvements in insulin sensitivity are blunted after such inactivation [27,41]. However, these metabolic benefits might also be at least partially ascribed to the decreased body weight associated with these nutritional situations.
Here, to assess the specific role of IGN in glucose homeostasis, i.e. independently of its activation by the nutritional environment, we used

[1]Institut National de la Santé et de la Recherche Médicale, U855, Lyon, F-69008, France [2]Université de Lyon, Lyon, F-69008, France [3]Université Lyon1, Villeurbanne, F-69622, France

*Corresponding author. Inserm U855, Faculté de Médecine Laennec, 7 rue Guillaume Paradin, 69372, Lyon cedex 08, France. Tel.: +33 478 77 10 28; fax: +33 478 77 87 62. E-mail: gilles.mithieux@univ-lyon1.fr (G. Mithieux).

Received December 5, 2014 • Revision received December 17, 2014 • Accepted December 18, 2014 • Available online 26 December 2014

http://dx.doi.org/10.1016/j.molmet.2014.12.009

 © 2014 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

mice with a tamoxifen-inducible and intestine-specific deletion of the catalytic subunit (*G6pc*) of glucose-6 phosphatase (G6Pase, the key enzyme of gluconeogenesis) (I-$G6pc^{-/-}$ mice) [34]. We first characterized glucose tolerance, insulin sensitivity and the pancreatic function in I-$G6pc^{-/-}$ mice, in the context of a normal chow diet. We then investigated the roles of the autonomic nervous system and the homeostatic hypothalamic function, a key target of IGN in normal animals, in the defects observed. Finally, we tested whether the absence of IGN could accelerate the development of T2D with a high fat/high sucrose diet (HF/HS).

2. METHODS

2.1. Animals, diets and treatments

I-$G6pc^{-/-}$ mice were generated as described previously [34] and the experiments were performed 5 weeks after gene deletion. We used only male adult I-$G6pc^{-/-}$ and I-$G6pc^{+/+}$ control (C57Bl/6J, Charles River Laboratories, France) mice for the present studies. All the mice were housed in the animal facility of Lyon 1 University ("Animalerie Lyon Est Conventionnelle" and "Specific Pathogen Free") under controlled temperature (22 °C) conditions, with a 12-h light/dark cycle. All mice had free access to water. The standard diet was from SAFE (Augy, France) and the High-Fat/High Sucrose diet (consisting of 36.1% fat, 35% carbohydrates (50% maltodextrine + 50% sucrose), 19.8% proteins) was produced at the "Unité de Préparation des Aliments Expérimentaux" (UE0300 INRA, Jouy-en-Josas, France). All the procedures were performed in accordance with the principles and guidelines established by the European Convention for the protection of Laboratory Animals. The regional animal care committee (CREEA, CNRS, Rhône-Alpes Auvergne, France) approved all the experiments. For yohimbine treatment, mice were subcutaneously injected with yohimbine (2 mg/kg body weight, TOCRIS Bioscience) or vehicule (saline) once a day for 15 days. A fresh sterile solution of yohimbine was prepared every day.

2.2. Glucose and insulin tolerance tests

Glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed in 16 h- or 6 h-fasted mice. Animals received an intraperitoneal injection of glucose (1 g/kg b.w.) or insulin (0.5 U/kg b.w., Insulatard, Novo Nordisk). Blood glucose was monitored for 120 min using a glucometer (Accu-Check, Roche) on blood samples collected from the tail vein. Insulin and glucagon were quantified using Mouse Insulin or Glucagon Elisa kits (Mercodia).

2.3. Insulin release

Animals were fasted for 16 h and then received an intraperitoneal injection of glucose (3 g/kg b.w., ip). Blood was withdrawn from the tail vein at 0, 3, 6 and 12 min after injection for insulin assessment.

2.4. Leptin tolerance tests

Animals were fasted for 16 h and then received an intraperitoneal injection of leptin (1 mg/kg b.w., Enzo Life Science®) or vehicle (Nacl). In the first procedure, animals were refed and food intake was measured at 1 and 3 h after refeeding. In the second procedure, mice were euthanized 30 min after injection by cervical dislocation before hypothalamus sampling.

2.5. Hyperinsulinemic euglycaemic clamp

Clamp was performed in conscious, unrestrained, catheterized mice. A catheter was inserted into the right jugular vein under anesthesia (2% isoflurane), and mice were allowed to recover for 7 days. After 6 h of fasting, blood was withdrawn from the tail vein to measure glycemia (with an Accu-Check glucometer, Roche). A bolus of [3-^{3}H] glucose (2.5 μCi, specific activity 0.74 TBq/mmol, Perkin Elmer, Boston, USA) and insulin (1.25 mU) was administered via the catheter to the mice. We then infused [3-^{3}H] glucose (0.17 μCi) and insulin (3 mU/kg/min) at a rate of 1 μL/min in the jugular vein. In the same way, euglycemia was maintained through a variable infusion of 15% (weight/volume) glucose. Glycemia was monitored every 20 min until the end of the clamp. After 120 min infusion, 20 μL of blood sampled from the tail vein was collected to determine [3-^{3}H] glucose specific activity, as described previously [23,27]. We checked that a steady state in plasma glucose and [3-^{3}H] glucose specific activity was established at this time, as previously reported [27]. Mice were euthanized by cervical dislocation before liver sampling for glycogen determination. GIR, EGP and Rd were determined using radioactivity and glycemia values, as described previously [27].

2.6. Portal glucose infusion

A catheter was inserted into the portal vein under isoflurane anesthesia (2%), and mice were allowed to recover for 7 days. A 0.9% saline or 20% glucose (Laboratoire Aguettant) was infused at 40 μmol/kg/min for 6 h. In the GTT experiments, mice received infusion in the portal vein during the last 7 h of fasting and GTT was performed at the end of the infusion as described above. In the clamp procedure, the portal infusion was performed during the prior fasting period and maintained during the clamp. In the insulin secretion experiments, pancreatic islets were isolated after 7 h of glucose infusion and insulin secretion was measured as described in the section "insulin secretion" [35]. In the study relating to leptin sensitivity, portal glucose infusion was carried out for 24 h. At the end of the infusion, mice received an intraperitoneal injection of leptin or vehicle and the leptin tolerance tests were performed as described above.

2.7. Western blot analysis

Tissues were rapidly sampled in liquid nitrogen and stored at −80 °C before analysis. Whole cell extracts from tissues were lysed in standard lysis buffer at 4 °C. Aliquots of 30 μg proteins were separated by 9%-SDS polyacrylamide gel electrophoresis and transferred to PVDF Immobilon membranes (Millipore). The membranes were probed with antibodies diluted in TBS/0.2% Tween/5% BSA against TH, pAkt Ser473, Akt, pSTAT3 and STAT3 (dilution 1:2000, Cell Signaling) and then with goat secondary anti-rabbit IgG linked to peroxidase (dilution 1:10 000, Biorad). The intensity of the spots was determined by densitometry with the VersaDoc™ system (Biorad) and analyzed using the Quantity One® software (Biorad).

2.8. Insulin secretion

Islets were obtained from mice, isolated through collagenase perfusion and Histopaque gradient (Sigma) and cultured, as described previously [10,35]. Eight islets were pre-incubated in HEPES-buffered Krebs–Ringer bicarbonate medium without glucose for 30 min and then replaced for 1 h in the same medium supplemented with 5.5 or 16.7 mM glucose at 37 °C with 5% CO2. At the end of the experiment, the supernatant was recovered to measure the insulin released, and islets were extracted with acid-ethanol solution to measure insulin content.

2.9. Immunohistochemistry

For the morphometric analysis, tissue samples were fixed in 10% buffered formalin and embedded in paraffin. Four μm-thick tissue sections were prepared according to conventional procedures.

 © 2014 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Download English Version:

https://daneshyari.com/en/article/3001619

Download Persian Version:

https://daneshyari.com/article/3001619

Daneshyari.com